European Journal of Cancer 197 (2024) 113472

Contents lists available at ScienceDirect

European Journal of Cancer

journal homepage: www.ejcancer.com

Original research

Midgut neuroendocrine tumor patients have a depleted gut microbiome with a discriminative signature

M.C.F. Mulders [a,*,1], A.S. Audhoe [a], P.M. Van Koetsveld [a], R.A. Feelders [a], L.J. Hofland [a], W.W. de Herder [a], R. Kraaij [b], J. Hofland [a]

[a] ENETS Center of Excellence, Section of Endocrinology, Department of Internal Medicine, Erasmus Medical Center and Erasmus Medical Center Cancer Institute, Rotterdam, the Netherlands

[b] Laboratory of Population Genomics, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
Gut microbiome
Neuroendocrine tumor
Carcinoid syndrome
Diagnosis
Biomarkers

ABSTRACT

Rationale: When compared to other types of cancer, the prevalence of midgut neuroendocrine tumors (NET) has disproportionally increased over the past decades. To date, there has been very little progress in discovering (epi) genetic drivers and treatment options for these tumors. Recent microbiome research has revealed that enteroendocrine cells communicate with the intestinal microbiome and has provided novel treatment targets for various other cancer types. Hence, our aim was to analyze the role of the gut microbiome in midgut NET patients.
Methods: Fecal samples, prospectively collected from patients and control subjects, were analyzed with next generation 16S sequencing. Patients with neuroendocrine carcinomas and recent antibiotics use were excluded. Relevant variables were extracted from questionnaires and electronic health records. Microbial composition was compared between patients and controls as well as between groups within the patient cohort.
Results: 87 midgut NET patients and 95 controls were included. Midgut NET patients had a less rich and diverse gut microbiome than controls ($p < 0.001$). Moreover, we identified 31 differentially abundant species and a gut microbial signature consisting of 17 species that was predictive of midgut NET presence with an area under the receiver operating characteristic curve of 0.863. Gut microbial composition was not directly associated with the presence of the carcinoid syndrome, tumor grade or multifocality. Nonetheless, we did observe a potential link between microbial diversity and the presence of carcinoid syndrome symptoms within the subset of patients with elevated 5-hydroxyindolacetic acid levels.
Conclusion: Midgut NET patients have an altered gut microbiome which suggests a role in NET development and could provide novel targets for microbiome-based diagnostics and therapeutics.

1. Introduction

Neuroendocrine tumors (NET) are a diverse group of neoplasms that predominantly arise from the embryonic gut, subdivided into foregut, midgut and hindgut NET [1]. NET incidence is estimated at 7.0–8.8 per 100,000 persons and has increased 3.7- to 6.4-fold over the previous 4 decades, which is a disproportionate increase when compared to the age-adjusted incidence of all malignant neoplasms [2–4]. Even though this disproportionate increase in NET incidence is in part attributable to increased awareness and improved diagnostic modalities, a role for environmental drivers of NET development cannot be excluded. This relationship is supported by the differences in the primary origin distribution of NET across the globe. While hindgut and foregut are the leading primary sites of NET in Asia, midgut NET are most prevalent in western countries [4,5]. Recent whole-genome analysis results provide further evidence for environmental drivers of NET development, as they revealed that advanced NET have the lowest mutational burden of a wide range of solid tumors [6].

Approximately one-third of midgut NET patients suffers from the carcinoid syndrome (CS), defined by chronic diarrhea and/or flushing in the presence of systemic elevated levels of serotonin or its metabolite 5-hydroxyindolacetic acid (5-HIAA) [7,8]. While the carcinoid syndrome

* Correspondence to: Section of Endocrinology, Department of Internal Medicine, Erasmus Medical Center, Dr. Molewaterplein 40, 3015 GD Rotterdam, the Netherlands.
E-mail address: m.mulders@erasmusmc.nl (M.C.F. Mulders).
[1] Twitter handle: @merijn_mulders

https://doi.org/10.1016/j.ejca.2023.113472
Received 3 July 2023; Received in revised form 28 November 2023; Accepted 30 November 2023
Available online 9 December 2023

is associated with a limited quality of life [9], its pathophysiology is incompletely understood. Unfortunately, very little progress has been made in the discovery of etiological factors and treatment options for patients with the CS.

