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ORIGINAL ARTICLE

Response of gut microbiota in type 2 diabetes to hypoglycemic agents

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Abstract

Purpose Accumulated evidence has indicated that the gut microbiome affected the pharmacology of anti-diabetic agents, and their metabolic products induced by the agents transformed the structure of gastrointestinal microbiota in return. However, the studies around heredity, ethnicity, or living condition, referring to human microbiome were mostly represented by an occidental pattern partial and rare studies that focused on the effect of several first-line hypoglycemic agents on the gut flora in a single medical center. Therefore, we aimed to explore the interaction between gut microbiome and type 2 diabetes (T2D) or hypoglycemics in Chinese population.

Methods A total of 130 T2D patients with a specific hypoglycemic treatment and 50 healthy volunteers were enrolled in this study. Gut microbiome compositons were analyzed by 16S ribosomal RNA gene-based sequencing protocol.

Results Hypoglycemic agents contributed to the alteration of specific species in gut bacteria rather than its total diversity. Metformin increased the abundance of *Spirochaete*, *Turicibacter*, and *Fusobacterium*. Insulin also increased *Fusobacterium*, and α-glucosidase inhibitors (α-GIs) contributed to the plentitude of *Bifidobacterium* and *Lactobacillus*. Both metformin and insulin improved taurine and hypotaurine metabolism, and α-GI promoted several amino acid pathways. Although the community of gut microbiota with metformin and insulin showed similarity, significant differences were available in each diabetic group with hypoglycemia.

Conclusions Gut microbiota is significantly associated with anti-diabetic agents. The gut microbiome and metabolism have shown respective characteristics in different T2D groups, which were also significantly different from the healthy group. This study provides some new insights for identification and exploration of the pathogenesis of T2D.

Keywords Microbiota · Type 2 diabetes · Metformin · Insulin · α-Glucosidase inhibitors.

Introduction

As a major public health issue, type 2 diabetes (T2D) is a complex metabolic disorder affected by both genes and the environment [1]. In China, the total number of individuals with diabetes had risen, significantly increasing from 0.9% in 1980 to 10.9% in 2013 [2–4]. Although associations between T2D and several risk factors, such as obesity, physical activity, and active genetic architecture, have been demonstrated [5], the mechanism of pathogenesis is still unknown. Recently, a series of studies have reported that gut microbiota is also involved in the pathogenesis and therapeutic responses of T2D [6–9].

The gut microbiota, which mainly consisted of bacteria, refers to a complicated assembly of trillions of microbes in the human gastrointestinal tract [10]. Gut bacteria exerted

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benign or malignant influence on metabolic disorders and its dysbiosis have been demonstrated to be related to several metabolic diseases including T2D [11, 12]. In addition, accumulated evidence has indicated that the gut microbiome affected the pharmacology of anti-diabetic agents, and their metabolic products induced by the agents transformed the structure of gastrointestinal microbiota in return [9, 13]. Numerous studies have identified that the species-specific therapeutic drug altered the gut microbiome in animal models or *Homo sapiens* [14, 15], revealing the potential for targeting the gut microbiota. However, it was observed in some occidental pattern partial studies that the number of factors, such as heredity, ethnicity, or living condition, was instrumental in the variants in composition and diversity of human microbiome [16]. Moreover, to our best knowledge, rare studies simultaneously refer to the effect of several first-line hypoglycemic agents on gut flora in the one regional population. Therefore, exploring the interaction between gut microbiome and hypoglycemics in a specific population not only clarify their association but also contribute to developing potential therapeutic strategies with individualization.

For this purpose, an assembly of patients with T2D and healthy volunteers was randomly selected in this study. T2D subjects with separate treatment of anti-diabetic agents, including metformin, insulin, and α-glucosidase inhibitors (α-GI), were collected to investigate the effect of therapeutic agents on the structure and function of microbiota in the human gastrointestinal tract. DNA extracted from each individual's fecal sample was subjected to the 16S ribosomal RNA (16s rRNA) gene sequencing and metagenomic analysis. Our results clarify and characterize the variation of gut bacterial community in the Chinese population with T2D, and the features of human gut microbiome altered by anti-diabetic drugs may offer a potential support in developing specific pharmacy strategies.

Material and methods

Human subjects

Written informed consent and questionnaire data sheets were obtained from all subjects who visited the Department of Endocrinology, Clinical Laboratory, and Core Research Laboratory. They agreed to serve as sample donors, in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki). All enrolled subjects with similar physical characteristics were achieved from Qingdao Central Hospital (Table 1), including 50 healthy volunteers and 130 patients diagnosed as T2D with blood glucose ≥7.0 mmol. Based on their personal drug-using history, subjects with T2D were classified into four groups: metformin, insulin, α-GI, and non-therapeutic (NT). Several collective criterions were implemented in the diabetic group including duration of hypoglycemic treatment >3 months, specific drug-using history, and without complications of other body systems. Ethical approval was granted by the medical ethics committee of Qingdao Central Hospital Ethics Committee (QCHEC) with the following reference number 2016-8-2602. All volunteers had received information regarding their participation in the study and gave written informed consent.

