Gut microbiota and metabolites of α-synuclein transgenic monkey models with early stage of Parkinson'sdisease

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Abstract

Background: Parkinson's diseas （ PD ） is the second most prevalent neurodegenerative disease. Gut microbes are susceptible to various external factors (such as living environment, diet, antibiotic use), our research avoids these interferences very well. Gut microbiota affect the physiological processes of the host by regulating metabolites. However, it is unclear whether microbiota and metabolites have demonstrated changes at early stages of PD due to the difficulty to diagnose and identify early stage PD in clinical practice.

Methods: In a previous study, we constructed A53T transgenic monkeys with early Parkinson's symptoms. Here we analyzed the gut microbiota by metagenomic sequencing and metabolites by untargeted chromatography, which represent the first effort to identify the association between intestinal microbiota, metabolites and early stage of PD.

Results: Compared with control monkeys, the gut microbiota of A53T monkeys is more diverse. Sybergistetes and *Eggerthella lenta* were significantly elevated in A53T monkeys. In monkeys with early Parkinson's symptoms, Glyceric acid, L-Aspartic acid and p-Hydroxyphenylacetic acid were significantly elevated, but Myristic acid and 3-Methylindole was significantly decreased. ABC transporters are associated with two decreased metabolites. Metabolic pathways are associated with three elevated metabolites. We found KO0131 and KO2147 from metabolic pathways are related to Glycolysis.

Conclusion: We identified differential gut microbiota coincides with the microbiota of the currently reported PD patients to some extent. We found these differential metabolites and KOs suggest that A53T monkeys may have Glycolysis problem, and Glycolysis problem may be associated with mitochondrial dysfunction. Our results may be a sign of early Parkinson's screening and diagnosis.

Keywords：Early stage of PD, A53T, gut microbiota, metabolites

Background

Parkinson’s disease (PD) is the second most prevalent neurodegenerative disease [1, 2]. The main characteristics of PD include the loss of dopaminergic neurons in the substantia nigra. As a neuronal protein, α-synuclein (α-syn) plays an important role in the formation of Lewy body, the aggregation of α-syn particularly impacting dopaminergic neurons of substantia nigra pars compacta (SNpc) and is closely related to the pathogenesis of PD. The aggregation of α-syn is a gradual process that leads to impaired motor features of PD including rigidity, tremors, postural instability and bradykinesia [3-5]. PD patients also universally suffer from non-motor symptoms including depression, anxiety, cognitive defects, and sleeping disorders. Moreover, gastrointestinal (GI) dysfunction is an additional common non-motor symptom in PD patients. GI dysfunction reflect changes in the composition of the intestinal microbiota [6, 7]. This mutually beneficial relationship between the host and the resident gut microbiota contributes to the fitness of the host [8]. A series of complex

and dynamic factors shape the microbial composition and diversity, which include genes, life style, antibiotic use, disease and so on. Proofs have indicated that neurologic symptoms may be a consequence of a primarily gastrointestinal pathology [9]. The most recent hypothesis is that PD may originate in the gut and spread to the brain through α-syn transmission, systemic inflammation and increased permeability of the blood-brain barrier [10, 11]. Translocation bacteria and inflammatory bacteria increase intestinal inflammatory response and oxidative stress, triggering the accumulation of α-syn in the enteric nervous system [12, 13]. The latest research also found that the injection of α-syn into the intestinal wall of rodents caused propagation of α-syn from the gut to the brain and leads to PD symptoms, the aggregated α-syn can reach brain via the vagus nerve [14]. Gram-negative bacterial infection in the intestine is associated with the onset of PD [15].

