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Deletion of poly(ADP-ribose) polymerase-1 changes the composition of the microbiome in the gut

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Abstract. Poly(adenosine diphosphate-ribose) polymerase (PARP)-1 is the prototypical PARP enzyme well known for its role in DNA repair and as a pro-inflammatory protein. Since PARP1 is an important co-factor of several other pro-inflammatory proteins, in the present study the possible changes in microbial flora of PARP1 knockout mice were investigated. Samples from the duodenum, cecum and feces from wild type and PARP1 knockout C57BL/6J male mice were collected and 16S ribosomal RNA genes were sequenced. Based on the sequencing results, the microbiome and compared samples throughout the lower part of the gastrointestinal system were reconstructed. The present results demonstrated that the lack of PARP1 enzyme only disturbed the microbial flora of the duodenum, where the biodiversity increased in the knockout animals on the species level but decreased on the order level. The most prominent change was the overwhelming abundance of the family *Porphyromonadaceae* in the duodenum of PARP1$^{-/-}$ animals, which disappeared in the cecum and feces where families were spread out more evenly than in the wild type animals. The findings of the present study may improve current understanding of the role of PARP1 in chronic inflammatory diseases.

Introduction

Poly[adenosine diphosphate (ADP)-ribose] (PAR) polymerase (PARP)-1 is the member of the PARP family that is considered to be the 'prototypical' PARP enzyme (1). PARP1 can be activated by DNA strand breaks and a set of posttranslational modifications [previously reviewed in (2)]. Active PARP1 cleaves aldehyde dehydrogenase into nicotinamide and ADP-ribose (ADPR), and forms ADPR polymers, also known as PAR, on different acceptor proteins (3,4). PAR chains can modify the function of the acceptors, enabling PAR-mediated regulation of protein function. PARP1 is responsible for >80% of all cellular PARP activities (5,6).

PARP1 is widely recognized as a pro-inflammatory protein in T helper1-mediated pathologies [previously reviewed in (7-9)]. The pro-inflammatory properties of PARP1 have numerous molecular roots. Firstly, PARP1 is a vital positive co-factor of several pro-inflammatory transcription factors [previously reviewed in (7)], of which the first to be identified was nuclear factor-κB (NF-κB) (10). In addition, PARP-mediated epigenetic changes also contribute to the pro-inflammatory transcriptional properties of PARP1 (11). The induction of these transcription factors facilitates the production of pro-inflammatory chemokines, cytokines and lipid mediators (7,11). These mediators in turn facilitate the chemotaxis of immune cells and also have a pivotal role in their activation (12,13). Adhesion factors (such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1) that help immune cells enter the site of inflammation are also expressed in a PARP1-dependent manner (14). Finally, there are other factors, including inducible nitric oxide synthase, cyclooxygenase-2 and certain matrix metalloproteinases that are activated in a PARP1-dependent manner as well (15,16). Taken together, immune cell activation, infiltration, cell migration and oxidative/nitrosative stress are PARP1-dependent. Notably, the administration of PARP inhibitors to humans also has an anti-inflammatory effect (17).

Recent advances in sequencing technology have largely increased current knowledge on the composition of the microflora (the collective microfloral genome often referred to as the microbiome) in various regions of the human body (18-22). Sequencing-based determination of the microbiome not only revealed novel bacterial species and enabled the study of the microflora, but also revealed that bodily cavities (such as lower airways), previously thought to be sterile, do contain bacteria in low numbers (18-22). Furthermore, these studies have shed light on the interactions between the host and the microbiome. Microbes produce metabolites that enter the systemic circulation, and can affect the cells of the

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host (23-29) and interact directly with the components of the innate immune system (30-32). In turn, the host influences the microbial communities through the immune system, feeding behavior and personal hygiene (18). Changes in the composition of the microbiome have been associated with particular diseases, including metabolic diseases, autism and cancer, where the reduction in the diversity of the microbiome frequently coincides with the onset of the disease (33).

The molecular determinants of the interaction between the host's immune system and the microbiome are largely unknown. Previous studies have revealed the role of the innate immune system, more precisely the Toll-like receptor (TLR) family (30,31,34,35). As PARP1 modulates the TLR-mediated signaling (32,36-40), the present study was performed to investigate the changes in the microbiome upon the deletion of PARP1.