Specimen processing and 16s rRNA gene sequencing

Fecal samples gathered from the healthy controls and patients by sterile centrifuge tube were immediately transferred into liquid nitrogen and then stored at −80 °C for preservation. About 400 mg stool ingredients were separately applied in DNA isolation by FastDNA Spin Kit for Feces (MP Biomedics) as described in the previous study

Table 1 Cohorts' information

	Metformin	Insulin	α-GI	NT	Health
Subject number	51	36	17	26	50
Age	58.1 ± 9.4	59.75 ± 13.0	62.3 ± 10.4	56.4 ± 10.6	58.5 ± 3.0
Gender ratio[a]	1.3	1.1	0.9	1.3	1
Height (cm)	168.3 ± 8.2	166.9 ± 9.0	169.8 ± 9.1	170.8 ± 8.7	166.0 ± 6.5
Weight (kg)	73.5 ± 10.5	70.6 ± 11.3	74.4 ± 9.7	74.3 ± 9.4	69.5 ± 12.0
Blood sugar (mmol/l)	9.5 ± 2.6	9.0 ± 3.3	9.9 ± 2.5	9.8 ± 2.9	5.3 ± 1.1
HbA1c (%)	8.7 ± 2.2	8.7 ± 2.0	8.6 ± 1.8	9.2 ± 2.1	6.0 ± 0.7
Insulin level (uIU/ml)	10.9 ± 6.0	11.3 ± 5.5	13.6 ± 8.2	10.7 ± 6.1	9.3 ± 4.3

α-GI α-glucosidase inhibitor, *NT* non-therapeutic, *HbA1c* hemoglobin A1c

[a]Gender ratio is the ratio of males/females

[17]. The quality of extracted DNA was estimated using NanoDrop 2000 (Thermo Scientific). The qualified bacterial composition obtained from the subject's stool was identified by amplicon generated from the V1–V2 region of 16s rRNA gene sequencing by universal primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 338R (5′-CTGCCTCCCGTAGGAGT-3′), and then purified using QIAquick PCR Purification Kit (Qiagen Co. Ltd). The pooled PCR products in equal concentration were followed by preparation of sequence libraries on the Illumina Hiseq 2500 platform with a standard protocol.

Sequencing data analyses

Paired-end data sets were matched and filtered by fast length adjustment of short reads (FLASH) to improve read assemblies as described before [18]. All sequences analysis was provided by the quantitative insights into microbial ecology (QIIME, version 1.9.1) software suite, according to the QIIME tutorial with several modified methods [19]. Chimeric sequences were removed using usearch61 with de novo models. Sequences were clustered against the 2013 Greengenes (13.8 release) ribosomal databases 97% reference dataset. Sequences that did not match any entries in this reference were subsequently clustered into de novo operational taxonomic units (OTUs) at 97% similarity with UCLUST. Taxonomy was assigned to all OTUs using the RDP classifier within QIIME and Greengenes reference dataset [20]. Rarefaction and rank abundance curves were calculated from OTU tables utilizing α-diversity and rank abundance scripts within the QIIME pipeline.

Statistical analyses

To account for any bias caused by uneven sequencing depth, the least number of sequences present in any given sample from a sample category was selected randomly prior to calculating community-wide dissimilarity measures (α-diversity and β-diversity); we rarefied the OTU table to a sequencing depth of 24,000 per sample for both diversity analyses. Statistical analyses were performed by R and STAMP. The graphical representation of the results was done with R or STAMP, and *P* values were calculated using the Mann–Whitney *U* test (significance threshold: $P < 0.05$). Principal coordinate analysis (PCoA) was based on unweighted UniFrac distances using evenly sampled OUT abundance. The prediction of the functional composition of metagenome using a database of reference genomes was done with phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) as described [21].

Results

Subjects and sequencing data

The sample bank included a cohort of healthy volunteers, untreated T2D patients, and patients treated with different hypoglycemic agents, including metformin, insulin, and α-GI (Table S1). After quality control and omitting low-quality reads of the sequencing data, a total of 3,834,095 and 1,431,378 qualified sequences were obtained from the fecal samples of the T2D patients ($n = 130$) and heathy controls ($n = 50$). Among the diabetic group (Table S2), 766,580 reads were gained in the NT group ($n = 26$), 1,502,444 reads were from the metformin group ($n = 51$), 1,064,474 reads were from the insulin group ($n = 36$), and 500,597 reads were from the α-GI group ($n = 17$). Acquired sequences of each individual were in the range of 24,292–29,895 with a mean of 29,252 (Table S2).

Gut microbial communities of patients with hyperglycemia

Significant discrepancies in species diversity of gut microbiota between each diabetic cohort and the healthy control were found in this study, which indicated (Fig. 1a–d) that the gut microbiota in the patients was associated with the T2D. Despite multiple mechanisms of metformin, insulin, and α-GI to release hyperglycemia, a few divergences were observed between NT and T2D groups with anti-diabetic drugs. The results demonstrated that agents had little effect on a variety of microbes in the human gastrointestinal tract.

Taxonomy of sequencing reads aligned to the 2013 Greengenes (13_8 release) ribosomal databases 97% reference data set were classified and mainly categorized to four phyla, including *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Fusobacteria* (Fig. 1e). Distinguished from that of the European cohort [22], the proportion of *Firmicutes* in Chinese population showed no significant fluctuation among each group (Fig. 1e). In addition, analogous tendencies in abundance of *Bacteroidetes* or *Proteobacteria* were also observed (Fig. 1e). However, several classes attributed to *Firmicutes* and *Proteobacteria* illustrated variances induced by special hypoglycemic agents (Fig. 1f). In the phylum *Proteobacteria*, the abundance of *Gammaproteobacteria* (10.50%, 9.11%) and *Betaproteobacteria* (4.39, 3.45%) was similar between the NT group and the healthy group (Fig. 1f); the abundance of *Gammaproteobacteria* (14.99%) was positively correlated with insulin treatment, while the abundance of *Betaproteobacteria* (1.73%) was negatively correlated with insulin treatment (Fig. 1f). Metformin caused a slight decrease in the diversity of *Betaproteobacteria* (3.20%) and *Gammaproteobacteria*

