Short Communication

Suleyman Nahit Sendur, Koray Ergunay, Yakut Akyon*, Annika Brinkmann, Muhittin Serdar, Selcuk Dagdelen, Tomris Erbas, Andreas Nitsche and Engin Yilmaz

Probable alterations in fecal bacterial microbiota by somatostatin receptor analogs in acromegaly

[Akromegali olgularında somatostatin reseptör analoglarına bağlı olası fekal mikrobiyota değişiklikleri]

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Abstract

Objective: Data on bacterial diversity and microbiota alterations in acromegaly are currently lacking. The effects of somatostatin receptor analogs on gut microbiota remain unknown. The objective of this study was to determine microbiota alterations in patients with acromegaly and to assess the effects of somatostatin receptor analogs on gut microbiota.

Methods: The study was designed as a cross-sectional case-control research and three cohorts, comprising individuals with acromegaly without medical therapy (n=5), acromegaly receiving octreotide acetate (OCT) (n=8) and healthy controls (n=5), were evaluated.

Results: No statistically-supported changes in *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria* abundance were observed. *Bacteroidaceae*, *Odoribacteraceae*, *Porphyromonadaceae*, *Prevotellaceae* and *Alistipes* families of *Bacteroidetes* and *Bifidobacterium* genus of the *Actinobacteria* phyla were detected, without overt differences. Variations in *Clostridia*, *Erysipelotrichaceae* and *Veillonellaceae* were not significant, while *Lactobacillales* were increased in individuals receiving OCT. Moreover, *Akkermansia mucinophila* was present in patients under OCT treatment.

Conclusion: Our preliminary results suggest that the bacterial community profile under OCT treatment may facilitate a colonic microenvironment for improved glucose metabolism. Alterations in the gut microbiota may be a factor affecting diabetes development during somatostatin analog treatment in acromegalic patients.

Keywords: acromegaly; *Firmicutes*; *Lactobacillus*; microbiota; somatostatin receptor analog.

Suleyman Nahit Sendur and Koray Ergunay have contributed equally and shared the first authorship.

***Corresponding author: Yakut Akyon**, Department of Medical Microbiology, Hacettepe University, Faculty of Medicine, Sihhiye 06100, Ankara, Turkey, E-mail: yakyon@yahoo.com. https://orcid.org/0000-0001-6449-5450

Suleyman Nahit Sendur, Selcuk Dagdelen and Tomris Erbas, Department of Endocrinology and Metabolism, Hacettepe University, Faculty of Medicine, Ankara, Turkey. https://orcid.org/0000-0001-6740-6708 (S.N. Sendur)

Koray Ergunay, Department of Medical Microbiology, Hacettepe University, Faculty of Medicine, Ankara, Turkey

Annika Brinkmann and Andreas Nitsche, Robert Koch Institute, Center for Biological Threats and Special Pathogens 1 (ZBS-1), Berlin, Germany

Muhittin Serdar, Department of Medical Biochemistry, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey. https://orcid.org/0000-0002-3014-748X

Engin Yilmaz, Department of Medical Biology, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkey. https://orcid.org/0000-0001-8873-7645

Öz

Amaç: Akromegali olgularında bakteri çeşitliliği ve mikrobiyota değişiklikleri konusunda bilgiler kısıtlıdır. Somatostatin reseptör analoglarının barsak mikrobiotası üzerine etkileri bilinmemektedir. Bu çalışmada akromegali olgularında mikrobiyota değişikliklerinin belirlenmesi ve somatostatin reseptör analoglarının bağırsak mikrobiyotası üzerindeki etkilerinin incelenmesi amaçlandı.

Gereç ve Yöntemler: Kesitsel bir vaka-kontrol araştırması olarak planlanan çalışmada, tıbbi tedavi altında olmayan akromegali olguları (n=5), oktreotid asetat (OCT) uygulanan akromegali olguları (n=8) ve sağlıklı kontroller (n=5) değerlendirildi.