Materials and methods

Animals. PARP1 knockout mice with a C57BL/6J background were used (41), and were generated in Het-to-Het breeding. A total of 6 mice were housed in each cage (standard block shape 365x207x140 mm, surface 530 cm^2; 1284 L Eurostandard Type II. L from Techniplast) with Lignocel Select Fine (J. Rettenmaierund Söhne, Germany) as bedding. Mice were housed under a 12 h light/dark cycle at 22±1˚C. Mice had *ad libitum* access to food and water (sterilized tap water). The animal facility was overseen by a veterinarian. Male mice (20 animals, 8-12 weeks old, 22-26 g body weight) were randomly selected from a larger pool of mice bred at the Animal Facility of the University of Debrecen (Debrecen, Hungary). Randomization between groups was not possible since group assignment was based on genotypes (n=20, 10 per experimental group). Animals were fasted 16 h prior to sampling to exclude the effect of potentially different eating periods and ingested food quantities. Following this, fresh fecal samples were collected and stored immediately in liquid nitrogen. Subsequently, animals were sacrificed by cervical dislocation. Subsequently the initial 15 mm segment of the duodenum and ~1/3 of the cecum was removed. Both intestinal samples in addition to a freshly collected fecal pellet were rapidly frozen in liquid nitrogen immediately following removal. For long term storage samples were kept at -80˚C (41). All animal experiments were approved by the local and national ethical board of the University of Debrecen (reg. 1/2015/DEMÁB).

DNA isolation and sequencing. Total DNA was isolated form each sample using the DNeasy PowerSoil kit according to the manufacturer's protocol (cat. no. 12888-100; Qiagen GmbH, Hilden, Germany). Subsequently identical amounts of DNA from each sample within the groups were pooled; the use of identical quantities of DNA ensures that each sample contributes equally to the abundance. From the pooled samples 16S ribosomal RNA genes were amplified and sequenced. Samples were assessed for quality and potential contaminants on a 1.5% agarose gel. DNA isolation and sequencing were performed by UD-GenoMed as a commercial service (UD-GenoMed, Debrecen, Hungary).

Analysis of the microbiome. Sequence fragments were uploaded to the metagenomics RAST server, MG-RAST (v4.0, metagenomics.anl.gov/) where paired end joining and microbiome reconstruction was performed (42). Subsequent analysis was performed with a specialized standalone software Taxamat (v1.04), which is freely available at www.taxamat.com. Using Taxamat, data representing food contaminants and host DNA (*Viridiplantae* and *Metazoa*) was removed. To produce the sequencing depth of each sample to a comparable level, data were downsampled so that samples with higher abundance matched the samples with the lowest abundance values. Source files for the sequencing raw and the curated data can be found at www.ncbi.nlm.nih.gov/bioproject/411773 (NCBI Bioproject PRJNA411773).

Statistical analysis. Diversity profiles were created using Palaeontological Statistics (PAST) (43). Diversity indices were calculated using PAST and Taxamat (www.taxamat.com). When comparing diversity profiles, the curve data points were downsampled to eight evenly distributed values over the whole range and statistical significance was determined using two tailed Student's t-test for paired samples. Family and order distributions across samples were compared using the 'prop.test' function (www.rdocumentation.org/packages/mosaic/versions/1.1.1/topics/prop.test) in RStudio (version 0.99.484; www.rstudio.com) (44,45).

Results

As a first step, changes to microbial diversity were assessed by comparing the gut samples of the $PARP1^{+/+}$ and $PARP1^{-/-}$ mice. When comparing diversity indices, the main pitfall is the arbitrary choice of the used index. To avoid this issue diversity profiles were plotted in addition to comparing individual indices (Fig. 1). These curves use the parameter (α) dependent exponent of the Renyi index (46). This function returns the number of taxa at $\alpha=0$, a number proportional to the Shannon index at $\alpha=1$ and a Simpson-like index at $\alpha=2$. When plotting these profiles, it was demonstrated that the duodenal samples of $PARP1^{+/+}$ and $PARP1^{-/-}$ animals were slightly different in terms of their diversity profiles (Fig. 1A and D; $P<0.001$ and $P<0.01$, respectively). Notably, on the species level the samples from $PARP1^{-/-}$ demonstrated higher diversity values while on order level this trend was the opposite. When comparing diversity profiles representing the lower gastrointestinal tract (cecum) and feces, the only significant differences were in the cecal samples on order level ($P<0.05$; Fig. 1E). However, even in that case, although statistically significant, profile curves were very similar. The same trend was also demonstrated by the traditional Shannon and Simpson indices (Table I).