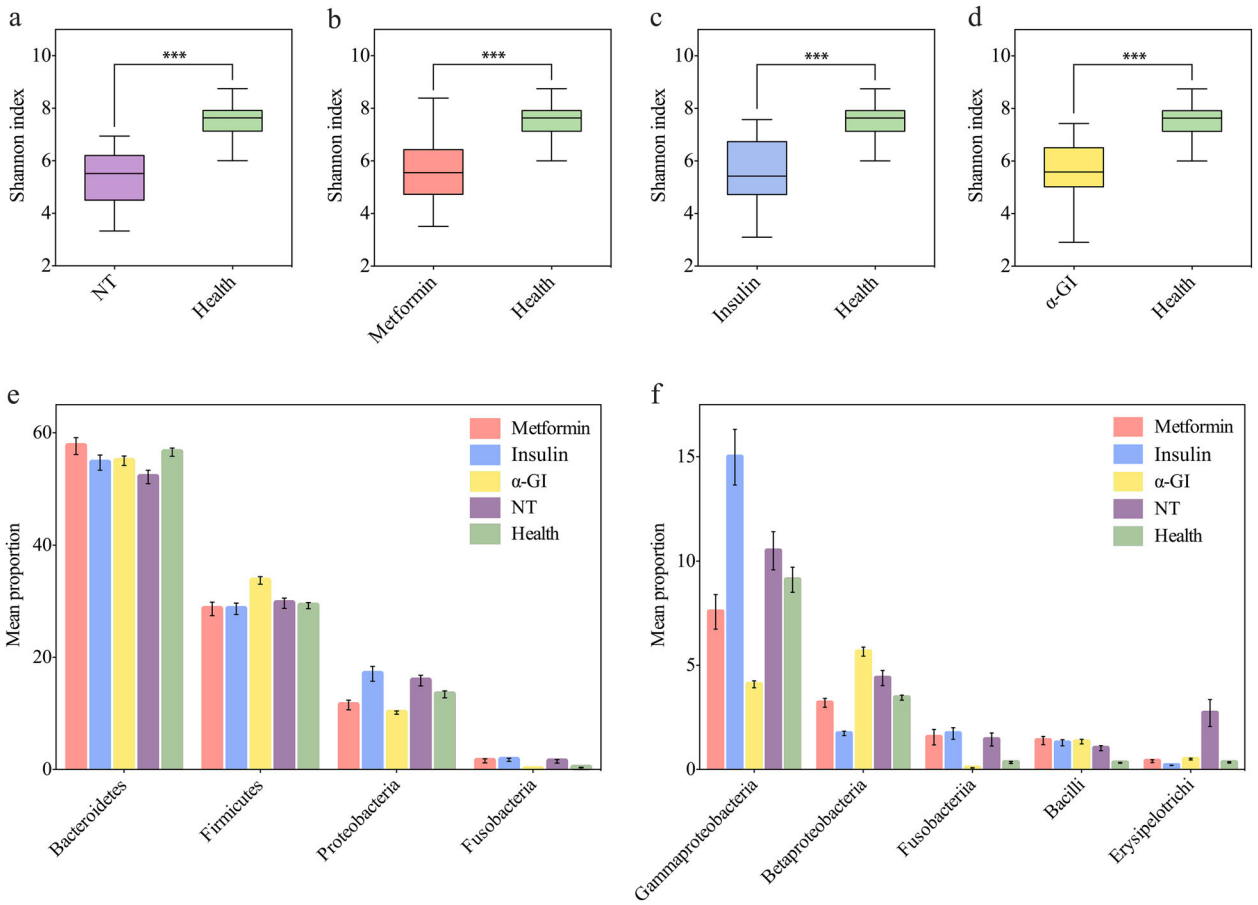

Fig. 1 Diversity of gut microbiota and abundance of several species. Indexes were calculated according to the optical transform unit (OTU) profile of each group, ***$P < 0.001$. **a** Diversity of the gut microbiota community in NT and healthy subjects. **b** Diversity of the gut microbiota community in metformin and healthy subjects. **c** Diversity of the gut microbiota community in insulin and healthy subjects. **d** Diversity of the gut microbiota community in α-GI and healthy subjects. **e** Alternation of gut microbiota induced by anti-diabetic agents based on taxonomy of phylum. The discrepancy based on taxonomy of phylum is shown for each group. **f** Alternation of gut microbiota induced by anti-diabetic agents based on taxonomy of class

(7.57%), while α-GI exerted a contrast impact in *Betaproteobacteria* (5.66%) and *Gammaproteobacteria* (4.09%) (Fig. 1f). In taxonomy of *Firmicutes*, compared to NT subjects, each therapeutic agent caused a decrease in *Erysipelotrichi*, which was positively linked to obesity in rodents or *Homo sapiens* [23, 24], and the abundance of *Bacili* was rarely changed in all the T2D groups (Fig. 1f). Despite an infrequent proportion, *Alphaproteobacteria* (not shown in figure) performed an obvious decreased tendency in metformin (0.24%), insulin (0.04%), and α-GI (0.01%) patients compared to the healthy cohort (0.67%). Meanwhile, *Fusobacteria* significantly decreased in the α-GI group (0.08%; healthy: 0.34%), but it was up-regulated about 1% in metformin, insulin, or NT groups. In contrast, with no heterogeneity, *Actinobacteria* (not shown in figure) significantly raised in cohorts of hypoglycemic agents with a mean of 0.26% (healthy: 0.01%).

In addition, to estimate the similarity of community of intestinal flora among each group, PCoA of unweight Unifrac metric based on phylogenetic distances (Fig. 2) was selected and each gut microbial component of diabetic cohorts was observed with a significant separation from that of the healthy cohort ($P < 0.01$) (Fig. 2). Moreover, therapeutic agents remarkably altered the community of gut microbiota, which was different from that in the NT group ($P < 0.01$) (Fig. 2). Although the community of metformin and insulin performed convergence, the distance among each high blood glucose with drug was significant ($P < 0.01$) (Fig. 2).