Across various cancer types, studies of microbial composition have uncovered novel insights and viable treatment targets [10]. However, only two studies have analyzed the microbial composition of midgut NET patients, one in a small, mixed population [11], the other through dated techniques [12]. There is substantial evidence that microbes and microbial metabolites regulate neuroendocrine cell function in the gastro-intestinal system [13]. Specifically, microbial metabolites directly influence hormonal secretion from enterochromaffin cells, which are the predominant neuroendocrine cell type of the small intestine [14,15]. Given the interrelationship between the microbiome and enteroendocrine cells, it is possible that the gut microbiome directly influences the development or behavior of NET. Therefore, our aim was to study the composition of the gut microbiome in a large series of midgut NET patients and its association with clinical outcomes.

2. Materials and methods

Samples were prospectively recruited from patients between May 2019 and January 2022 at the Erasmus MC (Rotterdam, The Netherlands), an ENETS Center of Excellence. Patients were deemed eligible if they were above 18 years old and had a histologically verified diagnosis of a midgut NET. Exclusion criteria included the use of antibiotics within 3 months before the sampling date and having a neuroendocrine carcinoma or a NET of appendiceal primary origin. Controls were recruited from the social network of the patient, preferably from the same household. Ethical approval for this study was obtained from the local Medical Ethics Committee (IRB protocol MEC-2018–1512) and written informed consent was acquired from all included patients.

Subjects were instructed to collect their stool samples with the OMNIgene Gut sample collection kit (OMR-200, DNA Genotek, Ottawa, Canada) at home and to send them to our laboratory by regular mail. Upon arrival, these samples were immediately stored at − 80 °C. Epidemiological and lifestyle data were collected from dedicated questionnaires and clinical data was retrieved from electronic patient records. Data was missing in 5.5% of cases and was imputed using a random-forest based algorithm for missing data imputation called missForest (n = 100 trees) [16]. The imputation accuracy was high according to the imputation error estimate (mean out-of-bag error = 0.08).

This cohort study was conducted and is reported according to the 'Strengthening The Organization and Reporting of Microbiome Studies' (STORMS) guideline [17]. The technical and statistical aspects of the analyses performed in this article will be provided in the supplemental materials. Model building and model evaluation was performed using the SIAMCAT R package v.1.12.0 [18]. All data analyses were conducted in R Studio v4.1 or higher [19]. A two-tailed p-value of < 0.05 was considered significant. For the sake of reproducibility, the analysis code and exact consensus sequence of our ASVs will be shared in a data repository. The data generated in this study are not publicly available due to patient privacy requirements but are available upon reasonable request from the corresponding author.

3. Results

3.1. Study population

218 subjects met our inclusion criteria. After exclusion of one appendix NET patient and samples with less than 15,000 reads, a total of 87 patients and 95 controls were included in the analysis. 90 subjects were eligible for the household-matched analysis. Baseline characteristics of the entire cohort are shown in Table S1.

3.2. Midgut NET patients have a less rich and diverse gut microbiome when compared to controls

Analysis of within-sample microbial diversity (α-diversity) as well as microbial community composition (β-diversity) revealed that the gut microbiome of midgut NET patients was less rich and diverse compared to that of controls ($p < 0.001$ for α-diversity and for β-diversity on genus and species level) (Fig. 1). This difference was upheld in the household-matched β-diversity analysis and after correction for the effect of BMI, age, gender, DNA extraction batch, previous gastrointestinal surgery or inflammatory bowel disease, and use of proton pump inhibitors, laxatives, motility inhibitors and statins ($p < 0.001$ for both the household matched and multivariate analysis). When studying individual bacterial species, 8 species were enriched and 23 species were depleted in midgut NET patients compared to controls. Species with consistently low p-values or strong effect sizes across all analyses included the *Veillonella (atypica and unknown strain)* and *Streptococcus (unknown strain)*, which were enriched in feces of midgut NET patients, and the *Clostridia UCG-014, Faecalibacterium (unkown strain)* and *Christensenellaceae R-7 group*, which were depleted (Table S2).

3.3. A gut microbial signature predictive of the presence of a midgut NET

Using a LASSO regression technique, 17 species were selected for development of a midgut NET-specific microbial signature (Fig. 2A). The resulting model accurately distinguished midgut NET patients from controls (AUROC = 0.863) (Fig. 2B). The most discriminative features for being a patient *versus* a control were enrichment of *Veillonella atypica* and *Erysipelatoclostridium ramosum* and depletion of *Faecalibacterium (unknown strain)* and *Paludicola (unknown strain)*. A model that only considered species that were either more abundant or depleted in patients did not improve discriminative performance (AUROC = 0.834 and 0.826, respectively). None of the ten included covariates were selected as predictive features by the model, indicating that the microbial signature was more discriminative than any other feature. Furthermore, none of these covariates were individually, *i.e.* independent of disease status, associated with the microbial species selected by the model, ruling them out as potential confounders (Fig. S1). These results suggest that the created microbial signature is likely independent of potential confounders of microbial composition.