The most abundant orders in all samples were then investigated. Clostridiales and Bacteroidales represented between ~70 and 90% of all taxa across all samples. The less abundant orders were Lactobacillales, Erysipelotrichales and Verrucomicrobiales accounting for a further 5-15% while the rest (~5-15%) were spread out almost evenly between a further ~30 orders (Fig. 2). On the order level all samples appeared to be similar, the only trend worth noting was the decreased ratio of Clostridiales in cecal and fecal samples when compared with duodenal ones (Fig. 2, middle panel). The statistical

Table I. Simpson and Shannon indices obtained in the present study.

	Species						Order					
Indices	Duodenum		Cecum		Feces		Duodenum		Cecum		Feces	
PARP1	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-
Simpson	0.883	0.948	0.955	0.933	0.942	0.931	0.651	0.528	0.726	0.693	0.710	0.727
Shannon	3.378	3.789	3.858	3.733	3.656	3.643	1.360	1.046	1.570	1.477	1.533	1.617

PARP1, poly(adenosine diphosphate-ribose) polymerase 1.

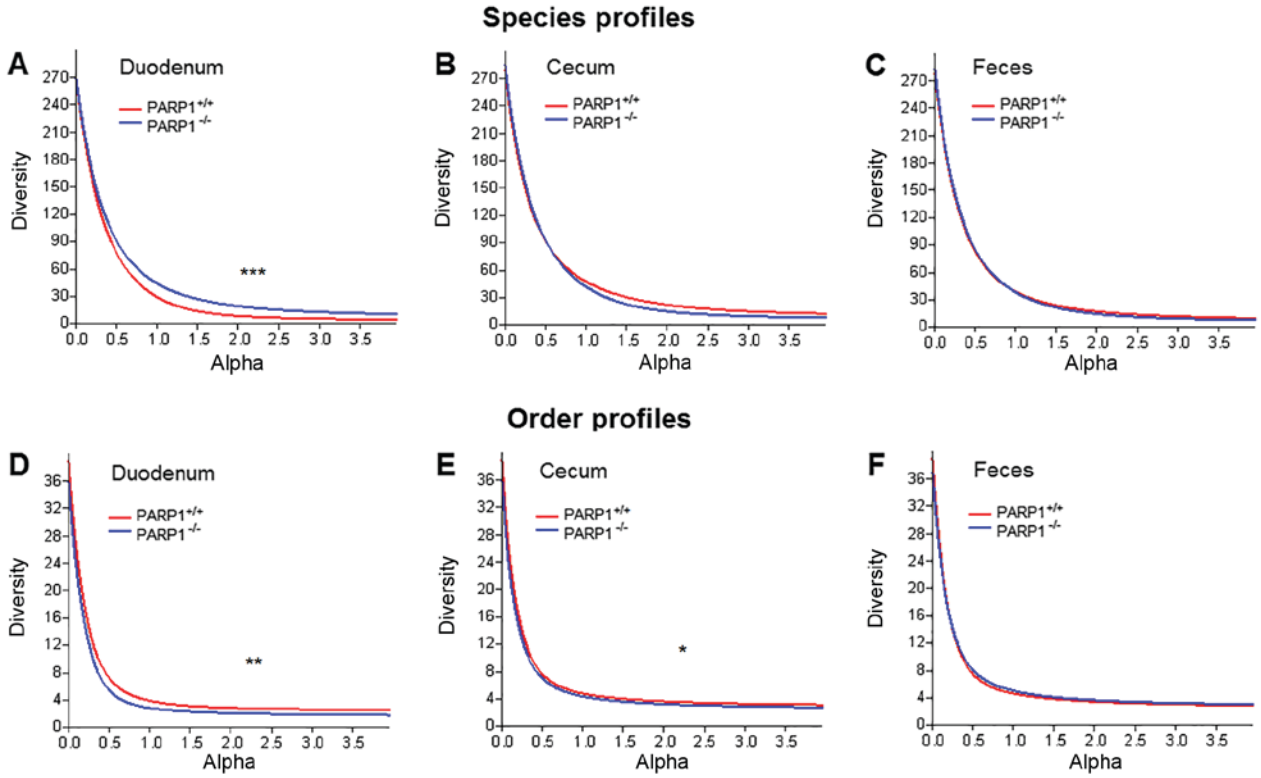

Figure 1. Absence of PARP1 reduces microbial diversity. (A-C) Species and (D-F) order profiles in the different parts of the gastrointestinal tract tested, including the (A and D) duodenum, (B and E) cecum and (C and F) feces based on the Rényi index. PARP1, poly(adenosine diphosphate-ribose) polymerase 1. *P<0.05, **P<0.01 and ***P<0.001 vs. PARP1$^{+/+}$.