Specific features in the gut microbiota altered by hypoglycemic agents

A composite of species filtered by linear discriminant analysis was transformed to a heatmap by R (Fig. 3a). As a member, *Firmicutes*, *Faecalibacterium*, and *Ruminococcus* exhibited a decreasing tendency in the NT group (Fig. 3a). The two bacteria were deemed to mainly butyrate-

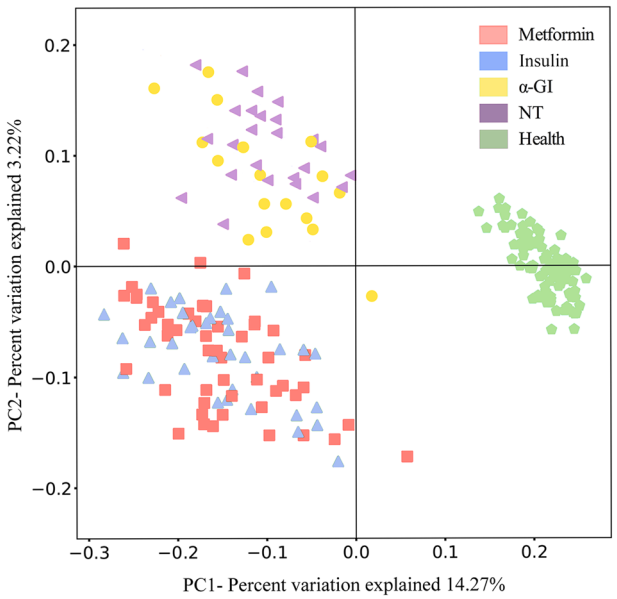

Fig. 2 Unweighted UniFrac PCoA plot. The community of gut flora in T2D patients varied from that in healthy individuals ($P < 0.01$). Although metformin and insulin showed convergence, significant discrepancies existed in each diabetic group ($P < 0.01$)

producing species, which is a kind of four-carbon short-chain fatty acid (SCFA) associated with obesity-related metabolic diseases and intestinal homeostasis. In contrast, the abundance of *Erysipelotrichaceae*, which positively refer to obesity in the NT group, was distinctly high when compared to other groups (Fig. 3a). Although its function remains to be elucidated, the proliferation of *Erysipelotrichaceae* has been enriched in mice fed with high fat diet or with non-alcoholic fatty liver disease.

Among therapeutic cohorts, the relative abundance of *Spirochaete* and *Turicibacter* were increased in the metformin treatment group (Fig. 3a), and the decrease of these genera revealed a positive relationship with hepatocirrhosis or inhibition of mechanistic target of rapamycin (mTOR). Meanwhile, metformin ameliorated the abundance of *Ruminococcus* (Fig. 3a). As a kind of butyrate-producing bacteria, *Ruminococcus* was related to the impaired glucose control with regard to T2D [22]. The plentitude of *Fusobacterium* was positively altered by insulin (Fig. 3a) and this increment would influence the up-regulation of genes involved in triglyceride and arachidonic acid metabolism in the colon and down-regulation in the small intestine, including bile acid and retinol metabolism. In addition, the content of *Lactobacillus* and *Bifidobacterium* was associated with the treatment of α-GI (Fig. 3a). As one of the component of ferritin related to insulin resistance [25], iron absorption was identified to be promoted by *Lactobacillus*. Although its function remain unclarified, *Bifidobacterium* was positively related to T2D [26].

Additionally, receiver operating characteristic (ROC) was applied to estimate the effects of the above species in distinguishing between healthy and patients with hyperglycemia in the anti-diabetic group or the NT group. Based on the area under curve of ROC, *Faecalibacterium* was reliable in making a distinction between the healthy and patients with T2D (Fig. 3b), and a similar conclusion was appropriate in the phylum *Spirochaete* with metformin in the NT group (Fig. 3c) or in the phylum *Fusobacterium* with insulin in the NT group (Fig. 3d). However, the effect of *Lactobacillus* or *Bifidobacterium* was unsatisfactory in measuring α-GI subjects.

The impact of therapeutic agents on potential functions of gut microbiota

A preliminary Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of bacterial flora reflected the differences generated by T2D or anti-diabetic drugs based on 16s rRNA sequencing data was analyzed with PICRUSt [21]. The graphical representation of the results was done with STAMP; the corrected *P* values of all presented pathways in this section was lower than 0.05 (Fig. 4).

Compared to the healthy control, catabolism pathways of glycan degradation including other glycan and glycosaminoglycan were down-regulated in NT patients (Fig. 4a). Meanwhile, several amino acid-, lipid-, and fatty acid-associated metabolic pathways were scant in the diabetic group with varying degrees (Fig. 4b–d). Among these pathways, the most metabolic pathways refer to the amino acid synthesis and metabolism, and almost all the pathways, except some in the insulin group, show down-regulating tendencies in groups with hypoglycemic drugs. When compared with the NT group, different anti-diabetic drugs up-regulated the abundance of certain functional pathways related to glycan, lipid, or fatty acid with the drugs specificity (Fig. 4e–g). For more detailed information, metformin promoted the lipid biosynthesis (Fig. 4e), and insulin enforced carbohydrate metabolism (Fig. 4f). Both agents contributed to the metabolism of taurine and hypotaurine, which have been demonstrated to significantly decrease blood sugar in diabetic animal models. Alternations of α-GI mainly referred to increasing amino acid pathways may reflect its benign impact on the function of gut microbiota (Fig. 4g), and the abundance of fatty acid metabolism and lipid synthesis was more in the NT group (Fig. 4g).