The gut microbes regulate the host's metabolites, which affect the host's biochemical and physiological processes, thereby increase the host's susceptibility to disease [16]. In the metabolism of food and xenobiotics, the host and its intestinal microbiota together produce a large number of small molecules that play a decisive role in the transmission of information between the host cell and the microbe. The gut microbiota-derived metabolite trimethylamine N-oxide is elevated in Alzheimer’s disease [17]. Another study proved that the altered transport of branch chain amino acids across the blood brain barrier led to dysregulation of amino acids levels and neurological impairments [18]. At present, it has been shown that short-chain fatty acids were significantly reduced in PD patients [19-21], and recently the

interconversion of methionine and cysteine via cystathionine was found that differed between PD patients and controls based on a computational modeling of gut microbiota[22]. However, currently no other metabolites have been identified and reported in Parkinson's patients, especially the patients at early stage of PD. Evidence from recent years also indicates that mitochondrial dysfunction also is central to the pathogenesis of both sporadic and familial PD [23, 24]. New data suggests that α-syn can interact with mitochondria by binding to the mitochondrial outer membrane [25, 26]. This also indicates a certain relationship between α-syn and the mitochondria. Alpha-syn can disrupt the introduction of mitochondrial proteins and localize to mitochondrial outer membranes in PD and post-mortem PD patients. The accumulation of α-syn in the outer membrane may interfere with the introduction mechanism of the protein, but may also interfere with the homeostatic pathway of other mitochondria [25, 27].

To the best of our knowledge, there are no reports about the gut metagenomics and metabolites in PD patients at early stages so far, which might be due to the difficulty to diagnose and identify early stage PD in clinical practice, the limited samples from early stage PD patients and ethics issues of human sampling. The lack of biomarkers is one of the challenges in identifying early stage of PD. Meanwhile, the study of the mechanism and the development of PD drugs and therapies are hampered by a lack of suitable animal models that replicate both the histological and metabolic features of human PD. The Parkinson models of mouse and non-human primates are based on chemical drug induction (rotenone and 1-Methyl-4-phenyl-1, 2, 3,

6-tetrahydropyridine) or transgenesis [28-31]. However, the chemical drug-induced model does not replicate both the histological and metabolic features of human diseases. In addition, limitations also exist in α-syn transgenic mouse models [32], and there are broad differences in the stability of different α-syn assemblies [33, 34]. In contrast, nonhuman primates are ideal models to recapitulate these key aspects including the non-motor behaviors and motor behaviors of PD patients.

In our previous study, we have generated α-syn (A53T) transgenic monkeys showing early Parkinson's disease symptoms including α-syn aggregation and cognitive impairment, but with no apparent motor deficits[35]. These specific monkey models provide a unique opportunity for us to analyze the gut microbiota and metabolites in transgenic rhesus monkeys at early stage of PD and disclose the relationship between gut microbiota, metabolites and PD. Since the occurrence of motor deficits in Parkinson's patients initializes at the middle and/or late stages of PD, we aim to reveal potential diagnostic markers for early stage Parkinson's disease before the onset of dyskinesia via the analysis of the gut microbes and metabolites of the α-syn mutant monkeys.

Materials and Methods

Animal

Ten adult rhesus monkeys individually caged and randomly assigned to two groups, which included five transgenic A53T monkeys generated in our previous study [35] and five wild monkeys as normal control (Table 1). All of the animals were

individually caged. The animal room was set on a 12 hours light: 12 hours darkness cycle. The temperature and the humidity of the animal room were kept at 18 °C to 26 °C and 40% to 70%, respectively. All procedures were approved by the Institutional Animal Care and Use Committee of Kunming University of Science and Technology, and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (8^{th} edition).

Sample collection and DNA extraction

Fresh fecal samples were collected in sterile tubes from the ten rhesus monkeys. Then, the fecal samples were transferred to the laboratory immediately in an ice bath and stored at −80°C. The isolation of purified microbial genomic DNA was performed from each fecal sample using a MoBioPowerSoil® DNA Extraction Kit (Arlsbad, CA, USA) according to the manufacturer's recommendation. The DNA concentration was measured using Qubit® DNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA).

Library preparation for sequencing

Each sample needed a total amount of 700ng DNA to be used as input material for the DNA sample preparations. According to the manufacturer's recommendation, sequencing libraries were generated using NEB Next® Ultra DNA Library Prep Kit for Illumina® (NEB, USA), and index codes were added to attribute sequences for each sample.

Clustering and sequencing

153 In the cBot Cluster Generation System, the clustering of the index-coded samples was
154 performed by HiSeq 4000 PE Cluster Kit (Illumina) according to the manufacturer's
155 instructions. After cluster generation, the library preparations were sequenced on an
156 Illumina Hiseq 4000 platform and 150 bp paired-end reads were generated.

