INTRODUCTION: Parkinson’s disease is a debilitating neurological condition affecting more than 1% of the global population aged 60 and above. The primary medication used to treat Parkinson’s disease is levodopa (L-dopa). To be effective, L-dopa must enter the brain and be converted to the neurotransmitter dopamine by the human enzyme aromatic amino acid decarboxylase (AADC). However, the gastrointestinal tract is also a major site for L-dopa decarboxylation, and this metabolism is problematic because dopamine generated in the periphery cannot cross the blood-brain barrier and causes unwanted side effects. Thus, L-dopa is coadministered with drugs that block peripheral metabolism, including the AADC inhibitor carbidopa. Even with these drugs, up to 56% of L-dopa fails to reach the brain. Moreover, the efficacy and side effects of L-dopa treatment are extremely heterogeneous across Parkinson’s patients, and this variability cannot be completely explained by differences in host metabolism.

RATIONALE: Previous studies in humans and animal models have demonstrated that the gut microbiota can metabolize L-dopa. The major proposed pathway involves an initial decarboxylation of L-dopa to dopamine, followed by conversion of dopamine to m-tyramine by means of a distinctly microbial dehydroxylation reaction. Although these metabolic activities were shown to occur in complex gut microbiota samples, the specific organisms, gene, and enzymes responsible were unknown. The effects of host-targeted inhibitors such as carbidopa on gut microbial L-dopa metabolism were also unclear. As a first step toward understanding the gut microbiota’s effect on Parkinson’s disease therapy, we sought to elucidate the molecular basis for gut microbial L-dopa and dopamine metabolism.

RESULTS: Hypothesizing that L-dopa decarboxylation would require a pyridoxal phosphate (PLP)-dependent enzyme, we searched gut bacterial genomes for candidates and identified a conserved tyrosine decarboxylase (TyrDC) in Enterococcus faecalis. Genetic and biochemical experiments revealed that TyrDC simultaneously decarboxylates both L-dopa and its preferred substrate, tyrosine. Next, we used enrichment culturing to isolate a dopamine-dehydroxylating strain of Eggerthella lenta, a species previously implicated in drug metabolism. Transcriptomics linked this activity to a molybdenum cofactor–dependent dopamine dehydroxylase (Dadh) enzyme. Unexpectedly, the presence of this enzyme in gut bacterial genomes did not correlate with dopamine metabolism; instead, we identified a single-nucleotide polymorphism (SNP) in the dadh gene that predicts activity. The abundance of E. faecalis, tyrDC, and the individual SNPs of dadh correlated with L-dopa and dopamine metabolism in complex gut microbiotas from Parkinson’s patients, indicating that these organisms, genes, enzymes, and even nucleotides are relevant in this setting.

We then tested whether the host-targeted AADC inhibitor carbidopa affected L-dopa decarboxylation by E. faecalis TyrDC. Carbidopa displayed greatly reduced potency toward bacteria and was completely ineffective in complex gut microbiotas from Parkinson’s patients, suggesting that this drug likely does not prevent microbial L-dopa metabolism in vivo. To identify a selective inhibitor of gut bacterial L-dopa decarboxylation, we leveraged our molecular understanding of gut microbial L-dopa metabolism. Given TyrDC’s preference for tyrosine, we examined tyrosine mimics and found that (S)-α-fluoromethyltyrosine (AFMT) prevented L-dopa decarboxylation by TyrDC and E. faecalis as well as complex gut microbiota samples from Parkinson’s patients. Co-administering AFMT with L-dopa and carbidopa to mice colonized with E. faecalis also increased the peak serum concentration of L-dopa. This observation is consistent with inhibition of gut microbial L-dopa metabolism in vivo.

CONCLUSION: We have characterized an interspecies pathway for gut bacterial L-dopa metabolism and demonstrated its relevance in human gut microbiotas. Variations in these microbial activities could possibly contribute to the heterogeneous responses to L-dopa observed among patients, including decreased efficacy and harmful side effects. Our findings will enable efforts to elucidate the gut microbiota’s contribution to treatment outcomes and highlight the promise of developing therapies that target both host and gut microbial drug metabolism.

Gut microbes metabolize the Parkinson’s drug L-dopa. Decarboxylation of L-dopa by E. faecalis TyrDC and human AADC likely limits drug availability and contributes to side effects. E. lenta dehydroxylates dopamine produced from L-dopa using a molybdenum-dependent enzyme. Although the host-targeted drug carbidopa did not affect gut bacterial L-dopa decarboxylation, AFMT inhibited this activity in complex human gut microbiotas.