Bulgular: *Bacteroidetes, Firmicutes, Proteobacteria* and *Actinobacteria* üyelerinde, gruplar arasında istatistiksel olarak anlamlı değişiklik saptanmadı. Örneklerde *Bacteroidetes* şubesi *Bacteroidaceae*, *Odoribacteraceae*, *Porphyromonadaceae*, *Prevotellaceae* and *Alistipes* ailelerinde ve *Actinobacteria* şubesi *Bifidobacterium* cinsinde bakteriler, belirgin bir farklılık göstermeden izlendi. *Clostridia*, *Erysipelotrichaceae* ve *Veillonellaceae* üyesi bakterilerde izlenen varyasyonlar anlamlı değilken, OCT tedavisi alan olgularda *Akkermansia mucinophila* saptandı ve *Lactobacillales* üyeleri artmış olarak izlendi.

Sonuç: Ön çalışmamız, OCT tedavisi alanlarda glukoz metabolizması yönünden olumlu olarak değerlendirilebilecek bir barsak bakteri profili varlığına işaret etmektedir. Akromegali olgularında diabet gelişiminde mikrobiota değişikliklerinin de etkisi söz konusu olabilir.

Anahtar Kelimeler: Akromegali; *Firmicutes*; *Lactobacillus*; mikrobiyota; somatostatin reseptör analogları.

Introduction

Acromegaly is a chronic, progressive disorder caused by growth hormone (GH) and insulin-like growth factor-1 (IGF-1) excess [1]. If left untreated, high GH and IGF-1 levels lead to gastrointestinal complications and various colonic diseases [2]. Current information suggests an association of diversity and composition of the microbiota with diverse colonic diseases [3]. However, data on probable alterations of the gut microbiota in individuals with acromegaly is currently lacking.

Somatostatin receptor analogs are the most commonly-used medication as second-line treatment in acromegaly [4]. Although somatostatin has substantial inhibitory effects on the gastrointestinal tract, the impact of somatostatin receptor analogs on gut microbiota has not been investigated previously. Hence, this study was undertaken to characterize bacterial populations in individuals with acromegaly, to evaluate alterations in the colonic microbiota and to investigate possible modifications during somatostatin receptor analog therapy.

Materials and methods

Ethics, consent and permissions

All individuals were included in the study with written informed consent. The study were approved by the Hacettepe University institutional non-interventional clinical research ethics board (GO18/608, 21.06.2018) and performed in accordance with ethical standards of the Helsinki declaration and its later amendments.

Study design and cohorts

The study groups comprised individuals with the diagnosis of acromegaly without octreotide acetate (OCT) therapy (n=5), receiving OCT therapy (n=8) and healthy controls (n=5) (Supplementary File 1). The participants, originating from same geographic region, had no history of antibiotic use or dietary change in the last six months.

Specimen processing, 16S rDNA amplification and deep sequencing

Fecal samples were collected and subjected to DNA purification, using Qiagen Stool Mini Kit (Qiagen, Hilden, Germany) with a final DNA amount of 50 ng/μL for each specimen. For the amplification of 16S rDNA sequences, primers targeting the V3-V4 region were employed [5]. Adapter attachment, amplification and library preparation were performed using the Nextera XT Index and Nextera DNA Library Prep kits (Illumina, San Diego, California, United States). The sequencing runs were performed in the Illumina MiSeq system (Illumina Inc.).

Data handling, phylogenetic and statistical analyses

Sequence data handling and taxonomic assignment were carried out using Geneious v11.1 (Biomatters Ltd, Auckland, New Zealand), MALT v0.3.8 and Megan v6.11 [6, 7]. Alpha diversity metrics Shannon–Weaver and Simpson's reciprocal indices were calculated using Megan. The trimmed bacterial reads were mapped to the NCBI-NT RefSeq 16S database. Relative abundances of different bacterial taxonomic levels were calculated using the reads numbers of the corresponding operational taxonomic units (OTUs).

Descriptive variables and total reads, reads assigned to bacteria and bacterial phyla were assessed via statistical tests. Student's T, Kruskal–Wallis and Mann–Whitney U tests were employed for comparisons among groups where appropriate. The linear correlation between two variables was assessed via the Spearman correlation coefficient. All analyses were performed using Analyse-it software v4.20.1 (Analyse-it Software, Ltd. Leeds, United Kingdom).