Discussion

In this study, the gut microbiota from the Chinese T2D patients taking different therapeutic agents and healthy

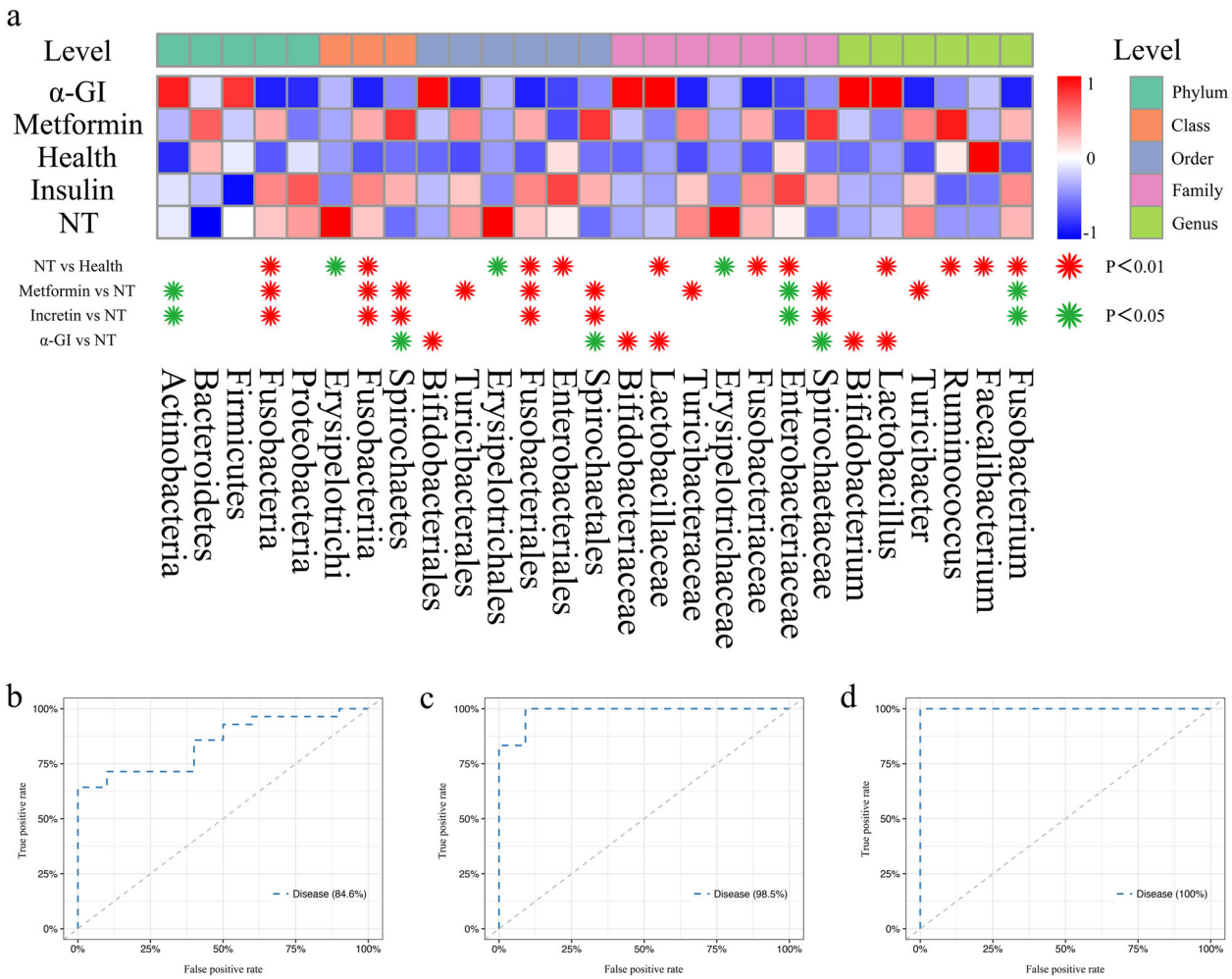

Fig. 3 Specific species altered by pathogenesis and therapeutic agents. **a** Relative abundance of species altered by pathogenesis and therapeutic agents. Several bacteria filtered by species of LDA (score > 2) were transformed to the heatmap. **b** ROC plot of healthy individuals and patients given NT for Faecalibacterium. **c** ROC plot of patients given metformin vs. NT for Spirochaete. **d** ROC plot of patients given insulin vs. NT for Fusobacterium

controls was compared and classified based on the data of the V1–V2 region of the 16s rRNA sequencing. The diversity of intestinal microbiome significantly declines and the number of butyrate-producing species including *Faecalibacterium* and *Ruminococcus* were scant in non-treated T2D patients. With few impacts on bacterial diversity, certain species and functional pathways related to the gut microbiota altered by hypoglycemic agents were further demonstrated. With the exception of α-GI, selected bacteria were reliable in distinguishing different group members based on our results.

Although no significant variations exist in the phylum of *Firmicutes*, *Fecalbacterium* attributed to *Firmicutes* exhibited higher abundance in healthy population compared to patients with T2D. *Fecalbacterium* genus was involved in butyrogenesis as well as in oligosaccharide membrane transport and pyruvate phosphate dikinase activity, and these metabolic functions were imperative to the intestinal homeostasis [27, 28]. As a kind of four-carbon SCFA, butyrate promoted biosynthesis enzymes generated by butyric bacteria, which is essential for the health of the colon. Several studies demonstrated the relationship between butyrate production and obesity-related metabolic diseases including T2D. Although up-regulation of butyrate synthesis pathway was not observed in this research, the scant *Fecalbacterium* would be a reliable marker for identifying patients with T2D, which would have the potential clinical applications in the diagnosis of T2D. In contrast, an increased abundance of *Erysipelotrichaceae* was observed in the NT group. Although its metabolic function is involved in hyperglycemia still remain unclarified, increased *Erysipelotrichi* exhibited in mice switched to diets high in fat was reported [24]. In addition, its derivatives (such as *Erysipelatoclostridium*) were treated as an opportunistic pathogen maintaining a relationship with intestinal diseases including colitis [29]. Moreover, a negative association between *Erysipelotrichaceae* and inosine has been observed [30], which