The list of author affiliations is available in the full article online.
**MICROBIOTA**

**Discovery and inhibition of an inter species gut bacterial pathway for Levodopa metabolism**

Vayu Maini Rekdal, Elizabeth N. Bess, Jordan E. Bisanz, Peter J. Turnbaugh*, Emily P. Balskus*

**INTRODUCTION:** Parkinson’s disease is a debilitating neurological condition affecting more than 1% of the global population aged 60 and above. The primary medication used to treat Parkinson’s disease is levodopa (L-dopa). To be effective, L-dopa must enter the brain and be converted to the neurotransmitter dopamine by the human enzyme aromatic amino acid decarboxylase (AADC). However, the gastrointestinal tract is also a major site for L-dopa decarboxylation, and this metabolism is problematic because dopamine generated in the periphery cannot cross the blood-brain barrier and causes unwanted side effects. Thus, L-dopa and dopamine metabolism.

**RATIONALE:** Previous studies in humans and animal models have demonstrated that the gut microbiota can metabolize L-dopa. The major proposed pathway involves an initial decarboxylation of L-dopa to dopamine, followed by conversion of dopamine to m-tyramine by means of a distinctly microbial dehydroxylase reaction. Although these metabolic activities were shown to occur in complex gut microbiota samples, the specific organisms, gene, and enzymes responsible were unknown. The effects of host-targeted inhibitors such as carbidopa on gut microbial L-dopa metabolism were also unclear. As a first step toward understanding the gut microbiota’s effect on Parkinson’s disease therapy, we sought to elucidate the molecular basis for gut microbial L-dopa and dopamine metabolism.

**RESULTS:** Hypothesizing that L-dopa decarboxylation would require a pyridoxal phosphate (PLP)-dependent enzyme, we searched gut bacterial genomes for candidates and identified a conserved tyrosine decarboxylase (TyrDC) in Enterococcus faecalis. Genetic and biochemical experiments revealed that TyrDC simultaneously decarboxylates both L-dopa and its preferred substrate, tyrosine. Next, we used enrichment culturing to isolate a dopamine-dehydroxylating strain of Eggerthella lenta, a species previously implicated in drug metabolism. Transcriptomics linked this activity to a molybdenum cofactor-dependent dopamine dehydroxylase (Dadh) enzyme. Unexpectedly, the presence of this enzyme in gut bacterial genomes did not correlate with dopamine metabolism; instead, we identified a single-nucleotide polymorphism (SNP) in the dadh gene that predicts activity. The abundance of *E. faecalis*, *TyrDC*, and the individual SNPs of *dadh* correlated with L-dopa and dopamine metabolism in complex gut microbiotas from Parkinson’s patients, indicating that these organisms, genes, enzymes, and even nucleotides are relevant in this setting.

We then tested whether the host-targeted AADC inhibitor carbidopa affected L-dopa decarboxylation by *E. faecalis* TyrdC. Carbidopa displayed greatly reduced potency toward bacteria and was completely ineffective in complex gut microbiotas from Parkinson’s patients, suggesting that this drug likely does not prevent microbial L-dopa metabolism in vivo. To identify a selective inhibitor of gut bacterial L-dopa decarboxylation, we leveraged our molecular understanding of gut microbial L-dopa metabolism. Given TyrDC’s preference for tyrosine, we examined tyrosine mimics and found that (S)-a-fluoromethyltyrosine (AFMT) prevented L-dopa decarboxylation by TyrdC and *E. faecalis* as well as complex gut microbiota samples from Parkinson’s patients. Coadministering AFMT with L-dopa and carbidopa to mice colonized with *E. faecalis* also increased the peak serum concentration of L-dopa. This observation is consistent with inhibition of gut microbial L-dopa metabolism in vivo.

**CONCLUSION:** We have characterized an interspecies pathway for gut bacterial L-dopa metabolism and demonstrated its relevance in human gut microbiota. Variations in these microbial activities could possibly contribute to the heterogeneous responses to L-dopa observed among patients, including decreased efficacy and harmful side effects. Our findings will enable efforts to elucidate the gut microbiota’s contribution to treatment outcomes and highlight the promise of developing therapies that target both host and gut microbial drug metabolism.
The human gut microbiota metabolizes the Parkinson’s disease medication Levodopa (L-dopa), potentially reducing drug availability and causing side effects. However, the organisms, genes, and enzymes responsible for this activity in patients and their susceptibility to inhibition by host-targeted drugs are unknown. Here, we describe an interspecies pathway for gut bacterial L-dopa metabolism. Conversion of L-dopa to dopamine by a pyridoxal phosphate-dependent tyrosine decarboxylase from Enterococcus faecalis is followed by transformation of dopamine to m-tyramine by a molybdenum-dependent dehydroxylase from Egerthella lenta. These enzymes predict drug metabolism in complex human gut microbiota. Although a drug that targets host aromatic amino acid decarboxylase does not prevent gut microbial L-dopa decarboxylation, we identified a compound that inhibits this activity in Parkinson’s patient microbiota and increases L-dopa bioavailability in mice.