Results

Clinical and descriptive data

A total of 18 individuals (14 males, 4 females) were enrolled in the study, with an age range of 28–68 years (mean: 47.8, median: 48). Patients under therapy were receiving 10–40 mg of OCT. Five of the acromegalic patients had colon polyps and none had colorectal cancer (Supplementary File 1).

Bacterial communities in control and acromegaly groups

The initial analysis of the trimmed sequences revealed no statistically-significant difference among study groups for total, bacterial and unassigned reads. In all groups, predominant bacterial species were *Bacteroidetes* and *Firmicutes* (Figure 1). Members of the *Proteobacteria* and *Actinobacteria* phyla were also identified in all specimens, despite varying relative abundances (Table 1). *Elusimicrobia*, *Spirochetes* and *Synergistetes* were identified in trace copies in patients with acromegaly.

Bacteroidetes sequences were observed to belong in *Bacteroidaceae*, *Odoribacteraceae*, *Porphyromonadaceae*, *Prevotellaceae* and *Alistipes* families, where *Bacteroidaceae* and *Porphyromonadaceae* predominate in the study groups (Figure 2). Members of these families were detected in all specimens, regardless of the study group except *Odoribacteraceae*, was absent in one individual of the control group (C3). The families with the phyla *Firmicutes* were identified as *Clostridia*, *Lactobacillales*, *Erysipelotrichaceae* and *Veillonellaceae* (Figure 3). Here, *Clostridia* were ubiquitous in all specimens (Figure 3). *Bifidobacterium* genus was evaluated separately, as presence and relative abundance of this genus also demonstrated variations in control and acromegaly groups (Table 1, Supplementary File 1). Furthermore, *Akkermansia mucinophila* was identified as the single bacterial species in the phylum *Verrucomicrobia* in the acromegaly groups.

Bacterial species detected in trace copies comprised *Elusimicrobium minutum* (phylum *Elusimicrobia*), *Treponema succinifaciens* (phylum *Spirochetes*), *Pyramidobacter piscolens* and *Cloacibacillus porcorum* (phylum *Synergistetes*) (Figure 1). These species were detected only in acromegaly groups, accompanied by *A. mucinophila* in three individuals (Supplementary File 1).

Comparative analysis of bacterial communities

Variations on the relative abundance of *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* phyla were not statistically-significant among study cohorts (Table 1). Similarly, *Bacteroidaceae*, *Odoribacteraceae*, *Porphyromonadaceae*, *Prevotellaceae* and *Alistipes* families and *Bifidobacterium* genus revealed no significant variations. At the family level, relative abundance variations of bacteria belonging in *Clostridia*, *Erysipelotrichaceae* and *Veillonellaceae* were not statistically-supported among study groups. On the contrary, *Lactobacillales* abundance was significantly elevated in patients receiving OCT (Table 1). Interestingly, when patients with detectable *A. mucinophila* sequences (n=6) were overviewed, five were observed to be receiving OCT, along with one individual without OCT and receiving metformin for diabetes mellitus (patient A5, Supplementary File 1). When this individual was omitted from the analysis, *A. mucinophila* detection became specific for the OCT group, with statistical significance (p=0.025). No significant variation was observed between Shannon diversity indices between study groups.

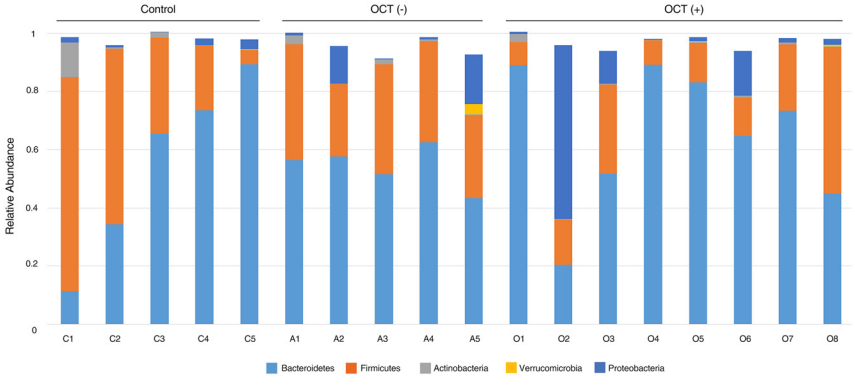

Figure 1: Bacterial community compositions are given as relative abundance at the phylum level. Study cohorts and enrolled individuals are indicated as control (C1–5), acromegaly without OCT (OCT–) (A1–5) and acromegaly with OCT (OCT+) (O1–8). Color codes assigned to each phylum are shown.