157 *Analysis of fecal metabolites*

158 The sample preparation and derivatization protocols with MCF were based on the
159 method used in previously published procedures [36, 37]. Samples were thawed on an
160 ice-bath to diminish sample degradation. Approximately 50 mg of the study materials
161 were homogenized with 300μL of NaOH (1M) solution using a homogenizer (BB24,
162 Next Advance, Inc., Averill Park, NY, USA) and centrifuged at 13, 500 rpm and 4 °C
163 for 20 min (Microfuge 20R , Beckman Coulter, Inc., Indianapolis, IN, USA). Each
164 200μL of the supernatant was transferred into an autosampler vial (Agilent
165 Technologies, Foster City, CA, USA), and the residue was further exacted with
166 200μL of cold methanol. After the second step of homogenization and centrifugation,
167 each 167μLof the supernatant was combined with the first supernatant in the
168 autosampler vial. The extracts in the autosampler vial was capped and submitted for
169 automated sample derivatization with a robotic multipurpose sample MPS2 with dual
170 heads (Gerstel, Muehlheim, Germany). Briefly, 20μL of MCF was added to the
171 mixture and the sample was vortexed vigorously for exactly 30s. Another 20μL of
172 MCF was added for the derivatization a second time. Four hundred microliters of
173 chloroform followed by four hundred microliters of sodium bicarbonate solution
174 (50mM) was added to achieve the separation. The prepared samples were centrifuged

at 4°C and 4000g for 20min, and the bottom chloroform layer was carefully transferred by the robotic preparation station to a capped empty autosampler vial preloaded with approximately 25 mg of an hydroussodium sulfate. The sample pretreated with sodium sulfate was shaken on a laboratory shaker at 1, 500 rpm and 4°C for 20 min and further transferred to a capped empty autosampler vial for injection.

A gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS) system (Pegasus HT, Leco Corp., St. Joseph, MO, USA) operated in electron ionization (EI) mode was used to quantify the microbial metabolites in this project. The optimized instrument settings are briefly described below. Instrument optimization was performed every 24 hours. The raw data generated by GC-TOFMS were processed using proprietary software XploreMET (v2.0, Metabo-Profile, Shanghai, China) for automatic baseline denoising, smoothing, peak picking, and peak signal alignment.

Microbial metagenomic sequencing and metabolites bioinformatics analysis

Quality of sequencing data was checked using FASTQC [1] (V 0.11.7) and MultiQC [2] (V 1.7). 16S ribosomal RNA from metagenomic data was filtered by SortMeRNA [3] (V 2.1), and OTU was clustered in the Mothur [4] (V 1.41.1) pipeline. Shotgun metagenomic reads were first trimmed and filtered to the host contamination using Trimmomatic [5] (V 0.36) and Bowtie2 [6] as part of the KneadData(V0.6.1) pipeline (https://bitbucket.org/biobakery/kneaddata/wiki/Home). Kraken [7] (V 1.0) and Bracken [8] (V 1.0) was used to classify metagenomic sequences. Metagenomic

sequences were assembled using Megahit [9] (V 1.1.3) and QUAST [10] (V 5.0.2) was used to check assembly quality. 40,461,161 coding sequences were predicted by Prokka [11] (V 1.12) from the metagenomic of the Megahit assembly. Among them, 1,817,945 sequences with amino acid lengths greater than 200 were merged by CD-HIT [12] (V 4.7), and finally the abundance calculation was performed using the Salmon [13] (V 0.12.0) software to obtain 1,709,060 non-redundant coding sequences. The gene functional annotation was determined through ortholog assignment by eggNOG-mapper [14, 15] (V 1.0.3) (Supplementary Fig. S1.).

Integrated analysis of microbial metagenomic sequencing and metabolites

To show the relation between enriched KO and the abundances of metabolites, as well as microbial species, genus, and phylum, we calculated the Spearman coefficients between the abundances of metabolites and the counts of KOs, the abundances of microbial species, the abundances of microbial genus, and the abundances of microbial phylum. Furthermore, we also calculated the Spearman coefficients between the counts of KOs and the abundances of microbial species, the abundances of microbial genus, and the abundances of microbial phylum to show the enrichments of KOs in different microbial species, genus and phylum.