Growing body of evidence links the trillions of microbes that inhabit the human gastrointestinal tract (the human gut microbiota) to neurological conditions, including the debilitating neurodegenerative disorder Parkinson’s disease (1, 2). Gut microbes from Parkinson’s patients exacerbate motor deficits when transplanted into germ-free mouse models of disease (2). This effect is reversed with antibiotic treatment, suggesting a causal role for gut microbes in neurodegeneration. Multiple studies have revealed differences in gut microbiota composition in Parkinson’s disease patients compared with healthy controls that may correlate with disease severity (3–9). However, the influence of the human gut microbiota on the treatment of Parkinson’s and other neurodegenerative diseases remains poorly understood.

The primary treatment for Parkinson’s disease is Levodopa (L-dopa) (10), which is prescribed to manage motor symptoms that result from dopaminergic neuron loss in the substantia nigra. After crossing the blood-brain barrier, L-dopa is decarboxylated by aromatic amino acid decarboxylase (AADC) to dopamine, the active therapeutic agent. However, dopamine generated in the periphery by AADC cannot cross the blood-brain barrier, and only 1 to 5% of L-dopa reaches the brain, owing to extensive presystemic metabolism in the gut by enzymes such as AADC (11–13). Peripheral production of dopamine also causes gastrointestinal side effects, including orthostatic hypotension through activation of vascular dopamine receptors, and may induce cardiac arrhythmias (14, 15). To decrease peripheral metabolism, L-dopa is coadministered with AADC inhibitors such as carbidopa. Despite this, 50% of L-dopa is metabolized peripherally (16), and patients display highly variable responses to the drug, including loss of efficacy over time (17). Multiple lines of evidence suggest that gut microbial interactions with L-dopa influence treatment outcomes (18). Administering broad-spectrum antibiotics improves L-dopa therapy, suggesting that gut bacteria interfere with drug efficacy (19, 20). The gut microbiota can also metabolize L-dopa, potentially reducing its bioavailability and leading to side effects (21–24).

The major proposed pathway involves an initial decarboxylation of L-dopa to dopamine followed by a distinct microbial dehydroxylation reaction that converts this neurotransmitter to m-tyramine by selectively removing the para hydroxyl group of the catechol ring (Fig. 1A) (25, 26). When we began our work, the gut microbial species, genes, and enzymes involved in these transformations were unknown because previous studies examined undefined and uncharacterized consortia. The clinical relevance of this pathway was also unclear given the potential effects of coadministered inhibitors of host peripheral L-dopa metabolism on these gut microbial activities.

### The human gut bacterium Enterococcus faecalis decarboxylates L-dopa

We sought to elucidate the genetic and biochemical bases for gut microbial L-dopa metabolism and understand how coadministered AADC inhibitors affect this pathway. Using a genome-mining approach, we first identified strains that encode candidate L-dopa decarboxylating enzymes. Aromatic amino acid decarboxylation is typically performed by enzymes using pyridoxal 5′-phosphate (PLP), an organic cofactor that provides an electron sink (27). Recently, a PLP-dependent tyrosine decarboxylase (TyrDC) from the food-associated strain Lactobacillus brevis CGMCC 1.2028 was shown to have promiscuous activity toward L-dopa in vitro (28). To locate TyrDC homologs in human gut bacteria, we performed a BLASTP (Protein Basic Local Alignment Search Tool) search against the complete set of Human Microbiome Project (HMP) reference genomes available through the National Center for Biotechnology Information (NCBI). The majority of hits were found in the neighboring genus Enterococcus, with some hits within lactobacilli and Proteobacteria (Fig. 1B, fig. S1, and data file S1). We selected 10 representative gut strains that contain TyrDC homologs (29 to 100% amino acid ID) and examined their ability to decarboxylate L-dopa in anaerobic culture. Although both Enterococcus faecalis and Enterococcus faecium displayed activity, only E. faecalis showed complete decarboxylation across all strains tested (Fig. 1C). All E. faecalis strains tested share the highly conserved four-gene tyrDC operon (fig. S2), and we found tyrDC in 98.4% of the E. faecalis assemblies deposited in NCBI with a median amino acid identity of 99.8 (range 97.0 to 100). This high degree of sequence conservation and prevalence is consistent with tyrosine decarboxylation being a common phenotypic trait of E. faecalis (29). We therefore chose this prevalent, genetically tractable gut organism as a model for characterizing L-dopa decarboxylation (30).