3.4. Association with clinical parameters in midgut NET patients

When comparing 53 patients with CS to 34 patients without this syndrome, no differences in α-diversity or β-diversity were observed ($p > 0.05$ for α-diversity and for β-diversity on genus and species level). Correcting for the effect of BMI, age, gender and DNA extraction batch did not impact these findings ($p > 0.05$ for both analyses). Moreover, no specific species were found to be differentially abundant in patients with CS in more than one analysis method. However, among the 66 patients with an elevated urinary excretion of 5-HIAA (>50 mcmol/24 h), we did observe a difference in microbial composition when comparing 42 patients with CS symptoms of diarrhea or flushing to 24 patients without any CS symptoms at baseline ($p = 0.019$ and $p = 0.041$ on genus and species level, respectively) (Fig. 3). Although no bacteria were marked as differentially abundant by all four analyses, depletion of *Parasutterella* and *Oscilibacter* was associated with the presence of CS symptoms in both the Maaslin2 and Wilcoxon analysis. The inclusion of urinary 5-HIAA levels and use of somatostatin analogs (SSA) as covariates did not alter these results.

No differences in microbial composition or species abundance were observed between patients with grade 1 and 2 NET or patients with and without a multifocal NET ($p > 0.05$ for α-diversity and for β-diversity on genus and species level).

Lastly, we explored the possibility that SSA use was confounding our analysis. Although the use of SSA was associated with a difference in

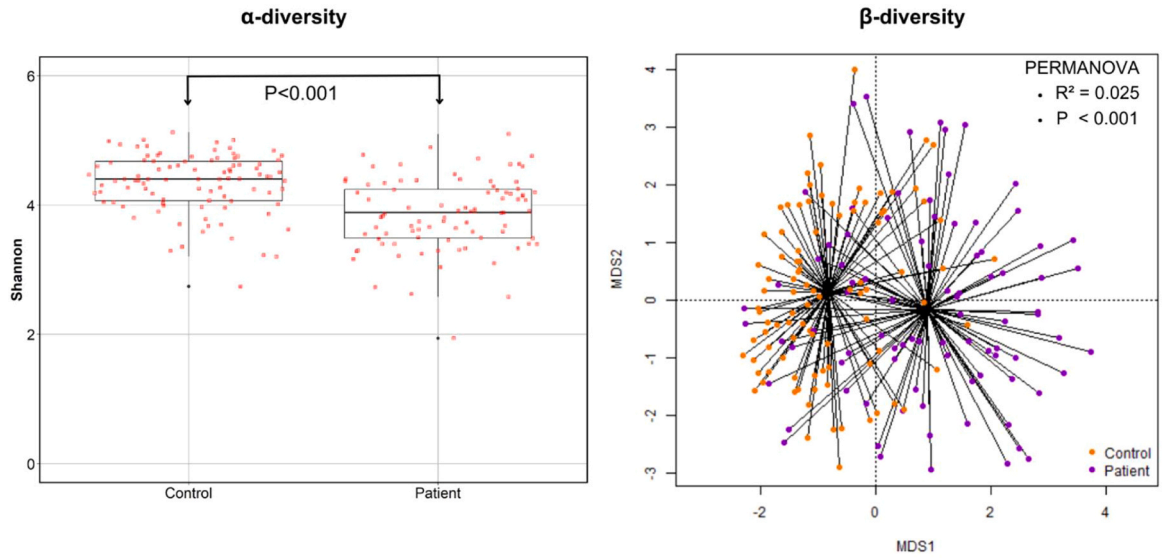


Fig. 1. Gut microbial composition of midgut NET patients compared to controls. p-values indicate the difference in Shannon index between the two groups for α-diversity (Wilcoxon ranked-sum test) and the difference in microbial community composition between the two groups for β-diversity (PERMANOVA test). R^2 indicates the variance explained by the covariate. PERMANOVA = permutational analysis of variance.

β-diversity ($p = 0.003$ on both genus and species level), no differences were observed in the α-diversity analysis ($p = 0.087$) and no differentially abundant species were identified (Fig. 3). Moreover, comparing the microbial composition of patients who did not use SSA with that of controls still resulted in similar differences ($p = 0.003$ for α-diversity and $p = 0.008$ and 0.003 for β-diversity on genus and species, respectively). Also, the use of SSA was not individually associated with any of the microbial species included our microbial signature.