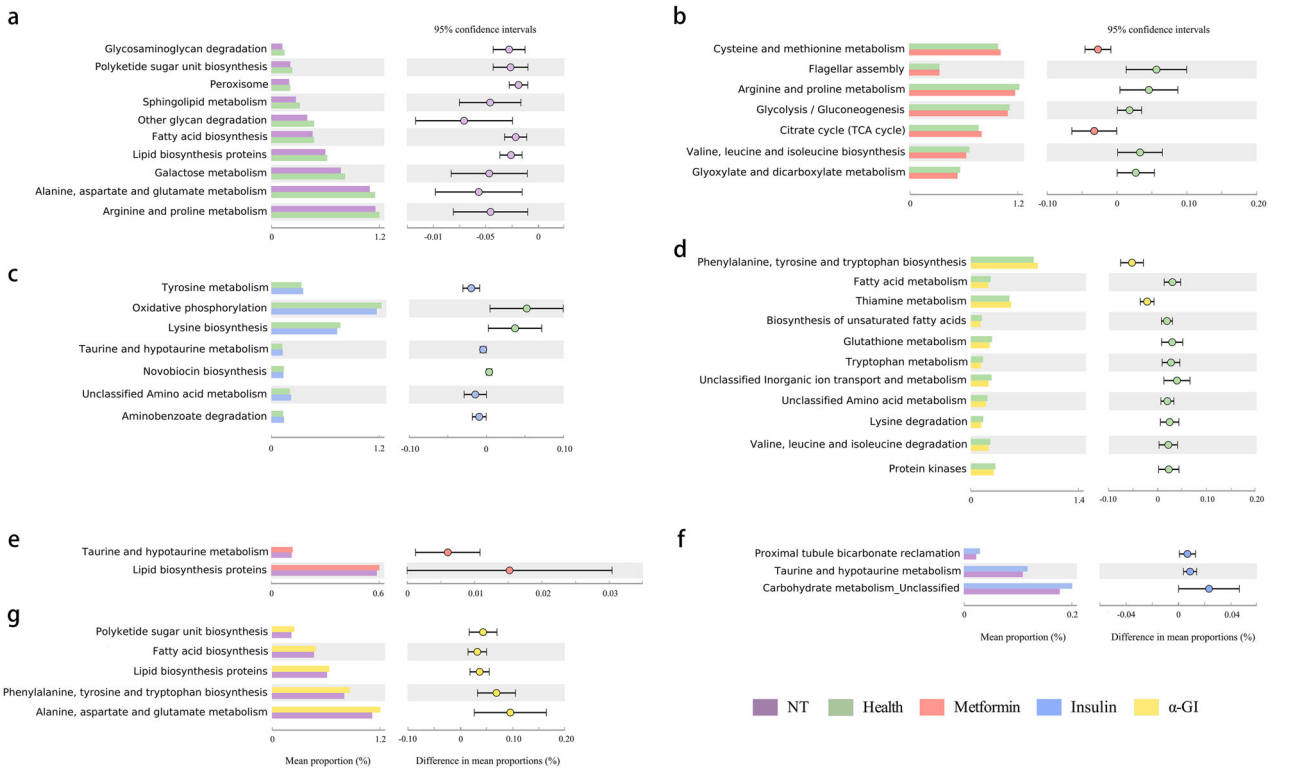

Fig. 4 Predictive analysis in the metabolic function of gut microbiota. **a** Metabolic function of gut microbiota altered by pathogenesis of T2D. **b** Differences in metabolic function of gut microbiota between patients given metformin and the healthy. **c** Differences in metabolic function of gut microbiota between patients given insulin and the healthy. **d** Differences in metabolic function of gut microbiota between patients given α-GI and the healthy. **e** Differences in metabolic function of gut microbiota between patients given metformin vs. NT. **f** Differences in metabolic function of gut microbiota between patients given insulin vs. NT. **g** Differences in metabolic function of gut microbiota between patients given α-GI vs. NT

may be implemented in the treatment of patients with gout. This relevance may offer microbial clues to unveil long-term complications of T2D involving stroke [31].

As a first-line hypoglycemic agent in the treatment for T2D, metformin has been demonstrated to modify the gut microbiota structure and alter the bile acid recirculation, suggesting that its primary action site may exist in the intestine [32]. Our results identified that metformin made a contribution to the relative abundance of *Turicibacter* and *Spirochaete*. The two bacteria were found to be enriched in patients with T2D after treatment with metformin, which provided clues for estimating the curative effect of metformin. It has been reported that the abundance of *Turicibacter* decreased in response to a high fat diet or rapamycin [33]. Rapamycin is an inhibitor of both mTOR complex 1 and 2. The mTOR signal pathway is a crucial regulator of energy intake associated with metabolic disorders such as obesity or T2D. Hence, increased *Turicibacter* may indicate the positive impact of metformin on energy balance through mTOR pathway in patients with metformin. In addition, it has been reported that hosts with cirrhosis had lower abundance of *Spirochaetes* [34]. Several functional pathways altered by metformin were also estimated in this article. Both metformin and insulin improved taurine and hypotaurine metabolism. Although the mechanism of promoted lipid biosynthesis still remain unclarified, it was reported that weight and blood sugar in diabetic rats was significantly decreased by taurine. Although some changes in the metabolic function of gut microbiota in the metformin group were predicted by the software, it is hard to identify this relationship directly in the human body.

Despite the existed difference between metformin and insulin group, to some extent, the composition and diversity of gut microbiota altered by insulin exhibited a tendency to metformin, which may be explicated by the improvement of metformin in insulin-mediated glucose uptake in the skeletal muscle and modulating the insulin pathway. Both insulin and metformin positively induced *Fusobacterium*, yet it is reliable to discriminate insulin and NT subjects in the genus *Fusobacterium* (Fig. 3d). The species-specific role of *Fusobacterium* spp. was demonstrated, such as potentiation tumor growth and modulating host immune responses during disease by inducing expansion of T-helper type 17 cells [35]. Moreover, it has been reported that *F. varium* (a species of *Fusobacterium*) influenced the

upregulation of triglyceride and arachidonic acid metabolism in the colon, which may be related to energy balance of hosts [36]. Besides taurine and hypotaurine metabolism, insulin also promotes carbohydrate metabolism, indicating its impact on balancing intake between consumption of energy source [37]. In addition, the analytical data reflect insulin increase proximal tubule bicarbonate reclamation, but little knowledge of this metabolic pathway has been reported.