Although lyophilized E. faecalis cells decarboxylate L-dopa (31) and the tyrDC operon’s role in tyrosine decarboxylation in E. faecalis is well-characterized (32), the connection between tyrDC and L-dopa decarboxylation was unknown. We used genetics and in vitro biochemistry experiments to confirm that TyrDC is necessary and sufficient for L-dopa decarboxylation by E. faecalis. E. faecalis MMH594 mutants carrying a 2-kb Tet-cassette disrupting tyrDC could not decarboxylate L-dopa (Fig. 1D and fig. S3) and displayed no growth defects compared with wild type (fig. S4). In vitro characterization of TyrDC revealed a fivefold higher catalytic efficiency toward L-tyrosine compared with L-dopa, suggesting that drug metabolism arises from promiscuous enzyme activity (Fig. 1E, fig. S5, and table S1). This selectivity contrasts sharply with that of AADC, which displays very low activity toward L-tyrosine (33). Although TyrDC from E. faecalis was previously shown to decarboxylate tyrosine and phenylalanine,
Eggerthella lenta dehydroxylates dopamine using a molybdenum-dependent enzyme

Having identified a gut bacterial l-dopa decarboxylase, we next examined the conversion of dopamine to m-tyramine because this activity may influence the side effects associated with peripheral l-dopa decarboxylation. E. faecalis did not further metabolize dopamine, indicating that this step was performed by a different microorganism. Dehydroxylase of dopamine has not been reported for any bacterial isolate, and a screen of 18 human gut strains failed to uncover metabolizers. Therefore, we used enrichment culturing to obtain a dopamine-dehydroxylating organism. Recognizing the chemical parallels between this reductive dehydroxylation and reductive dehalogenation of chlorinated aromatics, which enables anaerobic respiration in certain bacteria (43), we inoculated a stool sample from a human donor into a minimal growth medium containing 0.5 mM dopamine as the sole electron acceptor (figs S10 and S11). Passaging over multiple generations enriched for active strains, as assessed by means of a colorimetric assay for catechol dehydroxylation (fig. S11). This effort identified a strain of the gut Actinobacterium Eggerthella lenta (referred to herein as strain A2) that is capable of selectively removing the para hydroxyl group of dopamine to give m-tyramine (fig. S12). Because E. lenta also inactivates the cardiac drug digoxin, our finding suggests a wider role for this gut organism in drug metabolism (44, 45).

Catechol dehydroxylation is a chemically challenging reaction that has no equivalent in synthetic chemistry and likely involves unusual enzymology. To identify the dopamine-dehydroxylyzing enzyme, we first searched the E. lenta A2 genome for genes that encode homologs of the only characterized aromatic para-dehydroxylase, l-hydroxybenzoyl-CoA reductase (46), but found no hits. Assays with E. lenta A2 cell lysates showed dopamine dehydroxylation required anaerobic conditions and was induced by dopamine (fig. S13). We therefore used RNA-sequencing of E. lenta A2 to identify the dehydroxylase. This experiment revealed >2500-fold up-regulation of three colocalized genes in response to dopamine (Fig. 2A and table S2). These genes encode a predicted bis-molybdenopterin guanine dinucleotide cofactor (moco)-containing enzyme belonging to the dimethyl sulfoxide reductase family. Moco-dependent enzymes catalyze a wide variety of oxygen-transfer reactions but have not been demonstrated to catalyze catechol dehydroxylation in vitro (47). We therefore hypothesized that this enzyme was a dopamine dehydroxylase (Dadh).

Fig. 1. E. faecalis metabolizes l-dopa using a PLP-dependent tyrosine decarboxylase. (A) Proposed major pathway for l-dopa metabolism by the human gut microbiota and potential for interaction with host-targeted drugs. (B) Phylogenetic distribution of TyrDC in the human microbiota. Human Microbiome Project reference genomes were queried by means of BLASTP for homologs of the L. brevis TyrDC, and the results are visualized on a cladogram of phylogeny [based on 16S ribosomal RNA (rRNA) alignment]. TyDC homologs found sporadically within Lactobacillus spp. (Lb) are widely distributed among Enterococcus (Ec: average amino acid identity 67.8% over 976% query length). (C) Testing representative gut microbial strains encoding TyDC reveals that E. faecalis strains reproducibly convert l-dopa to dopamine. Strains were cultured for 48 hours anaerobically. Bar graphs represent the mean ± SEM of three biological replicates. (D) Deletion of tyrDC abolishes l-dopa decarboxylation by E. faecalis. Dopamine was detected in culture supernatants after 48 hours of anaerobic growth with 0.5 mM l-dopa. Bar graphs represent the mean ± SEM of three biological replicates. (E) Kinetic analysis of E. faecalis TyrDC reveals a preference for tyrosine. Error bars represent the mean ± SEM of three biological replicates. ND, not detected. (F) l-dopa and tyrosine are simultaneously decarboxylated in anaerobic cultures of E. faecalis MMH594 grown at pH 5 with 1 mM l-dopa and 0.5 mM tyrosine. Bar graphs represent the mean ± SEM of three biological replicates.