Table 1: Relative bacterial abundances and alpha diversity metrics in study groups.

	Control (n=5)		Acromegaly – OCT(–) (n=5)		Acromegaly – OCT(+) (n=8)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-Value**
Bacteroidetes	0,5480	0,3151	0,5433	0,0724	0,6456	0,2425	0,3986
Bacteroides	0,6760	0,2407	0,3548	0,3774	0,4795	0,3397	0,8273
Odoribacteraceae	0,0136	0,0209	0,0052	0,0046	0,0066	0,0043	0,7558
Porphyromonadaceae	0,0619	0,0586	0,0296	0,0171	0,0707	0,0620	0,5052
Prevotellaceae	0,1502	0,2698	0,5787	0,3964	0,3667	0,3899	0,7558
Alistipes	0,0756	0,0834	0,0111	0,0165	0,0537	0,0596	0,8242
Firmicutes	0,3888	0,2800	0,3314	0,0623	0,2032	0,1433	0,0685
Clostridia	0,7026	0,1254	0,7561	0,1110	0,7429	0,0867	0,7863
Lactobacillales	0,0100	0,0065	0,0081	0,0061	0,0223	0,0174	**0,0373**
Erysipelotrichaceae	0,0018	0,0029	0,0073	0,0104	0,0018	0,0025	0,3937
Veillonellaceae	0,2179	0,1332	0,1532	0,1263	0,1206	0,0697	0,2142
Actinobacteria	0,0283	0,0508	0,0114	0,0118	0,0062	0,0086	0,5636
Bifidobacterium	0,2590	0,2767	0,2862	0,3919	0,2525	0,3540	0,3365
Verrucomicrobia	0,0008	0,0011	0,0072	0,0161	0,0013	0,0020	0,3800
Proteobacteria	0,0162	0,0129	0,0639	0,0794	0,1156	0,2023	0,5052
Shannon–Weaver index							
Species	3,0720	0,1964	2,5706	0,4637	2,8000	1,1871	0,6251
Genus	3,8612	0,7303	3,3788	0,3774	3,0275	0,7753	0,0867
Simpson's reciprocal index							
Species	3,9514	1,0262	2,9730	0,8517	5,2435	4,9907	0,6251
Genus	6,3998	3,2705	5,3804	1,5699	4,6554	2,0375	0,2735

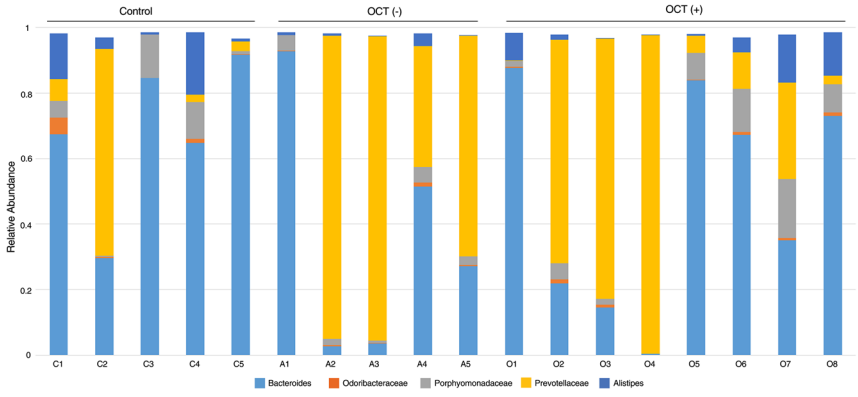

Figure 2: Bacterial community compositions of the phyla bacteroidetes, given as relative abundance at the family level. Study cohorts and enrolled individuals are indicated as control (C1–5), acromegaly without OCT (OCT–) (A1–5) and acromegaly. with OCT (OCT+) (O1–8). Color codes assigned to each family are shown.