By delaying the digestion of carbohydrates to reduce postprandial hyperglycemia, it has been demonstrated that α-GI prevented starch processing and absorption and enhanced starch fermenting [15]. According to our results, also supported by several articles, α-GI increased the abundance of *Bifidobacterium* and *Lactobacillus*, which were considered as probiotics in human intestine. α-GI control the absorption of carbohydrates in the small intestine, allowing saccharolytic bacteria to thrive. As saccharolytic organisms, *Bifidobacterium* and *Lactobacillus* genomes remain specific genes involving degradation of various carbohydrates and bile acid composition [13]. Representing part of the anaerobic gut community, *Bifidobacterium* may possess a specialized fermentation metabolic pathway to produce growth substrates for other members in the gut microbial community [38]. Attributed to a few subjects, the feasibility of *Bifidobacterium* and *Lactobacillus* in discriminating patients receiving treatment of α-GI with NT patients is unsatisfactory.

Although, based on clinical samples, our study has investigated the impacts of several anti-diabetic agents on gut microbiome and compares their similarities and differences, some further in vivo studies based on mice models are warranted in order to reflect the effect of anti-diabetic agents on gut microbiome in another aspect. However, in this study, we still identify the interaction between gut microbiome and hypoglycemics in a specific population, which will contribute to developing potential therapeutic strategies with individualization and provide new insights for exploration of the pathogenesis of T2D in in vivo studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Research involving human participants and/or animals: this article does not contain any studies with animals performed by any of the authors.

Informed consent All the subjects signed informed consent beforehand and belonged to the same geographical area. Data were collected by using a standardized questionnaire, including basic information, medical history, and examination results.

References

1. K.E. Wellen, J. Clin. Invest. **115**, 1111 (2005)
2. Lancet. **388**, 2566 (2016). http://www.chinadaily.com.cn/china/2016-11/15/content_27382697.htm
3. L. Wang, P. Gao, M. Zhang, Z. Huang, D. Zhang, Q. Deng, Y. Li, Z. Zhao, X. Qin, D. Jin, M. Zhou, X. Tang, Y. Hu, L. Wang, JAMA **317**, 2515 (2017)
4. Y. Xu, L. Wang, J. He, Y. Bi, M. Li, T. Wang, L. Wang, Y. Jiang, M. Dai, J. Lu, M. Xu, Y. Li, N. Hu, J. Li, S. Mi, C.-S. Chen, G. Li, Y. Mu, J. Zhao, L. Kong, J. Chen, S. Lai, W. Wang, W. Zhao, G. Ning, JAMA **310**, 948 (2013)
5. R.C.W. Ma, X. Lin, W. Jia, Lancet Diabetes Endocrinol. **2**, 980 (2014)
6. J. Qin, Y. Li, Z. Cai, S. Li, J. Zhu, F. Zhang, S. Liang, W. Zhang, Y. Guan, D. Shen, Y. Peng, D. Zhang, Z. Jie, W. Wu, Y. Qin, W. Xue, J. Li, L. Han, D. Lu, P. Wu, Y. Dai, X. Sun, Z. Li, A. Tang, S. Zhong, X. Li, W. Chen, R. Xu, M. Wang, Q. Feng, M. Gong, J. Yu, Y. Zhang, M. Zhang, T. Hansen, G. Sanchez, J. Raes, G. Falony, S. Okuda, M. Almeida, E. LeChatelier, P. Renault, N. Pons, J.-M. Batto, Z. Zhang, H. Chen, R. Yang, W. Zheng, S. Li, H. Yang, J. Wang, S.D. Ehrlich, R. Nielsen, O. Pedersen, K. Kristiansen, J. Wang, Nature **490**, 55 (2012)
7. K.E. Bouter, D.H. van Raalte, A.K. Groen, M. Nieuwdorp, Gastroenterology **1**, (2017)
8. Y. Vázquez-Baeza, C. Callewaert, J. Debelius, E. Hyde, C. Marotz, J.T. Morton, A. Swafford, A. Vrbanac, P.C. Dorrestein, R. Knight, Annu. Rev. Pharmacol. Toxicol. **58**, 253 (2018)
9. N.M. Koropatkin, E.C. Martens, Cell Metab. **26**, 456 (2017)
10. T.H. M. P; Consortium. Nature **486**, 207 (2012)
11. H.K. Pedersen, V. Gudmundsdottir, H.B. Nielsen, T. Hyotylainen, T. Nielsen, B.A.H. Jensen, K. Forslund, F. Hildebrand, E. Prifti, G. Falony, E. Le Chatelier, F. Levenez, J. Doré, I. Mattila, D.R. Plichta, P. Pöhö, L.I. Hellgren, M. Arumugam, S. Sunagawa, S. Vieira-Silva, T. Jørgensen, J.B. Holm, K. Trošt, M. Consortium, K. Kristiansen, S. Brix, J. Raes, J. Wang, T. Hansen, P. Bork, S. Brunak, M. Oresic, S.D. Ehrlich, O. Pedersen, Nature **535**, 376 (2016)
12. H. Herrema, R.G. IJzerman, M. Nieuwdorp, Diabetologia **60**, 613 (2016)
13. Y. Gu, X. Wang, J. Li, Y. Zhang, H. Zhong, R. Liu, D. Zhang, Q. Feng, X. Xie, J. Hong, H. Ren, W. Liu, J. Ma, Q. Su, H. Zhang, J. Yang, X. Wang, X. Zhao, W. Gu, Y. Bi, Y. Peng, X. Xu, H. Xia, F. Li, X. Xu, H. Yang, G. Xu, L. Madsen, K. Kristiansen, G. Ning, W. Wang, Nat. Commun. **1**, 1785 (2017)
14. H. Wu, E. Esteve, V. Tremaroli, M.T. Khan, R. Caesar, L. Mannerås-Holm, M. Ståhlman, L.M. Olsson, M. Serino, M. Planas-Fèlix, G. Xifra, J.M. Mercader, D. Torrents, R. Burcelin, W. Ricart, R. Perkins, J.M. Fernàndez-Real, F. Bäckhed, Nat. Med. **1**, 850–858 (2017)