(34-37), its ability to accept l-dopa had not been demonstrated. A recent, independent report also corroborates this finding (38).

We next tested whether tyrosine, which is the preferred substrate for TyrDC and is present in the small intestine, could interfere with l-dopa decarboxylation by E. faecalis (39, 40). In competition experiments, purified TyrDC (fig. S6) and anaerobic E. faecalis cultures decarboxylated l-dopa and tyrosine simultaneously (500 μM tyrosine, approximating the resting small intestinal concentration) (fig. 1F and fig. S7) (40). This observation sharply contrasts with previ-ous investigations of phenylalanine, which is metabolized by E. faecalis only when tyrosine is completely consumed (36). Simultaneous decarboxylation of l-dopa and tyrosine also occurred in E. faecalis MMH594 cultures that contained higher tyrosine concentrations (1.5 mM, approximating the small intestinal post-meal concentration) (fig. S8) and in three human fecal suspensions (fig. S9). As observed previously for tyrosine, l-dopa decarboxylation occurred more rapidly at lower pH across all strains tested (figs S7 and S8), suggesting that this metabolism is likely accelerated at the lower pH of the upper small intestine (41, 42). Because the Michaelis constant (Km) of TyrDC for l-dopa (1.5 mM) is below the estimated maximum in vivo small intestinal l-dopa concentration even at its lowest clinically administered dose (5 mM), these data strongly suggest that peripheral decarboxylation is performed by both host and gut bacterial enzymes.
To assess Dadh’s role in dopamine dehydroxylation, we first explored whether this activity was molybdenum-dependent by culturing *E. lenta* A2 in the presence of tungstate. Substitution of molybdate with tungstate during moco biosynthesis generates an inactive metallocofactor (fig. S14) (49). Treating cultures of *E. lenta* A2 with tungstate inhibited dopamine dehydroxylation without affecting growth (Fig. 2B and fig. S15), whereas incubating cell lysates with tungstate had no effect, which is consistent with inhibition requiring active moco biosynthesis (fig. S16). We next confirmed the activity of Dadh in vitro. Heterologous expression of >20 constructs in multiple hosts failed to provide active enzyme, prompting us to pursue native purification. Anaerobic activity-guided fractionation of *E. lenta* A2 cell lysates yielded a dopamine-dehydroxylating fraction containing four proteins as assessed by means of SDS–polyacrylamide gel electrophoresis (Fig. 2C, fig. S17, and table S3). Dehydroxylation activity correlated with a 115-kDa band that was confirmed with mass spectrometry (MS) to be Dadh. Dadh was the only isolated protein up-regulated in the presence of dopamine (tables S2 and S3). Together, these data strongly support the assignment of this enzyme.

We next assessed whether the presence of *dadh* in microbial genomes correlated with dopamine dehydroxylation. A BLASTP search revealed that this enzyme is restricted to *E. lenta* and its close Actinobacterial relatives (table S4), prompting us to screen a collection of 26 gut Actinobacterial isolates (49) for their ability to dehydroxylate dopamine in anaerobic culture. Although Dadh appeared to be encoded by 24 of the 26 strains (92 to 100% amino acid ID) (fig. S18 and table S5), only 10 *Eggerthella* strains quantitatively converted dopamine to m-tyramine, with low (<1%) or no metabolism in the others (Fig. 2D). This strain-level variability in dopamine metabolism reinforces that gut microbial species identity is often not predictive of metabolic functions (49, 50).

To better understand this variation, we first performed RNA-sequencing experiments with metabolizing (*E. lenta* 28B) and nonmetabolizing (*E. lenta* DSM2243) strains in the presence and absence of dopamine. Surprisingly, *dadh* was up-regulated in response to dopamine in both strains, indicating that lack of activity in *E. lenta* DSM2243 did not arise from differences in transcription (tables S6 and S7). Aligning the Dadh protein sequences, we instead found a single amino acid substitution that almost perfectly predicts metabolizer status: Position 506 is an arginine in metabolizing strains and a serine in inactive strains (Fig. 2D and fig. S19). This change arises from a single-nucleotide polymorphism (SNP) in *dadh*. The only exception, *E. lenta* W1BH16, has the Arg<sup>506</sup> variant and an additional substitution nearby (Cys<sup>500</sup>) (fig. S19). Thus, specific amino acid residues in the Dadh enzyme, rather than presence or transcription of *dadh*, predict dopamine dehydroxylation among gut bacterial strains. The Dadh variants do not correlate with *E. lenta* phylogeny (Fig. 2D). This reinforces that gut microbial species identity is often not predictive of metabolic functions (49, 50).