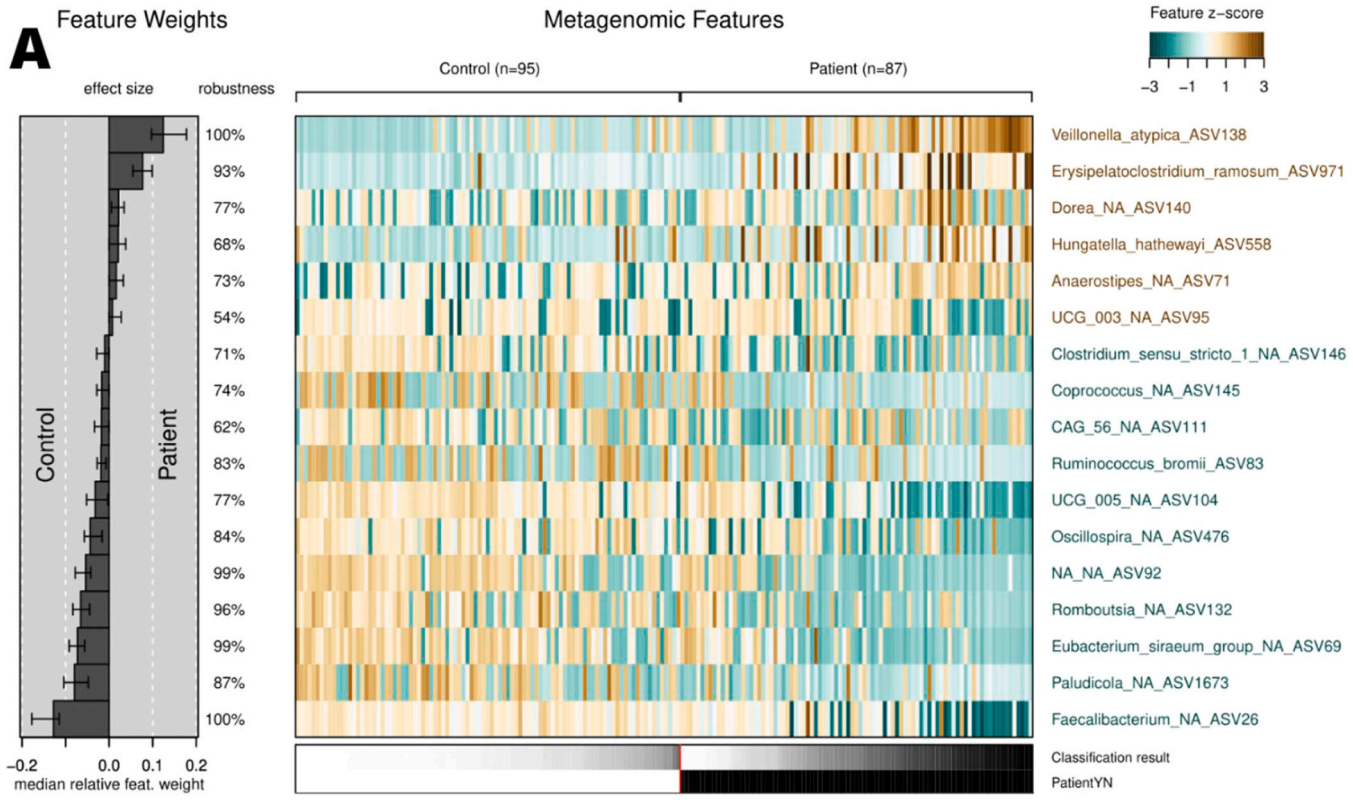
4. Discussion

As recent epidemiologic and genetic studies point towards a role of environmental drivers of NET development, this study set out to analyze the composition of the gut microbiome in midgut NET patients. We found that the gut microbiome of midgut NET patients was less rich and diverse compared to that of controls, while a total of 31 differentially abundant species could be identified. A gut microbial signature consisting of 17 species was found to be predictive of the presence of a midgut NET with high discriminative performance.

