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·微生物与生命健康专题·

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中国人群肠道微生物鸟枪法宏基因组测序研究进展

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摘 要: 肠道微生物与人类健康的相关性研究仍然是当前生命科学研究领域的前沿热点之一。不依赖培养的16S rRNA基因高通量测序是当前的主要研究手段。但随着测序成本的降低和数据分析方法的日渐成熟，宏基因组鸟枪法测序因具有信息量更大、更全等优势，将逐渐成为今后一段时间内研究肠道微生物组的重要手段。美国在人类微生物组计划的资助下，对30 805份样品进行了肠道微生物宏基因测序分析。通过NCBI PubMed和SRA数据库检索，共发现72项研究收集了约10 000份中国人的肠道样品用于宏基因组测序。但到目前为止，仅56项研究进行了公开发表，其中与代谢性疾病相关的文献16篇，与感染和免疫性疾病相关的文献16篇，与心脑血管疾病相关的文献12篇。由于采样地点以北京、广州、上海等大城市为主，测序平台和测序分析方法均存在较大差异，且大部分研究仍以相关性分析为主，相关研究成果在临床疾病诊疗中所发挥的作用仍非常有限。规范采样方法、标准化测序平台和数据分析流程，开展多中心平行研究将有助于数据整合和比较分析。同时，结合使用转录组、蛋白质组和培养组学等多组学方法开展功能验证和分子作用机制研究，将有利于更好地将肠道微生物研究成果服务于临床疾病诊疗。

关键词: 中国人，肠道微生物，鸟枪法测序，宏基因组

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Shotgun metagenome sequencing of Chinese gut microbiota: a review

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Abstract: The research on the relationship between gut microbiota and human health continues to be a hot topic in the field of life science. Culture independent 16S rRNA gene high-throughput sequencing is the current main research method. However, with the reduction of sequencing cost and the maturity of data analysis methods, shotgun metagenome sequencing is gradually becoming an important method for the study of gut microbiome due to its advantages of obtaining more information. With the support from the human microbiome project, 30 805 metagenome samples were sequenced in the United States. By searching NCBI PubMed and SRA databases, it was found that 72 studies collecting about 10 000 Chinese intestinal samples were used for metagenome sequencing. To date, only 56 studies were published, including 16 related to metabolic diseases, 16 related to infectious and immune diseases, and 12 related to cardiovascular and cerebrovascular diseases. The samples were mainly collected in Beijing, Guangzhou, Shanghai and other cosmopolitan cities, where great differences exist in sequencing platforms and methods. The outcome of most studies are based on correlation analysis, which has little practical value in guiding the diagnosis and treatment of clinical diseases. Standardizing sampling methods, sequencing platform and data analysis process, and carrying out multi center parallel research will contribute to data integration and comparative analysis. Moreover, insights into the functional verification and molecular mechanism by using the combination of transcriptomics, proteomics and culturomics will enable the gut microbiota research to better serve the clinical diagnosis and treatment.

Keywords: Chinese, gut microbiota, shotgun sequencing, metagenome

肠道居住着数量庞大的、能与人类宿主相互作用的微生物，包括细菌、真菌、古菌和病毒，是一个复杂动态的生态系统。所有肠道微生物组编码的基因数量比人类基因组高一个数量级[1]。在某种程度上，也被认为是人体的“功能器官”。正常情况下，肠道菌群与人类宿主和谐共生，具有帮助宿主获取营养和促进免疫系统发育等功能。但当菌群出现紊乱时，也会与多种疾病的发生发展密切相关，如帕金森病、阿尔茨海默病、高血压、动脉粥样硬化、肥胖、糖尿病、炎症性肠病、结肠癌等[2]。

自 2008 年美国启动人类微生物组计划 (Human microbiome project, HMP, http://hmpdacc.org/)、欧盟启动人类肠道宏基因组计划 (MetaHIT, http://www.metahit.eu/) 以来，各国相继启动或设立了人类肠道微生物组相关研究项目。但除了美国人类微生物组计划研究进展较快、成果突出、数据更新较及时外，其他研究进展均相对缓慢。