**E. faecalis** and *E. lenta* metabolize l-dopa in human gut microbiota

Having identified organisms and enzymes that perform the individual steps in the l-dopa pathway, we next tested whether *E. faecalis* and *E. lenta* generated m-tyramine in coculture. Wild-type *E. faecalis* grown with *E. lenta* A2...
Fig. 3. E. faecalis and E. lenta Dadh predict L-dopa metabolism in complex human gut microbiotas. (A) Metabolism of L-dopa by cocultures of E. faecalis and E. lenta was measured for 48 hours with 1 mM d3-phenyl-L-dopa or 1 mM dopamine. Results are mean ± SEM (n = 3 replicates). (B) Metabolism of d3-phenyl-L-dopa by 19 unrelated human gut microbiota samples ex vivo. Samples were cultured anaerobically with d3-phenyl-L-dopa (1 mM) for 72 hours. Results are mean concentration ± SEM (****P < 0.0001, one-tailed Mann-Whitney test). (C) The abundance of tyrDC predicts L-dopa decarboxylation in human gut microbiota samples. Data represent the average tyrDC abundance (as assessed with qPCR) across the three replicates for samples in (B). Results are mean ± SEM (****P < 0.0001, one-tailed Mann-Whitney test). (D) The abundance of E. faecalis (as assessed with qPCR) predicts L-dopa decarboxylation in human gut microbiota samples. Each data point is the average abundance across three biological replicates for each sample shown in (B). Results are mean ± SEM (****P < 0.0001, one-tailed Mann-Whitney test). (E) Dopamine dehydroxylation by gut microbiota samples of 15 unrelated individuals. Samples were cultured for 48 hours with 0.5 mM dopamine. Bars are mean ± SEM of n = 6 for low reducers (≥50%) and n = 9 for high reducers (>50%) (****P = 0.0002, one-tailed Mann-Whitney test). (F) Dadh abundance does not correlate with dehydroxylation by human gut microbiotas. Data represent qPCR with Dadh-specific primers. Each data point is the dadh abundance in each sample shown in (E). Bars represent the mean and SE. (G) Dadh sequence variants predict dopamine dehydroxylation ex vivo. Full-length dadh from each culture in (E) was sequenced by using primers specific for the region containing position 506. Samples in which a mix of variants were present (n = 5) were removed. Bars represent the mean and SEM [n = 3 for samples encoding the Arg506 Dadh variant, n = 7 for samples encoding the Ser506 Dadh variant, n = 3 for DSM2243, and n = 3 for A2] (**P = 0.0083, one-tailed Mann-Whitney test, CGC samples versus AGC samples).
metabolized dopamine, whereas the activity of samples that carried the Ser<sup>506</sup> variant was indistinguishable from the nonmetabolizing E. lenta DSM2243 strain (Fig. 3G). These findings indicate that a single amino acid residue in a gut microbial enzyme predicts dopamine metabolism in complex communities. Given that dadh is highly prevalent (>70%) in gut microbiomes from human subjects and the two dadh variants are present among this population (figs. S22 and S25), we speculate that SNPs may influence xenobiotic metabolism in the context of both the host genome (52) and the human gut microbiome (53).

To further explore the clinical relevance of our findings, we assessed the metabolism of dopamine and l-dopa by fecal suspensions from Parkinson’s disease patients ex vivo. Similar to control subjects, these individuals displayed substantial disease patients ex vivo. Similar to control subjects, these individuals displayed substantial