significance levels are presented in Table II. This was true for samples from wild type and from PARP1$^{-/-}$ animals, albeit the duodenal samples from the latter demonstrated a higher ratio of Clostridiales (~63% in PARP1$^{-/-}$ animals vs. ~44% in PARP1$^{+/+}$ animals; Fig. 2A). As these results indicated, the majority of the other orders proportionally advanced to fill in the gap left by Clostridiales throughout the gastrointestinal system.

Further investigation on the family level revealed that there was little to no difference in the main composition in Clostridiales (Fig. 2, right-hand panel). The main families were *Lachnospiraceae, Clostridiaceae, Eubacteriaceae* and *Ruminococcaceae*. More than half of the Clostridiales were comprised of members of the *Lachnospiraceae* family (54-65%), while the next two, *Clostridiaceae* and *Ruminococcaceae*, were responsible for a further ~20%. When comparing the family composition of the different samples, there was little to no changes in these ratios throughout the gastrointestinal system, nor were any differences detected between samples from wild type and PARP1$^{-/-}$ animals, except for a slight elevation of the *Ruminococcaceae* ratio in the fecal samples of PARP1$^{-/-}$ animals (Fig. 2, right-hand panel; Table II; P<0.05).

When investigating the family composition of the order Bacteroidales the most abundant species were *Porphyromonadaceae* representing ~50-90% of all taxa (Fig. 2, left-hand panel). The other families included *Rikenellaceae, Bacteroidaceae* and *Prevotellaceae*. Other taxa not including those already mentioned reached only a combined ratio of a maximal 0.20% across all samples. PARP1$^{-/-}$ originated samples demonstrated little change throughout the gastrointestinal system. The most noteworthy was the ~14% decrease in the family *Porphyromonadaceae's* ratio, which was almost exclusively made up for by the increase in *Prevotellaceae* and *Bacteroidaceae* between the duodenal and fecal samples. This

Table II. Taxon proportion significance levels between compared samples.

Compared samples	Significance levels		
	Order	Bacteroidales	Clostridiales
$PARP^{+/+}$ duodenum vs. $PARP^{+/+}$ cecum	P<0.05	P<0.001	ns
$PARP^{+/+}$ duodenum vs. $PARP^{+/+}$ feces	P<0.001	P<0.001	ns
$PARP^{+/+}$ cecum vs. $PARP^{+/+}$ feces	ns	P<0.001	ns
$PARP^{-/-}$ duodenum vs. $PARP^{-/-}$ cecum	P<0.001	P<0.01	ns
$PARP^{-/-}$ duodenum vs. $PARP^{-/-}$ feces	P<0.001	P<0.01	ns
$PARP^{-/-}$ cecum vs. $PARP^{-/-}$ feces	ns	P<0.05	ns
$PARP^{+/+}$ duodenum vs. $PARP^{-/-}$ duodenum	P<0.001	P<0.001	ns
$PARP^{+/+}$ cecum vs. $PARP^{-/-}$ cecum	ns	P<0.001	ns
$PARP^{+/+}$ feces vs. $PARP^{-/-}$ feces	ns	P<0.001	P<0.05

ns, non-significant; PARP1, poly(adenosine diphosphate-ribose) polymerase 1.