15. Y. Kishida, H. Okubo, H. Ohno, K. Oki, M. Yoneda, J. Gastroenterol. **52**, 1180 (2017)
16. V.K. Gupta, S. Paul, C. Dutta, Front. Microbiol. **8**, 229 (2017)
17. M.M. Rodríguez, D. Pérez, F.J. Chaves, E. Esteve, P. Marin-Garcia, G. Xifra, J. Vendrell, M. Jové, R. Pamplona, W. Ricart, M. Portero-Otin, M.R. Chacón, J.M.F. Real, Nat. Publ. Group **1**, 14600 (2015) https://www.nature.com/articles/srep14600
18. T. Magoc, S.L. Salzberg, Bioinformatics **27**, 2957 (2011)
19. J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Peña, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, Nat. Methods **7**, 335 (2010)
20. J.R. Cole, Q. Wang, E. Cardenas, J. Fish, B. Chai, R.J. Farris, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, T. Marsh, G.M. Garrity, J.M. Tiedje, Nucleic Acids Res. **37**, D141 (2009)
21. M.G.I. Langille, J. Zaneveld, J.G. Caporaso, D. McDonald, D. Knights, J.A. Reyes, J.C. Clemente, D.E. Burkepile, R.L. Vega Thurber, R. Knight, R.G. Beiko, C. Huttenhower, Nat. Biotechnol. **31**, 814 (2013)
22. A. Tanca, M. Abbondio, A. Palomba, C. Fraumene, V. Manghina, F. Cucca, E. Fiorillo, S. Uzzau, Microbiome **5**, 79 (2017)
23. T. Greiner, F. Bäckhed, Trends Endocrinol. Metab. **22**, 117 (2011)
24. J. Henao-Mejia, E. Elinav, C. Jin, L. Hao, W.Z. Mehal, T. Strowig, C.A. Thaiss, A.L. Kau, S.C. Eisenbarth, M.J. Jurczak, J.-P. Camporez, G.I. Shulman, J.I. Gordon, H.M. Hoffman, R.A. Flavell, Nature **482**, 179 (2012)
25. R. Jiang, J.E. Manson, J.B. Meigs, J. Ma, N. Rifai, F.B. Hu, JAMA **291**, 711 (2004)
26. B. Su, H. Liu, J. Li, Y. Sunli, B. Liu, D. Liu, P. Zhang, X. Meng, J. Diabetes **7**, 729 (2015)
27. K. Machiels, M. Joossens, J. Sabino, V. De Preter, I. Arijs, V. Eeckhaut, V. Ballet, K. Claes, F. Van Immerseel, K. Verbeke, M. Ferrante, J. Verhaegen, P. Rutgeerts, S. Vermeire, Gut **63**, 1275 (2014)
28. L.K. Brahe, A. Astrup, L.H. Larsen, Obes. Rev. **14**, 950 (2013)
29. D. Gevers, S. Kugathasan, L.A. Denson, Y. Vázquez-Baeza, W. Van Treuren, B. Ren, E. Schwager, D. Knights, S.J. Song, M. Yassour, X.C. Morgan, A.D. Kostic, C. Luo, A. González, D. McDonald, Y. Haberman, T. Walters, S. Baker, J. Rosh, M. Stephens, M. Heyman, J. Markowitz, R. Baldassano, A. Griffiths, F. Sylvester, D. Mack, S. Kim, W. Crandall, J. Hyams, C. Huttenhower, R. Knight, R.J. Xavier, Cell Host Microbe **15**, 382 (2014)
30. L. Chen, J.E. Wilson, M.J. Koenigsknecht, W.-C. Chou, S.A. Montgomery, A.D. Truax, W.J. Brickey, C.D. Packey, N. Maharshak, G.K. Matsushima, S.E. Plevy, V.B. Young, R.B. Sartor, J.P.-Y. Ting, Nat. Immunol. **18**, 541–551 (2017)
31. T. Shao, L. Shao, H. Li, Z. Xie, Z. He, C. Wen, Front. Microbiol. **8**, 596 (2017)
32. J.B. Buse, R.A. DeFronzo, J. Rosenstock, T. Kim, C. Burns, S. Skare, A. Baron, M. Fineman, Diabetes Care dc150488 (2015)
33. M.-J. Jung, J. Lee, N.-R. Shin, M.-S. Kim, D.-W. Hyun, J.-H. Yun, P.S. Kim, T.W. Whon, J.-W. Bae, Sci. Rep. **6**, 30887 (2016)
34. A.N. Sarangi, A. Goel, A. Singh, A. Sasi, R. Aggarwal, BMC Gastroenterol. **17**, 125 (2017)
35. T.G. Tan, E. Sefik, N. Geva-Zatorsky, L. Kua, D. Naskar, F. Teng, L. Pasman, A. Ortiz-Lopez, R. Jupp, H.J. Wu, Proc. Natl. Acad. Sci. USA **113**, E8141 (2016)
36. N. Geva-Zatorsky, E. Sefik, L. Kua, L. Pasman, T.G. Tan, A. Ortiz-Lopez, T.B. Yanortsang, L. Yang, R. Jupp, D. Mathis, C. Benoist, D.L. Kasper, Cell **168**, 928–943.e11 (2017)
37. K. Winiarska, K. Szymanski, P. Gorniak, M. Dudziak, J. Bryla, Biochimie **91**, 261 (2009)
38. F. Turroni, C. Milani, S. Duranti, L. Mancabelli, M. Mangifesta, A. Viappiani, G.A. Lugli, C. Ferrario, L. Gioiosa, A. Ferrarini, J. Li, P. Palanza, M. Delledonne, D. van Sinderen, and M. Ventura, ISME J. **10**,1656–1668 (2016)

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