---

**Fig. 4.** l-dopa decarboxylation by E. faecalis is inhibited by AFMT but not the host-targeted drug carbidopa. (A) Carbidopa and AFMT. (B) Carbidopa preferentially inhibits human AADC over TyrDC. AADC or TyrDC were incubated with inhibitor, and reaction rates were measured with LC-MS/MS. “% Activity” represents the rate relative to a no inhibitor (vehicle) control. Results are mean ± SEM (n = 3 replicates). (C) Activity of carbidopa and AFMT in cultures of E. faecalis grown for 16 hours anaerobically with 0.5 mM l-dopa. Error bars represent the mean ± SEM for three biological replicates. (D) Activity of carbidopa in a human fecal microbiota from a Parkinson’s patient. The sample was cultured anaerobically with carbidopa and 1 mM d<sub>2</sub>-phenyl-l-dopa for 72 hours. Error bars represent the mean ± SEM for three biological replicates. (E) AFMT preferentially inhibits TyrDC over AADC in vitro. AADC or TyrDC were incubated with inhibitor, and reaction rates were measured with LC-MS/MS. “% Activity” represents the rate relative to a no inhibitor (vehicle) control. Error bars represent the mean ± SEM for three biological replicates. (F) Detection of an AFMT-PLP covalent adduct after incubation of TyrDC or AADC with AFMT for 1 hour. The data shown is the extracted ion chromatogram of the mass of the predicted covalent adduct. (G) Action of AFMT in human fecal microbiota from Parkinson’s patients incubated anaerobically with AFMT and 1 mM d<sub>2</sub>-phenyl-l-dopa for 72 hours. Error bars represent the mean ± SEM for three biological replicates. (H) Pharmacokinetic analysis in gnotobiotic mice colonized with E. faecalis and given l-dopa + carbidopa + AFMT demonstrates higher serum l-dopa relative to vehicle controls. Error bars represent the mean ± SEM. (I) The maximum serum concentration (C<sub>max</sub>) of l-dopa is significantly higher with AFMT relative to vehicle controls. In (H) and (I), *P < 0.05, Mann-Whitney U test; n = 4 to 5 mice per group.
predicted gastrointestinal concentration of carbidopa (0.4 to 9 mM), these data suggest that this drug does not fully inhibit gut bacterial l-dopa decarboxylation in Parkinson’s patients. We found that 2 mM carbidopa did not alter the kinetics of l-dopa degradation (fig. S3I) or endpoint m-tyramine production in stool samples from both Parkinson’s patients and neurologically healthy controls (Fig. 4D and fig. S32). These observations support previous findings that carbidopa administration does not affect m-tyramine production in patients (55).

Our results also highlight the possibility of therapeutically targeting gut microbial l-dopa decarboxylation to increase l-dopa efficacy. To selectively manipulate gut bacterial TyrDC in complex microbiotas, we turned to α-fluoromethyl amino acids, which are known mechanism-based inhibitors of PLP-dependent decarboxylases (39). A survey of potential amino acid substrates revealed that TyrDC requires a p-hydroxy group for robust activity, whereas AADC prefers a m-hydroxyl substituent (fig. S33), leading us to hypothesize that the L-tyrosine analog (S)-α-fluoromethyltyrosine (AFMT) (Fig. 4A) might selectively inhibit the microbial enzyme. In vitro, AFMT strongly inhibited l-dopa decarboxylation by TyrDC (IC₅₀ = 4.7 μM) but not AADC (~20% inhibition at solubility limit of 650 μM) (Fig. 4E and table S8). Consistent with this selectivity, AFMT formed a covalent PLP adduct only in the presence of TyrDC (Fig. 4F). AFMT was also effective in E. faecalis cultures (EC₅₀ = 1.4 μM) (Fig. 4C), outperforming carbidopa by 1000-fold without affecting growth (table S8 and fig. S29). It also reduced l-dopa decarboxylation by cocultures of E. faecalis and E. lenta without affecting growth or metabolism of E. lenta (figs. S29, S30, and S34). Last, AFMT completely inhibited l-dopa decarboxylation in gut microbiota samples from Parkinson’s disease patients and neurologically healthy control subjects (Fig. 4G and fig. S35) and was nontoxic to eukaryotic cells (fig. S36).

To investigate AFMT activity in vivo, we administered either AFMT (25 mg/kg) or a vehicle control in combination with l-dopa (30 mg/kg) and carbidopa (30 mg/kg) to gnotobiotic mice colonized with E. faecalis MMH594 (Fig. 4H). We found that AFMT significantly increased the peak serum concentration (C_max) of l-dopa compared with vehicle (P < 0.05, two-tailed Mann Whitney test) (Fig. 4I), which is consistent with inhibition of brain and gut microbial metabolism in the intestine. Although we cannot rule out the possibility that AFMT modulates additional, uncharacterized targets, this observation is consistent with our in vitro inhibition data. This result also aligns with a recent report that small intestinal tyrDC abundance negatively correlates with plasma l-dopa levels in conventional rats receiving l-dopa and carbidopa (38). Overall, these data suggest that AFMT could be a promising tool compound for the study of bacterial l-dopa metabolism (56) and highlight the promise of developing l-dopa-based combination therapies containing drugs that target both host and gut microbial decarboxylation.