美国人类微生物组计划旨在探索人类宿主的微生物群落，并描述其在人类健康和疾病中的作用。HMP 第一阶段 (最初 5 年) 的主要目标是建立一个大样本、大数据的健康受试者的人体微生物组成基线。HMP 第二阶段 (为期 3 年)，主要研究肠道菌群在 2 型糖尿病、炎症性肠病和妊娠/早产发生发展和疾病防治中的作用。到目前为止，HMP 计划已对 30 805 份样品进行了宏基因组分析，对 3 687 份样品进行了宏转录组分析，对 2 699 份样品进行宏蛋白质组分析[3]。

中国除了国家自然科学基金委员会和各省自然科学基金委员会立项支持人类肠道微生物组相

关研究项目外，科技部于 2013 年立项 973 计划课题支持浙江大学牵头开展“肠道细菌微生态与感染及代谢的研究”；中国科学院于 2017 年部署了“人体与环境健康的微生物组共性技术研究”暨“中国科学院微生物组计划”项目；然而，到目前为止，针对中国人肠道样品进行的宏基因组测序还不到 10 000 份，其他组学相关的研究更是凤毛麟角。

2021 年 5 月，中国科技部发布了国家重点研发计划“生物大分子与微生物组”重点专项申报指南，其中“标准微生物组及其与宿主/环境作用对生命活动影响的原理与机制研究”项目设置了一部分与肠道微生物研究相关的课题。如健康人微生物组库和特征解析；人体肠道微生物组稳态平衡及其失衡调控重大疾病的分子机制研究；微生物组与药物交互作用影响疗效及安全性的分子机制研究；微生物组学新技术及实验动物体系研究；病原微生物感染过程中的宿主免疫机制研究。该专项的实施将为建立健康中国人群肠道菌群基线、进一步研究肠道菌群在疾病发生发展中的作用及其分子机制奠定基础。相关研究成果也将为更好靶向肠道菌群开展新药研发和疾病防治提供理论依据和技术支撑。

截止到 2021 年 7 月 1 日，本研究通过 NCBI (National Center for Biotechnology Information) PubMed 文献检索和 NCBI SRA 数据库搜索，共找到 72 项研究对中国人肠道微生物菌群采用 shotgun 宏基因组测序方法进行了研究。现分析总结如下，希望能对下一步中国人肠道微生物宏基因组研究尤其是肠道微生物与疾病的相关性研究提供借鉴和指导。

1 中国人肠道微生物宏基因组测序进展

1.1 总体进展概述

在检索到的 72 项研究中，除 1 项研究使用了肠洗液样品外，其他研究均以粪便样品为研究对象。到目前为止，56 项研究进行了公开发表，其中与代谢性疾病相关文献 16 篇，与感染和免疫性疾病相关文献 16 篇，与肿瘤和癌症相关文献 5 篇，与心脑血管疾病相关文献 12 篇，与双胞胎儿童、百岁老人和不同民族菌群特征相关文献 7 篇 (表 1)。从发表时间来看，2017 年以后相对较多；从样品采集城市来看，主要以北京 (14 篇) 和广州 (8 篇) 为主，占总数的 40%，只有 1 篇进行了多医学中心采样分析，其他同一城市相关研究最多不超过 4 篇 (上海、深圳各 4 篇，杭州、苏州、香港各 3 篇，海口、唐山、长沙、重庆、西安各 2 篇，聊城、呼和浩特、锡林郭勒、昆明、乌鲁木齐、岳阳各 1 篇)。从使用的测序平台来看，除 7 项研究使用了国产的 BGISEQ-500 外，其他 49 项均选择使用了 Illumina 测序平台，其中 Hiseq2500 和 Hiseq2000 使用较广泛。

由表 1 可知，目前研究的疾病种类比较分散，很多疾病仅有 1 项相关研究报道。研究目的也存在较大差异，有些研究疾病与正常对照之间的菌群差异，有些研究疾病在发病过程中的菌群演变，有些研究药物治疗前后的菌群变化等。另外研究过程中样品的数量、收集的方法、测序的平台、数据处理的模型等均存在一定的差异。这些因素严重影响了相关研究结果的可比较性。本研究仅对在中国发病率较高、有较多公开发表文献的研究与国外相关研究进行比较分析。