A depleted gut microbiome of patients as compared to controls has been frequently described for a wide variety of cancers, including gastric, colorectal, biliary and liver cancer [20]. Although many cancer microbiome studies are associative in nature, emerging evidence from *in vitro* and *in vivo* studies strongly suggests that an aberrant gut microbiome contributes to oncogenesis by negatively affecting either host immune responses or host metabolism [20]. To the best of our knowledge, only three studies exist that have analyzed the role of the gut microbiome in NET patients. Although all three studies reported aberrant depletion of microbial species, dated techniques [12], heterogeneous cohorts [11,12] and small sample sizes [11,21] limit the comparison of their results to ours. Yet, similar to our results, depletion of the *Faecalibacterium prausnitzii* has been described in two of these studies [12,21]. This butyrate-producing species has shown potent anti-cancer activity *via* histone deacetylase inhibition and, therefore, might play a role in preventing NET development [22]. *Veillonella parvula*, a potential oncogenic species, has been shown to be enriched in lung cancer patients and to drive oncogenesis in lung cancer models [23]. In our study, both species were marked as differentially abundant in all analyses except for the ANCOM-BC. We speculate that the choice of four methods may have been too conservative and, therefore, a potential protective effect of the *Faecalibacterium prausnitzii* and an oncogenic effect of the *Veillonella parvula* in the development of midgut NET cannot be excluded. Although associative in nature, our results enable the development of *in vivo* and *in vitro* studies to further characterize microbial drivers of NET development. Once a causal link is established, clinical trials can be initiated to introduce treatment options that modulate this pathway. Currently, several interventional strategies have shown early promise as modulators of the gut microbiome, including fecal microbiome transplant, dietary interventions, prebiotic, probiotic and antibiotic treatments [24].

Another exciting development in recent microbiome research is the finding that many disease states seem to have their own specific microbial signature [18,25]. Likewise, we detected a microbial signature that was highly predictive of the presence of a midgut NET. Screening patients for such a microbial signature could lead to early disease detection through non-invasive diagnostics, increasing the chances of curative treatment and prolonged survival of midgut NET patients. However, it is important to bear in mind possible causes of bias before implementing such a signature. First, due to the single center nature of this study, our signature needs to be validated in a geographically separated cohort to ensure that it is not confounded by technical or location-specific factors. Second, the abundance or depletion of several species may also be representative of other disease states. For instance, depletion of *Faecalibacterium* spp. is common in inflammatory bowel disorders and abundance of *Veillonella atypica* has been described for pancreatic cancer [25,26]. For this reason, it is important to include disease states other than midgut NET in the validation cohort. Lastly, the relatively low prevalence of diseases like NET can lead to an increased likelihood of false positives. Therefore, further validation will require a cut-off point with near-maximum specificity and inclusion of a population with an increased *a priori* chance of having a midgut NET, such as patients with unexplained diarrhea or abdominal discomfort. In conclusion, while the future of early disease detection is within reach, multi-center collaboration is essential for the creation and validation of microbial signatures.

This microbiome study comprises of the largest cohort of midgut NET patients to date and is the first to describe a potential link to the CS. While there is sufficient evidence that hormonal secretion of enterochromaffin cells is modulated by microbial metabolites [14,15], the microbial composition of our cohort of patients with CS did not differ from those without CS. However, a potential role of the gut microbiome in the CS cannot be excluded, as we did observe differences in microbial