Results

Phylogenetic profiles of gut microbes in A53T transgenic monkeys

Gut bacteria was characterized by metagenomics sequencing. Alpha diversity analysis including the Chao, Ace, and Shannon index showed that the fecal microbiota of

A53T transgenic monkeys was more diverse compared to the controls, and Shannon index have significant difference (p=0.005) (Fig. 1A-1C). The value of Firmicutes versus Bacteroidetes (F/B value) was increased in the A53T transgenic monkeys (Fig. 1D). Beta diversity by examining the unweighted Unifrac distance expounded that microbiota composition has an obvious separation between both groups (Fig. 1E).

Phylotypes with a median relative abundance larger than 0.01% of the total abundance in either the healthy control group or A53T transgenic group were included for comparison. At the phylum level, Bacteroidetes and Firmicutes dominated the fecal microbial communities of both groups. Compared with the control groups, A53T monkeys had higher levels of Synergistetes, Acidobacteria and Nitrospirae, but lower level of Bacteroidetes. At the genus level, 20 genera were observed to have significantly increased and decreased in the A53T monkeys compared with controls group. Prevotella and Bacteroides were the dominant phylotype in both groups at genus level, but significantly decreased in the A53T group. Of the remaining enriched genera, Intestinimonas, Flavonifractor, Oscillibacter, Streptomyces and others were observed to have significantly increased. In contrast, Odoribacter, Barnesiella and others significantly decreased in the A53T transgenic monkeys. In the A53T transgenic monkeys, among the most increased species in abundance, four of them belong to Clostridiales of Firmicutes, six specieswere Eggerthellales of Actinobacteria, and four specieswere Alphaproteobacteria of Proteobacteria (Supplementary Table S1). Of the top 20 decreased species in the A53T monkeys, ten were Prevotella spp. and nine were Bacteroides spp., which

suggests that the two genera (Prevotella and Bacteroides) might play an important role in the onset of PD in monkeys (Fig 1F-1K).

Microbiota from A53T transgenic and control monkeys produce discrete metabolite profiles and correlation analysis

We performed untargeted gas chromatography analyses of colon contents from A53T transgenic and control monkeys, and a total of 93 metabolites were found, mainly including amino acids and fatty acids (Supplementary Table S2). The samples are separated on the PCoA plot into two groups suggesting that the abundance patterns of metabolites have significant differences between the two groups (Fig. 2A). Five different metabolites were found between A53T transgenic and control monkeys that include 3-Methylindole, Glyceric acid, L-Aspartic, Myristic acid and p-Hydroxyphenylacetic acid. Among the 5 different metabolites, 3-Methylindole and Myristic acid significantly decreased (multiple test corrected $p< 0.05$) (Fig. 2B, 2C) and Glyceric acid, L-Aspartic, and p-Hydroxyphenylacetic acid significantly increased (multiple test corrected $p< 0.05$) in A53T group compared to the control group (Fig. 2D, 2E, 2F). The results suggest that these metabolites might play important roles in the transgenic A53T monkeys.

Correlation analysis found that Glyceric acid and p-Hydroxyphenylacetic acid are positively correlated with Sybergistetes, which is significantly increased in A53T transgenic monkeys (multiple test corrected $p<0.05$). Myristic acid and Bacteroidetes are significantly positively correlated. However, p-Hydroxyphenylacetic acid is significantly negatively correlated with Bacteroidetes, and Bacteroidetes is observed

that significantly decreased in A53T transgenic monkeys (multiple test corrected $p<0.05$). *Candidatus Korarchaeota* and 3-Methylindole are significantly positively correlated, but no microbiota related to L-Aspartic acid was found (Fig. 2G).

Integrated analysis of intestinal microbiota and metabolites

Since the metagenomics and metabolite profiles were produced from the same batch of samples, we therefore want to explore whether there are some kinds of relations between the gut microbes and metabolites. We performed a correlation analysis for the microbiota, metabolites and functions. Significant differences in the microbiota, metabolites and functions between the two groups were observed, and the changes of the microbiota, metabolites and functions showed similar trend with good consistency (Fig.3A). We made a network of six phyla, which are the most abundant in the two groups with consistence. Acidobacteria, Sybergistetes, Nitrospirae significantly increased in A53T transgenic monkeys. In contrast, Bacteroidetes significantly decreased in A53T transgenic monkeys. Proteobacteria and Actinobacteria were not significantly different between the two groups, but 80 genera from Proteobacteria (about 80%) and 36 genera from Actinobacteria (about 70%) were significantly elevated in A53T transgenic monkeys (Fig.3B-3G).