1.2 2 型糖尿病相关人肠道微生物宏基因组研究

糖尿病是一种多器官、异质性、多因子疾病，临床上主要分为 1 型、2 型和妊娠期糖尿病，其中 2 型糖尿病 (Type 2 diabetes，T2D) 占发病率的 90%以上。据国际糖尿病联合会统计，2019 年，全世界有 4.63 亿年龄在 20–79 岁的成年人患有糖尿病，导致 420 万人死亡，相关医疗支出至少 7 200 亿美元；预计到 2045 年，糖尿病患病人数

表 1 中国人肠道微生物 **shotgun** 宏基因组测序研究统计

Table 1 Statistical analyses of shotgun metagenome sequencing-based Chinese gut microbiota studies

Classification	No.	Objective	Sample grouping	Data accession	References
Metagenomic analysis of the association between metabolic diseases and Chinese gut microbiota	1	Type 2 diabetes (T2D)	Probiotics + berberine group 106, probiotics group 102, berberine group 98, control group 103	PRJNA643353	[4]
	2	T2D	Diabetes group 171, not-diabetes group 174	PRJNA422434	[5]
	3	T2D	T2D group 77, pre-T2D group 80, normal glucose tolerant group 97	CNP0000175	[6]
	4	T2D	Acarbose treatment group 51, glipizide treatment group 43	PRJEB12124	[7]
	5	T2D	T2D group 16, fiber intervened T2D group 27	PRJEB15179	[8]
	6	T2D	T2D group 30, pre-T2D group 33, control 66	PRJEB30611	[9]
	7	T2D	Metformin treatment group 22	PRJNA486795	[10]
	8	Gestational diabetes mellitus (GDM)	GDM group 43, healthy pregnant women 81	PRJEB18755	[11]
	9	GDM	GDM group 23, non-GDM pregnant women 26	Unpublished	[12]
	10	Obesity	Obese group 95, lean controls 105	PRJEB12123	[13]
	11	Obesity	Prader-Willi syndrome 17, simple obesity 21	PRJNA256106	[14]
	12	Obesity	Obese 128, lean controls 101	PRJNA648796 PRJNA648797	[15]
	13	Polycystic ovary syndrome (PCOS)	PCOS group 14, control group 14	PRJNA549764	[16]
	14	PCOS	PCOS patients 14	PRJNA513209	[17]
	15	Renal failure	Renal disease group 223, control group 69	PRJNA449784	[18]
	16	Menopausal	Premenopausal 24, postmenopausal 24	PRJNA530339	[19]
Metagenomic analysis of the association between infectious-immune diseases and Chinese gut microbiota	1	Ankylosing spondylitis (AS)	AS group 127, control group 123	Unpublished	[20]
	2	AS	AS group 97, control group 114	PRJNA353560 PRJNA375935	[21]
	3	AS	AS group 113, control group 37	PRJEB28545	[22]
	4	AS	AS group 85, control group 62	PRJEB29373	[23]
	5	Rheumatoid arthritis (RA)	RA group 77, unrelated control 80, RA paired control 17, DMARD treated RA group 21	PRJEB6997	[24]
	6	Systemic lupus erythematosus (SLE)	Untreated SLE group 117, posttreatment SLE group 52, control group 115	PRJNA532888	[25]
	7	Ulcerative colitis (UC)	UC group 8, control group 8	Unpublished	[26]
	8	Crohn’s disease (CD)	CD group 49, control group 54	PRJEB15371	[27]
	9	Immune thrombocytopenia (ITP)	ITP group 99, control group 52	Unpublished	[28]
	10	Behcet’s disease (BD)	Active BD group 32, control group 74	PRJNA431482-PRJNA356225	[29]
	11	Vogt-Koyanagi-Harada disease (VKH)	Active VKH group 71, inactive VKH group 11, control group 67	PRJNA356225	[30]
	12	Segmented filamentous bacteria (SFB)	SFB positive group 7, SFB negative group 4	PRJNA299342	[31]

(待续)

(续表 1)