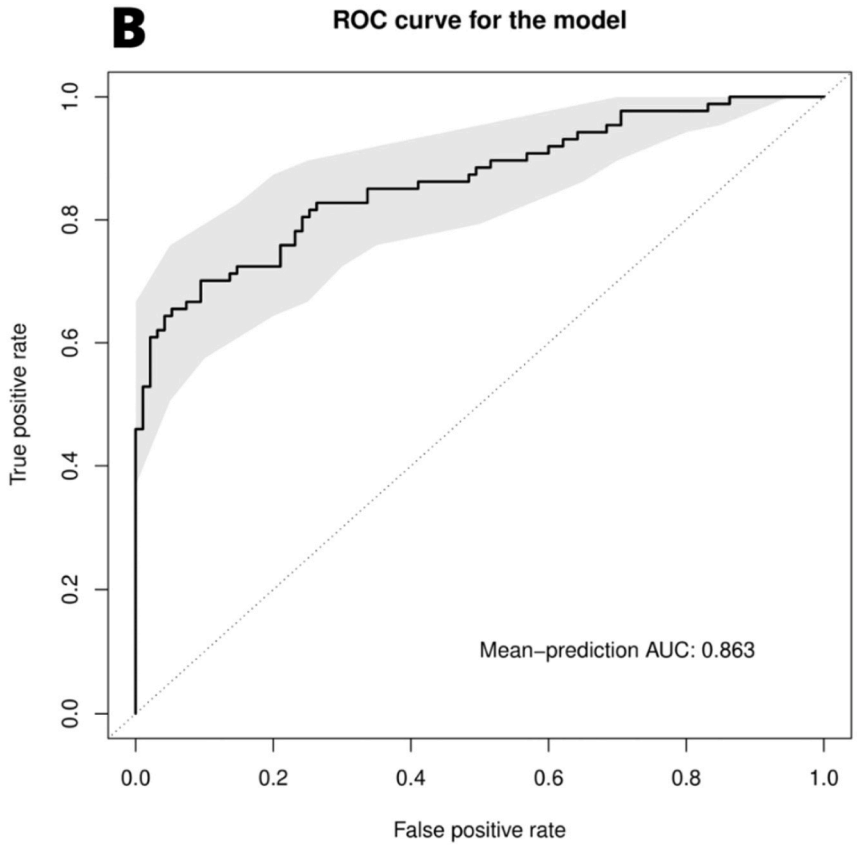


Fig. 2. Gut microbial signature predictive of a midgut NET. A: The centered panel displays the normalized abundance of the 17 selected species shown as a heat map, colors are indicative of the Z-score. The left panel represents the contribution of each selected species to the model and the robustness (percentage of models in which the feature is included as predictor) of each species. Classification scores for each individual are displayed at the bottom. ASV = amplicon sequence variant (consensus sequences are shared in the appendix), NA = not available (indicating that the exact strain name of this species was not available in the Silva database). B: Internal cross-validation results of the model shown as ROC curve. ROC = receiver operator characteristics, AUC = area under the curve.