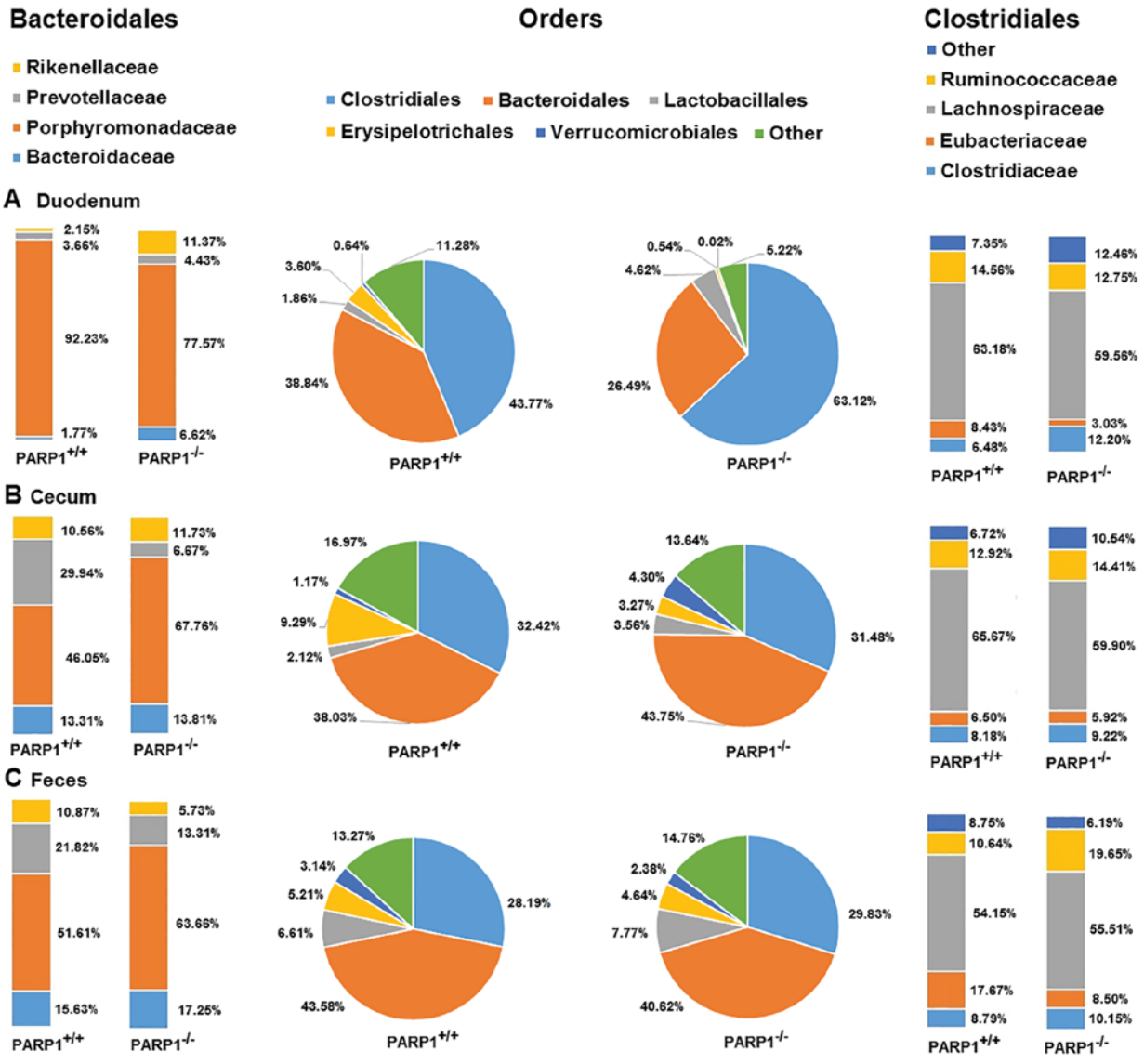

Figure 2. Composition of the bacterial flora changes on order and family ranks in the absence of PARP1. The ratio of the most abundant orders, as indicated in the middle pie charts and the family composition of the two most occurring families: Bacteroidales (left-hand panel, bar charts) and Clostridiales (right-hand panel, bar charts) for (A) duodenal, (B) cecal and (C) fecal samples. Microbiome taxon composition was created using the metagenomics RAST server, MG-RAST, and taxon lists, and were analyzed using PAST and Taxamat. PARP1, poly(adenosine diphosphate-ribose) polymerase 1.

14% decrease in the ratio of *Porphyromonadaceae* was much less prominent in PARP$^{-/-}$ animals than in their PARP1$^{+/+}$ counterpart, where a 46 and 40% decrease was detected in cecal and fecal samples respectively, when compared with duodenal samples. In fact, the little change of *Porphyromonadaceae* in PARP1$^{-/-}$ animals kept this family the most abundant one in the Bacteroidales order of the knockout strain across all samples, while in wild type animals only the duodenal samples were dominated (92%) by *Porphyromonadaceae* (Fig. 2A, left-hand panel). Cecal and fecal samples in the PARP1$^{+/+}$ group, consisted only 46 and 51% of *Porphyromonadaceae* respectively (Fig. 2B and C, left-hand panel; statistical significance levels are provided in Table II).