Conclusions

We have used chemical knowledge and interdisciplin ary tools to decipher the molecular mechanisms by which gut bacteria interfere with the treatment of Parkinson’s disease. The decarboxylation of l-dopa by E. faecalis mirrors host drug metabolism and, together with human AADC, likely limits drug availability and contributes to interindividual variation in efficacy. Together with recent work dissecting host and gut microbial contributions to the antiviral drug brivudine (57), our findings show that gut bacterial metabolism need not be chemically distinct from host activities to alter drug efficacy and suggest that such interactions may be underappreciated. Moreover, carbidopa’s failure to prevent l-dopa decarboxylation by E. faecalis implies that additional host-targeted drugs may lack efficacy toward activities also present in the gut microbiota. Although a recent, independent study also characterized E. faecalis TyrDC’s role in l-dopa decarboxylation and its lack of susceptibility to carbidopa (38), it did not show that this activity occurs in human gut microbiota or identify strategies for inhibiting the bacterial enzyme. By contrast, we demonstrate that TyrDC predicts drug metabolism in Parkinson’s patient microbiotas and use an understanding of its substrate specificity to identify a small molecule that prevents l-dopa decarboxylation in patient samples and increases l-dopa bioavailability in vivo. Through discovery of predictive biomarkers for l-dopa metabolism and identification of an inhibitor of this activity, this work will enable efforts to elucidate the contribution of the gut microbiota to drug availability, patient drug response, and treatment outcomes.

We also show that E. lenta further metabolizes the dopamine produced by l-dopa decarboxylation using a distinctly microbial reaction, catechol dehydroxylation. It is possible that this transformation influences the multiple side effects of l-dopa administration linked to dopamine production. This discovery also raises questions about the biological consequences of gut microbial metabolism of endogenous dopamine, which is present in the gastrointestinal tract and has been linked to phenotypes ranging from gut motility to pathogen colonization (58–60). The biological activity of the gut microbial metabolite m-tyramine in the host and the benefits of this metabolite with both E. faecalis and E. lenta are also poorly understood. Our findings will enable further study of these phenomena. Given that gut microbes dehydroxylate catechol groups found in numerous aromatic drugs and dietary compounds (38, 61–63), the discovery of Dadh will enable identification of additional catechol dehydroxylases and help to elucidate the biological role of this enigmatic transformation. Uncovering the unexpected effect of SNPs on gut microbial dopamine metabolism suggests that simply detecting functional genes may not accurately predict the activities encoded by the human gut microbiome and underscores the importance of studying enzymes from this community.

Materials and methods summary

Our methods for the identification and biochemical characterization of E. faecalis TyrDC; characterization of anaerobic l-dopa metabolism by E. faecalis and gut microbiota samples; enrichment culturing for dopamine dehydroxylating organisms; RNA-sequencing; culture-based assays; purification of Dadh; assays of anaerobic dopamine metabolism by Actinobacteria and complex gut microbiota samples; PCR and qPCR assays; liquid chromatography–MS (LC-MS) methods; and assays for evaluating inhibitors in vitro are provided in the supplementary materials. Additional information about our protocols, including references to the supplementary materials, can be found throughout the main text.
Competing interests: E.P.B. has consulted for Merck, Novartis, and Kintai Therapeutics; is on the Scientific Advisory Boards of Kintai Therapeutics and Caribou Biosciences; and is an Associate Member of the Broad Institute of Harvard and MIT. P.J.T. is on the scientific advisory board for Kaleido, Seres, uBiome, and WholeBiome. Data and materials availability: The *E. lenta* A2 genome has been deposited into GenBank (PRJNA412637). RNA-sequencing data has been deposited into the Sequence Read Archive available by way of BioProject PRJNAS07796. The small-molecule AFMT was obtained under a materials transfer agreement with Merck.

SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/364/6445/eaau6323/suppl/DC1

Materials and Methods

Figs. S1 to S36
Tables S1 to S8
References (64–84)
Data File S1

29 June 2018; resubmitted 18 April 2019
Accepted 2 May 2019
10.1126/science.aau6323
The dope on l-dopa metabolism

The efficacy of l-dopa treatment for Parkinson's disease is hugely variable between individuals, depending on the composition of their microbiota. L-Dopa is decarboxylated into active dopamine, but if the gut microbiota metabolize l-dopa before it crosses the blood-brain barrier, medication is ineffective. Maini Rekdal et al. found that different species of bacterium are involved in l-dopa metabolism (see the Perspective by O'Neill). Tyrosine decarboxylase (TDC) from *Eggerthella lenta* sequentially metabolizes l-dopa into m-tyramine. The microbial l-dopa decarboxylase can be inactivated by (S)-α-fluoromethyltyrosine (AFMT), which indicates possibilities for developing combinations of Parkinson's drugs to circumvent microbial inactivation.

*Science*, this issue p. eaau6323; see also p. 1030