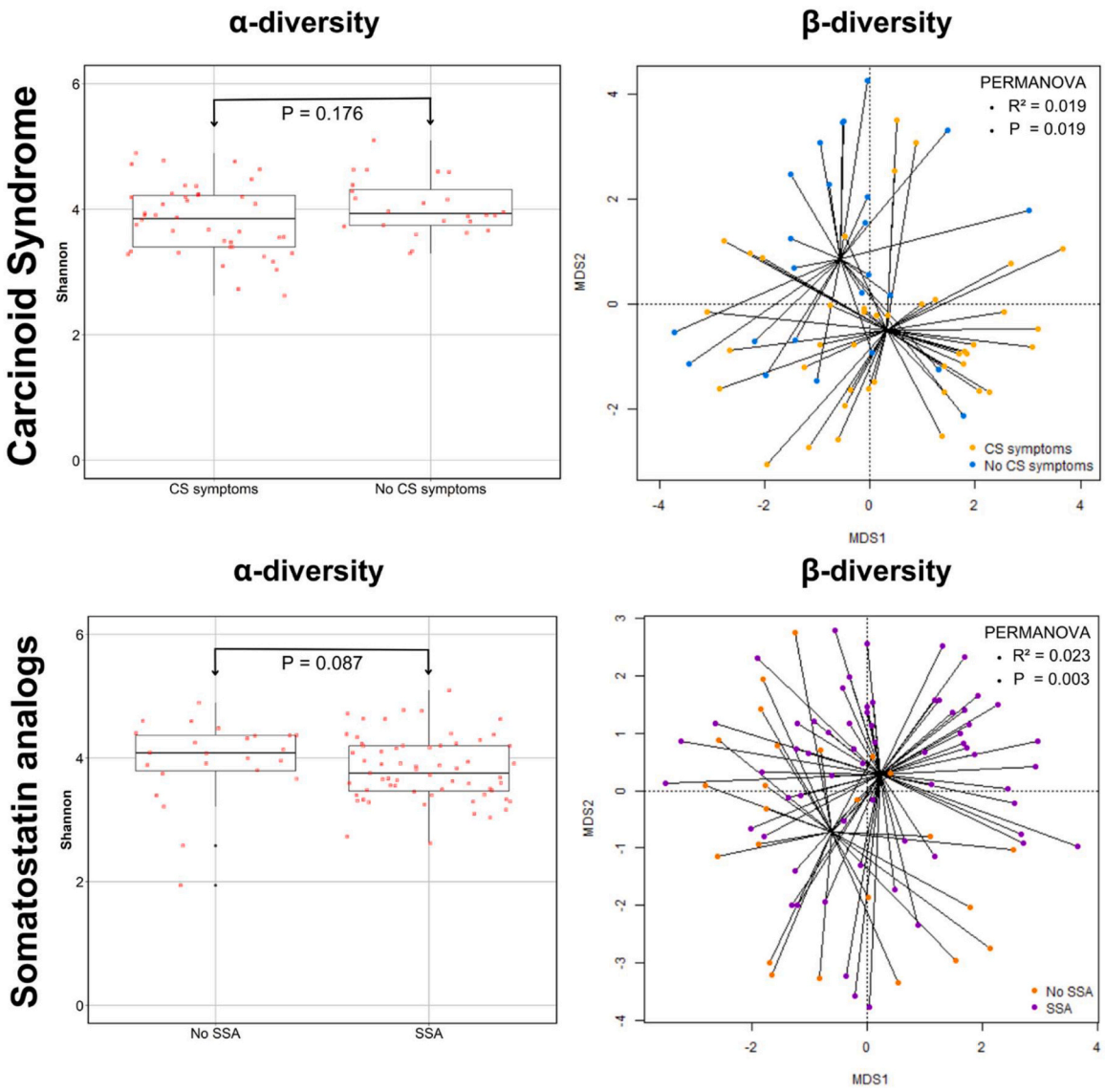


Fig. 3. Gut microbial composition of patients with versus without CS symptoms (upper panels) and patients with versus without use of SSA (lower panels). p-values indicate the difference in Shannon index between the two groups for α-diversity (Wilcoxon ranked-sum test) and the difference in microbial community composition between the two groups for β-diversity (PERMANOVA test). R^2 indicates the variance explained by the covariate. CS = carcinoid syndrome, SSA = somatostatin analogs, PERMANOVA = permutational analysis of variance.

composition when comparing patients with elevated 5-HIAA levels with and without CS symptoms at baseline. Further microbiome research, *e.g.* investigating the interaction between microbes and enterochromaffin cells, is therefore warranted to elucidate the pathogenesis of the CS. A potential target might be the *Parasutterella* genus, which was depleted in our cohort of patients with CS symptoms. Increasing the abundance of this genus by probiotics has been shown to inhibit gut motility and cause serotonin transporter upregulation in mice and intestinal epithelial cells [27].

Surprisingly, no differences were found in microbial composition of patients with and without multifocal NET. As whole genome analysis of 61 multifocal midgut NET revealed that they derive from multiple clonally independent cells, it has been suggested that these NET are caused by local, oncogenic factors [28]. Based on our results, these factors do not appear to be associated to the gut microbiome. However, due to the fact that we only included 13 patients with multifocal NET, some of whom had already received surgical or medical treatment, these results should be interpreted with caution.

5. Conclusions

Midgut NET patients have an altered gut microbiome which could suggest a role in NET development and provide novel targets for microbiome-based diagnostics and therapeutics. Multi-center collaboration is essential in order to validate these findings and translate the outcomes to the clinical setting.

CRediT authorship contribution statement

M.C.F. Mulders collected data from electronic patient records, handled the collected samples, carried out the formal data analysis and wrote the original draft of the manuscript. A.S. Audhoe collected data from electronic patient records and handled the collected samples. R. Kraaij participated in the study conceptualization and assisted with the data analysis. P.M. Van Koetsveld handled the collected samples. R.A. Feelders, L.J. Hofland and W.W. de Herder participated in the study conceptualization. R.A. Feelders, W.W. de Herder and J. Hofland performed patient recruitment. J. Hofland carried out the conceptualization

of the study. All authors reviewed the results, critically reviewed and edited the manuscript and approved the final version.

Funding

This work was supported by a pilot grant from the Neuroendocrine Tumor Research Foundation and by the Erasmus MC Foundation.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to proofread the completed manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

AA, PK, RF, LH and RK have no potential conflict of interest. MM has received a travel fee from Ipsen. JH has received speaker or consultancy fees from Novartis, Ipsen and Serb. WWH has received travel or speaker fees from Novartis, Ipsen, Camurus and Advanced Accelerator Applications, research funds from Ipsen and is on the Advisory Boards of Novartis and of Ipsen.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.113472.