Samples isolated from wild type animals demonstrated notable changes too. Duodenal samples harbored ~92% *Porphyromonadaceae* in the order Bacteroidales, leaving barely any room for the other families in this order, the second most abundant being *Prevotellaceae* (3.6%) followed by *Rikenellaceae* (2.1%). Notably, *Porphyromonadaceae* contributed to only ~50% of the Bacteroidales family, while the other most prominent families indicated a ~10-fold increase when compared with duodenal samples (*Bacteroidaceae*: 13-17%; *Prevotellaceae*: 21-29%; Fig. 2A-C, statistical significance levels are provided in Table II).

Discussion

A recent study demonstrated PARP1-mediated changes in the fecal microbiome in regard to mucosal injury (47). Following that thread, the composition of the microbiome on the lower part of the gastrointestinal tract and feces was assessed in the present study.

The most prominent result of the present study was that in the duodenum, in the absence of PARP1, the order of diversity decreased. These results were similar to those of Larmonier *et al* (47) who also reported a decrease in diversity.

Reference strains of mouse gut bacteria are practically unavailable and very few studies have attempted to provide a broad overview. One of these attempts was made in 2016 by Lagkouvardos *et al* (48) who aimed to establish the Mouse Intestinal Bacterial Collection. Their results demonstrated that certain species are specific to the mouse intestine. The present results are based on direct sequencing only, while Lagkouvardos *et al* (48) utilized culturing in parallel. Despite the differences in methodology, the present results on order and family level are very similar with the ones mentioned in Lagkouvardos *et al* (48), thus validating them.

It is of note that the present experimental system did not challenge the microbiome; in other words, the absence of PARP1 alone led to visible changes in the microbiome in the absence of a disease. Furthermore, direct sequencing of 16S ribosomal DNA was used, which may add a bias to the chemistry prior to the *in silico* evaluation as compared with shotgun sequencing; however, in the upper parts of the gastrointestinal tract the number of the bacterial DNA is low as compared with the host DNA making shotgun sequencing cumbersome (20).

What could cause these changes in the microbiome? Innate immunity is already implicated in the regulation of gut bacteria through TLRs (22-24). PARP1 is a positive co-factor of several key inflammatory transcription factors (such as NF-κB and activator protein-1) (6) and through that PARP1 may modulate TLR function (36,37,40,49). Although, there is no direct evidence, the present study proposed that the interdependence of PARP1 and TLRs is a likely explanation for changes in the microbiome in the PARP1$^{-/-}$ mice. PARP1 is responsible for the majority of the cellular PARP activity (5,50,51), therefore, its absence often resembles to PARP inhibitor treatment. However, there is no evidence for the capability of PARP inhibitors to influence the microbiome.

At present it is difficult to assess the physiological relevance of these findings. A body of evidence has indicated that PARP1 serves a key role in inflammatory pathologies [such as arthritis (52,53) or type I diabetes (54,55)] or metabolic diseases [such as type II diabetes (56-58)], where the microbiome has a pivotal pathogenic role (26,57,58). Similarly to these, changes in the duodenal flora serve a dominant role in the pathogenesis of type II diabetes (59). These possibilities require further assessment in order to verify causal association. Damage to the gut flora, similarly to certain antibiotics, may contribute to the diarrhea observed as a side effect of PARP inhibitor treatment in humans (60). This link between diarrhea and changes in the microbiome also suggests that the application of PARP inhibitors may predispose to or aggravate antibiotic-induced diarrhea in PARP inhibitor-treated patients. Taken together, understanding the link between PARPs and the microbiome has importance for the clinical application of these inhibitors.

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Availability of data and materials

The primary data for microbiome sequencing is available in the NCBI repository www.ncbi.nlm.nih.gov/bioproject/411773 (NCBI BioProject PRJNA411773).

Authors' contributions

AV and TK conducted the collection of fecal samples. AV conducted sample collection and performed data analysis. AV, GK, BLB and PB wrote the manuscript. PB conceptulized the study and drafted the manuscript. BLB and GK provided their medical expertise in understanding and discussing the observed changes. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the local and national ethical board of the University of Debrecen (reg. 1/2015/DEMÁB; Debrecen, Hungary).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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