In order to find the associated pathways, we performed functional analysis of the five differential metabolites found in the A53T transgenic monkeys. The main relevant pathways for the two metabolites (Myristic acid and 3-Methylindole) that were significantly higher in the normal control group were two-component system and bacterial chemotaxis (Fig.4A-4B). Metabolism pathways were the most significantly

285 associated pathway in the three metabolites (Glyceric acid, p-Hydroxyphenylacetic
286 acid, L-Aspartic) which was significantly increased in the A53T transgenic group
287 (Fig.4C-4E). We found four common KOs from the metabolic pathway including
288 glyceraldehyde-3-phosphate dehydrogenase (KO0131), pyrimidine metabolism
289 (KO0756), oxidative phosphorylation (KO2147) and benzoate degradation (KO4112)
290 (Fig.4F-4I).
291
292 **Discussion**
293 By analyzing the gut microbiota of the five A53T transgenic monkeys with early
294 Parkinson's symptoms and normal control monkeys, we found that the composition
295 and diversity of the gut bacterial in A53T transgenic monkeys is different. The results
296 also confirm previous reports that changes in gut microbes are associated with
297 changes in host genes [38]. In the present study, the alpha diversity significant
298 elevated in A53T transgenic monkeys compared to control monkeys, though there
299 was no significant difference. Similarly, significant elevation of alpha diversity is
300 observed in human PD patients compared to healthy humans [39, 40]. However, the
301 alpha diversity significantly reduced in the rotenone-induced PD mouse model [41].
302 The contrast between rodent and primates indicates that chemical drug-induced PD
303 rodent model is significantly inconsistent compare to PD patients in human, and the
304 A53T transgenic monkeys may present the features of human PD. There is a clear
305 separation in the composition of the gut bacteria and metabolites between the two

groups by PCoA analyze, which indicates that the composition of gut bacteria and metabolites in A53T transgenic monkeys and normal monkeys is very different.

In previous studies, Proteobacteria, Actinobacteria, Eggerthellaceae and Sphingomonas have been observed to be significantly elevated in PD patients [40, 42, 43]. This kind of trend is also observed in the A53T transgenic monkeys with early Parkinson's symptoms in the present study. Furthermore, in PD patients, Cupriavidus showed higher abundance in the non-tremor subtype of PD than in the tremor subtypes[39], and the same case about of Cupriavidus also was observed in A53T transgenic monkeys at early stage of PD in our study. Therefore, Cupriavidus may be a biomarker candidate for the diagnosis of early stage PD diagnosis. Prevotella and Bacteroides have been reported to be significantly decreased in PD patients [42, 43]. These two genera also decreased significantly in A53T transgenic monkeys with early Parkinson's symptoms in our study. Prevotella is a bacterium that produces hydrogen. H_2 bubbled water improved ratings for typical PD symptoms including a clinician scored motor evaluation among hospitalized patients [44]. We also observed that *Eggerthella lenta* significantly elevated in A53T transgenic monkeys, and this bacterium is a dopamine dehydroxylating strain that can convert dopamine to m-tyramine. M-tyramine is a chemical that regulates neurotransmitters, which was significantly elevated in the urine of patients with neurosis [45]. Odoribacter and Enterococcus were reported that increased in PD patients [39], but the abundance of these two genera were lower in A53T transgenic monkeys compared to control monkeys. Since the A53T transgenic monkeys only show early Parkinson's symptoms,

we speculate that these two genera may be associated with advanced PD. In Table 2, we summarized a comprehensive comparison of intestinal microbiota between Parkinson's patients and A53T transgenic monkeys, and in general, the monkeys at early stage of PD showed consistent gut microbiota with human PD patients (Table 2).