References

[1] Hofland J, Kaltsas G, de Herder WW. Advances in the diagnosis and management of well-differentiated neuroendocrine neoplasms. Endocr Rev 2020;41(2):371–403.
[2] Dasari A, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol 2017;3(10):1335–42.
[3] White BE, Rous B, Chandrakumaran K, Wong K, Bouvier C, Van Hemelrijck M, et al. Incidence and survival of neuroendocrine neoplasia in England 1995-2018: A retrospective, population-based study. Lancet Reg Health Eur 2022;23:100510.
[4] Fraenkel M, Kim M, Faggiano A, de Herder WW, Valk GD, Knowledge N. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. Endocr Relat Cancer 2014;21(3):R153–63.
[5] Das S, Dasari A. Epidemiology, incidence, and prevalence of neuroendocrine neoplasms: are there global differences? Curr Oncol Rep 2021;23(4):43.
[6] Priestley P, Baber J, Lolkema MP, Steeghs N, de Bruijn E, Shale C, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. Nature 2019;575(7781):210–6.
[7] Halperin DM, Shen C, Dasari A, Xu Y, Chu Y, Zhou S, et al. Frequency of carcinoid syndrome at neuroendocrine tumour diagnosis: a population-based study. Lancet Oncol 2017;18(4):525–34.
[8] Grozinsky-Glasberg S, Davar J, Hofland J, Dobson R, Prasad V, Pascher A, et al. European Neuroendocrine Tumor Society (ENETS) 2022 Guidance Paper for Carcinoid Syndrome and Carcinoid Heart Disease. J Neuroendocr 2022;34(7):e13146.
[9] Halperin DM, Huynh L, Beaumont JL, Cai B, Bhak RH, Narkhede S, et al. Assessment of change in quality of life, carcinoid syndrome symptoms and healthcare resource utilization in patients with carcinoid syndrome. Bmc Cancer 2019;19(1):274.
[10] Sepich-Poore GD, Zitvogel L, Straussman R, Hasty J, Wargo JA, Knight R. The microbiome and human cancer. Science 2021;371(6536).
[11] Mohamed A, Asa SL, McCormick T, Al-Shakhshir H, Dasari A, Mauricio R, et al. The Role of the Microbiome in Gastroentero-Pancreatic Neuroendocrine Neoplasms (GEP-NENs). Curr Issues Mol Biol 2022;44(5):2015–28.
[12] Dörffel Y, Swidsinski A, Loening-Baucke V, Wiedenmann B, Pavel M. Common biostructure of the colonic microbiota in neuroendocrine tumors and Crohn's disease and the effect of therapy. Inflamm Bowel Dis 2012;18(9):1663–71.
[13] Lerner A, Neidhöfer S, Matthias T. The Gut Microbiome Feelings of the Brain: A Perspective for Non-Microbiologists. Microorganisms 2017;5(4).
[14] Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015;161(2):264–76.
[15] Bellono NW, Bayrer JR, Leitch DB, Castro J, Zhang C, O'Donnell TA, et al. Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. Cell 2017;170(1):185–98.
[16] Stekhoven DJ, Bühlmann P. MissForest–non-parametric missing value imputation for mixed-type data. Bioinformatics 2012;28(1):112–8.
[17] Mirzayi C, Renson A, Genomic Standards C, Massive A, Quality Control S, Zohra F, et al. Reporting guidelines for human microbiome research: the STORMS checklist. Nat Med 2021;27(11):1885–92.
[18] Wirbel J, Zych K, Essex M, Karcher N, Kartal E, Salazar G, et al. Microbiome meta-analysis and cross-disease comparison enabled by the SIAMCAT machine learning toolbox. Genome Biol 2021;22(1):93.
[19] R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: Foundation for Statistical Computing,; 2021.
[20] Liu L, Shah K. The potential of the gut microbiome to reshape the cancer therapy paradigm: a review. JAMA Oncol 2022;8(7):1059–67.
[21] Hu W, Chen ZM, Li XX, Lu L, Yang GH, Lei ZX, et al. Faecal microbiome and metabolic signatures in rectal neuroendocrine tumors. Theranostics 2022;12(5):2015–27.
[22] Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc Natl Acad Sci USA 2014;111(6):2247–52.
[23] Tsay JJ, Wu BG, Sulaiman I, Gershner K, Schluger R, Li Y, et al. Lower Airway Dysbiosis Affects Lung Cancer Progression. Cancer Discov 2021;11(2):293–307.
[24] Park EM, Chelvanambi M, Bhutiani N, Kroemer G, Zitvogel L, Wargo JA. Targeting the gut and tumor microbiota in cancer. Nat Med 2022;28(4):690–703.
[25] Kartal E, Schmidt TSB, Molina-Montes E, Rodríguez-Perales S, Wirbel J, Maistrenko OM, et al. A faecal microbiota signature with high specificity for pancreatic cancer. Gut 2022;71(7):1359–72.
[26] Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 2019;7(1).
[27] Gu Y, Wang C, Qin X, Zhou B, Liu X, Liu T, et al. Saccharomyces boulardii, a yeast probiotic, inhibits gut motility through upregulating intestinal serotonin transporter and modulating gut microbiota. Pharm Res 2022;181:106291.
[28] Elias E, Ardalan A, Lindberg M, Reinsbach SE, Muth A, Nilsson O, et al. Independent somatic evolution underlies clustered neuroendocrine tumors in the human small intestine. Nat Commun 2021;12(1):6367.