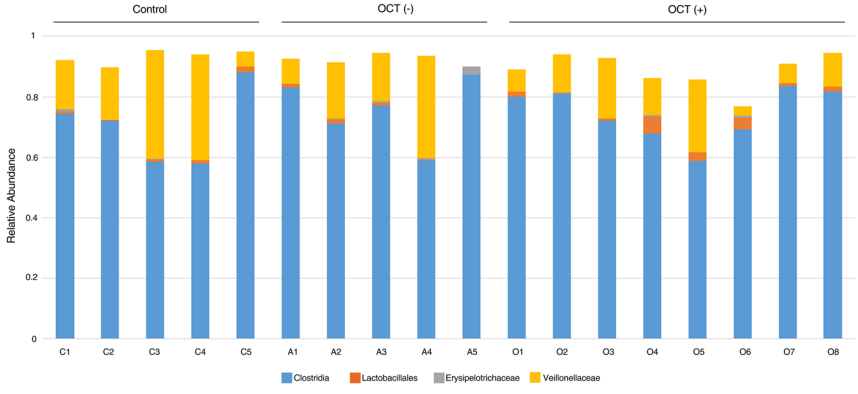

Figure 3: Bacterial community compositions of the phyla Firmicutes, given as relative abundance at the family level. Study cohorts and enrolled individuals are indicated as control (C1–5), acromegaly without OCT (OCT–) (A1–5) and acromegaly with OCT (OCT+) (O1–8). Color codes assigned to each family are shown.

Discussion

In this cross-sectional case-control study, we investigated bacterial communities in acromegaly patients with or without OCT therapy. We observed *Bacteroidetes* and *Firmicutes* phyla to predominate in acromegaly patients as well as controls. Moreover, members of the *Proteobacteria* and *Actinobacteria* phyla were detected in all individuals. Our findings suggest that the general composition of the fecal microbiota was not overtly altered in acromegaly cohorts or controls evaluated in the study, with no statistically significant variations in particular phyla/families [8, 9].

We observed a statistically-supported elevation in *Lactobacillales* abundance in individuals receiving OCT (Table 1). *Lactobacillus* species are used as growth promoters in poultry, associated with serum leptin and ghrelin levels in rats and have recently been linked to obesity in humans [10, 11]. Moreover, *Lactobacillus* and *Bifidobacterium* can interact with other bacteria or the host to modulate the immune system and the development of a tolerogenic response by interacting with dendritic cells [12, 13]. We could not demonstrate significant variations in *Bifidobacterium* genus or *Actinobacteria* phyla among cohorts. Among the minor components of the gut microbiota, we characterized *A. mucinophila* as the only species of the *Verrucomicrobia* phylum in acromegaly patients. Despite lack of statistically-supported variation in detection or relative abundance, the majority of the individuals with detectable *A. mucinophila* had been observed as receiving OCT. *A. mucinophila* has been associated with regulating effects on glucose metabolism and considered as an anti-inflammatory resident of the gut microbiota [14]. It correlates strongly with the lipid metabolism and inflammation in adipose tissue, as well as circulating glucose, insulin, triglycerides and leptin levels in a mice diet-induced-obesity model [15]. Moreover, metformin treatment has been reported to enhance *Akkermansia* species in gut bacterial communities, leading to improved glucose homeostasis. However, we could not observe a correlation between *A. mucinophila* detection and metformin therapy in our study. Nevertheless, frequent detection of *A. mucinophila* in the OCT cohort in this study indicate a favorable effect on glucose metabolism via microbiota alteration. Therefore, the bacterial changes during OCT treatment require further investigation as a potential marker of glucose metabolism alterations. The seemingly-controversial increase in *Lactobacillus* species in this group also requires further investigation.

In conclusion, our preliminary evaluation revealed distinct microbiota alterations associated with OCT treatment in patients with acromegaly.

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Informed consent: Informed consent was obtained from all individuals included in this study.

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Supplementary Material: Information on the medical history of the individuals enrolled in the study.

The online version of this article offers supplementary material (https://doi.org/10.1515/tjb-2020-0293).