Metabolites are the ultimate embodiment of cellular activity. Gene expression, splicing, and neuronal function in the brain can be regulated by small molecule metabolites. A few previous studies have indicated that short-chain fatty acids are associated with the onset of PD [46, 47]. Our results also indicate that short-chain fatty acids (butyric acid, isovaleric acid, propionic acid, valeric acid, isobutyric acid) in the A53T transgenic monkeys showed the trend of reduction compared to wild monkeys, though no significant difference was detected. This may be due to the limited number of A53T transgenic monkeys involved and the correlation between short-chain fatty acids and PD symptoms may be related to the severity of PD. In the A53T transgenic monkeys, the content of glyceric acid in the colonic contents is significantly higher than that of normal monkeys. Glyceric acid synthesized by 3-phosphoglycerute produced during glycolysis may accelerate the glycolysis of A53T transgenic monkeys. The significant increase of KO0131 also supports our hypothesis. A study has proven that the glyceric acid in the colonic contents of autistic mice by transplanting stool from autistic patients also significantly elevated[48]. We speculate that the glycolysis of A53T monkeys are accelerated, so a large amount of pyruvic acid may be produced, and pyruvic acid will be converted to

acetyl-CoA. In order to ensure the smooth progress of the TCA cycle, a corresponding amount of oxalacetic acid is required. A part of oxalacetic acid may be converted to L-aspartic acid, which was evaluated in our A53T transgenic monkeys. Accelerated glycolysis and TCA cycle may indicate mitochondrial dysfunction in the A53T transgenic monkeys. p-hydroxyphenylacetic acid also significantly elevated in A53T transgenic monkeys. Tyrosine is decomposed into dopamine and p-hydroxyphenylpyruvate, and *Eggerthella lenta* that elevated in A53T transgenic monkeys can convert dopamine to m-tyramine, p-hydroxyphenylpyruvate is oxidized to p-hydroxyphenylacetic. This may cause loss of tyrosine in A53T transgenic monkeys, and the speculated possible metabolic pathway and correlation are summarized in Figure 5.

Analyses of metagenomic data enabled exploring possible pathways for three differential metabolites of significantly increased in the A53T transgenic monkeys. KO0131, KO0756, KO2147, KO4112 were present at higher abundance in the A53T group. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (KO0131) is a key enzyme in glycolysis. The increase in concentration of this enzyme may accelerate the process of glycolysis and cause mitochondrial burden [49]. At same time, GAPDH can trigger neuronal cell death by oxidative stress. Overexpression of GAPDH was also found in a mouse model of rotenone-induced Parkinson's disease, it can promotes neuron apoptosis in several neurodegenerative disorders [50, 51]. The oxidative phosphorylation reaction (KO2147) in monkeys is carried out in the mitochondria. The increase in oxidative phosphorylation also suggests that the mitochondrial energy

supply accelerates and causes mitochondrial burden [52, 53]. Previous study have shown that mitochondrial dysfunction caused by aberrant mitochondrial dynamics play an essential role in the pathogenesis of both sporadic and familial PD [54]. The speculated possible pathway and correlation between differential metabolites, KOs and bacterium related to PD are summarized in Figure 5.

Furthermore, in our study, compared with the wild monkeys, Myristic acid and 3-Methylindole were significantly decreased in the A53T transgenic monkeys. Myristic acid was observed that significantly increased diacylglycerol kinase (DGK) δ protein expression in mouse. DGK is a lipid-metabolizing enzyme that phosphorylates diacylglycerol to produce phosphatidic acid, and the deficiency of DGKδ induces obsessive-compulsive disorder (OCD)-like behavior through enhancing axon/neurite outgrowth in DGKδ-KO mice [55-57]. Myristic acid and 3-Methylindole were associated with ABC transporters, ABC transporters are the largest class of transporters widely found in bacteria and humans can hydrolyze ATP and provide energy to cells.

Conclusion

Our research has evaded the factors (living environment, diet, antibiotic use, and age.) that could interfere with the study of gut microbiota and metabolites by using nonhuman primate PD models, therefore, the results of the study will be more credible. We identified differential gut microbiota coincides with the microbiota of the currently reported PD patients. Furthermore, we found these differential

metabolites and the associated KOs suggest that A53T monkeys may have mitochondrial dysfunction, and mitochondrial dysfunction may be associated with α-syn aggregation and early Parkinson's symptoms. Our results may be a sign of early Parkinson's screening and diagnosis.

Declarations

Ethics approval and consent to participate

All procedures were approved by the Institutional Animal Care and Use Committee of Kunming University of Science and Technology, and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (8^{th} edition).

Consent for publication

Not applicable

Availability of data and materials

The obtained metagenomic profiles have been uploaded into the NCBI SRA database and are accessible via the accession number: PRJNA574851.

Competing interests

The authors declare no competing interests.

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Authors' contributions

YY conceived and designed the experiments, performed the experiments, wrote the paper, RS prepared figures and/or tables. DY performed the experiments, LC and WZ did the statistical analyses and gut microbiome. NY was responsible for the establishment and evaluation of the A53T transgenic monkeys. BI edited the grammar and spelling. JW and ZY reviewed drafts of the paper. SW conceived and designed the experiments, contributed reagents, materials, and analysis tools, wrote the paper, reviewed drafts of the paper. All authors reviewed the manuscript.

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Fig. 1 Phylogenetic profiles of gut microbes in A53T transgenic monkeys. (A, B, C) α diversity including Chao index, Ace index, Shannon index increased in A53T transgenic group compared to the control group, and Shannon index have significant difference ($p<0.005$). (D) The ratio of Firmicutes to Bacteroidetes increased in the A53T transgenic group. (E) An obvious separation between the A53T transgenic group and the control group was observed by β diversity analysis. The phylotypes significantly increased ($p<0.05$) in the A53T transgenic monkeys at the phylum (F), genus (G) and species (H) levels. The phylotypes significantly decreased in the A53T transgenic monkeys at the phylum (I), genus (J) and species (K) levels. Red and blue represent controls group and A53T group, respectively. The phylotypes of the control and A53T group were compared with Welch's t-tests and the obtained p-values were corrected with the Benjamini Hochberg method.

Fig. 2 Microbiota from A53T transgenic and control monkeys produce discrete metabolite profiles and correlation analysis. (A) Metabolites had obviously divided between A53T transgenic group and control group. (B-C) Myristic acid, 3-Methylindole significant decreased in A53T group. (D-F) p-Hydroxyphenylacetic acid, Glyceric acid and L-Aspartic acid significant increased in A53T transgenic group. The concentrations of different metabolites in the A53T transgenic and control groups were compared with Welch's t-tests and the obtained p-values were corrected with the Benjamini Hochberg method. (G) Analyze the correlation between bacteria and metabolites. Glyceric acid and p-Hydroxyphenylacetic is positively correlated

with Sybergistetes. Myristic acid and Bacteroidetes are significant positive correlated. *Candidatus Korarchaeota* and 3-Methylindole are positive correlated.

Fig. 3 Integrated analysis of intestinal microbiota and metabolites. (A) The yellow/blue labels on the left side of the figure indicate that the microbe showed significantly different (t-test with p-value <0.05) enrichment in the control/A53T group. (B-G) This difference has a distinct transmission relationship in the classification of part microbe (The direction of the phylum-genus-species is indicated by blue/yellow arrows). Yellow present increased in A53T group. Blue present increased in control group.

Fig.4 Analysis of enriched pathways for the five differential metabolites. (A-B) Metabolite- enriched pathways significantly associated with control monkeys. (C-E) Metabolite-enriched pathways significantly associated with A53T transgenic monkeys. (F-I) 4 KOs from metabolic pathways that are significantly elevated in the transgenic A53T monkeys.

Fig. 5 We speculate a possible metabolic response analysis of three differential metabolites, in which an increase of glyceric acid and L-aspartic acid may indicate an acceleration of glycolysis and TCA cycle, and functional analysis of differential metabolites also illustrates this speculated (KO2147, KO0131). In addition, tyrosine is decomposed into dopamine and p-hydroxyphenylpyruvate, *Eggerthella lenta* can convert dopamine to m-tyramine, p-hydroxyphenylpyruvate is oxidized to

657 p-hydroxyphenylacetic. G-3-P: oxidation of plyeradehyde-3-phosphate; 1, 3-BPG: 1,
658 3-triglyceric acid diphosphate; 3-PG: 3-phosphoglycerate. Yellow: metabolites,
659 microbiota, KO increased significantly in A53T monkeys.
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