Classification	No.	Objective	Sample grouping	Data accession	References
	13	*Helicobacter pylori* (HP)	C14 breath test 313, HP positive group 128, HP negative group 185	Unpublished	[32]
	14	HIV-1	HIV group 61, control group 30	PRJNA391226	[33]
	15	Tuberculosis	TB group 46, control group 31	PRJNA401385	[34]
	16	*Clostridium difficile* (CD)	CD-positive group 5, CD-negative group 4, control group 5	PRJNA591064	[35]
Metagenomic analysis of the association between cancer and Chinese gut microbiota	1	Colorectal cancer (CRC)	Health subject 536	PRJNA557323	[36]
	2	CRC	CRC group 74, control group 54	PRJEB10878	[37]
	3	Rectal cancer	Rectal cancer patients 37	PRJNA484031	[38]
	4	Liver cirrhosis	Liver cirrhosis group 98, control group 83	PRJEB6337	[39]
	5	Lung cancer	Chemotherapy responder group 33, chemotherapy nonresponder group 31	Unpublished	[40]
Metagenomic analysis of the association between cardiovascular and cerebrovascular diseases and Chinese gut microbiota	1	Hypertension	Pre-hypertension 56, hypertension 99, control 41	PRJEB13870	[41]
	2	Hypertension	Hypertensive patients 60, healthy controls 60	ERP023883	[42]
	3	Atherosclerotic cardiovascular disease (ACVD)	ACVD group 218, control group 187	PRJEB21528	[43]
	4	Coronary heart disease (CHD)	CHD group 59, control group 43	PRJEB15111	[44]
	5	Atrial fibrillation	Atrial fibrillation group 50, control group 50	PRJEB28384	[45]
	6	Attention deficit hyperactivity disorder (ADHD)	ADHD group 17, control group 17	Unpublished	[46]
	7	Autistic (ASD)	ASD group 39, control group 40	CRA001746	[47]
	8	Warfarin response	Warfarin low responder (LR) 5, high responder (HR) 5, normal responder (NR) 5	PRJNA520777	[48]
	9	Schizophrenia	Medication-free schizophrenia patients 90, control group 81	PRJEB29127	[49]
	10	Multiple system atrophy (MSA)	MSA group 15, control group 15	PRJNA532538	[50]
	11	Gastrointestinal complications	Thoracic aortic dissection patients 40，control group 10	PRJNA379884	[51]
	12	Pompe disease	Eight members including two pompe siblings both had cerebral infarction	CNP0000237	[52]
Metagenomic analysis of other gut microbiota related to Chinese	1	Centenarians	Centenarians 75	Unpublished	[53]
	2	Urbanization	Urban group 20, rural group 20	PRJNA349463	[54]
	3	Ethnic specificity	Han Chinese 48, kazaks 48, uyghurs 96	NODEP00000053	[55]
	4	Ethnic specificity	Mongolians 63, Inner Mongolia of China 47	PRJNA328899	[56]
	5	Infant twins	Monozygotic group 5, dizygotic group 5	PRJEB12669	[57]
	6	Bioregenerative life support system	Healthy Chinese subjects 4	CNP0000408	[58]
	7	Fecal sample storage	Volunteers 8	PRJEB23662	[59]
Unpublished studies	PRJNA493884, PRJNA613947, PRJNA401977, PRJNA563508, PRJNA530971, PRJNA551354, PRJNA565546, PRJNA530620, PRJNA300602, PRJNA492158, PRJNA360177, /PRJNA638405, PRJNA565268, PRJNA674522, PRJNA474776, PRJNA597371				

会上升到 7 亿[60]。糖尿病已经成为严重危害人民健康和带来巨大医疗开支的“万病之源”。

2020 年 Gurung 等在 Google scholar 和 PubMed 搜索筛选到 42 项针对糖尿病与肠道菌群相关性进行的临床研究，其中大部分使用了 16S rRNA 基因高通量测序分析的方法，只有 7 篇研究选用了宏基因组测序方法[61]。综合这 42 篇研究，大多数研究报道双歧杆菌属 *Bifidobacterium*、拟杆菌属 *Bacteroides*、粪杆菌属 *Faecalibacterium*、阿克曼菌属 *Akkermansia* 和罗斯氏菌属 *Roseburia* 的细菌丰度与 T2D 呈负相关，而瘤胃球菌属 *Ruminococcus*、梭杆菌属 *Fusobacterium* 和布劳特氏菌属 *Blautia* 的细菌丰度与 T2D 呈正相关[61]。

由于宏基因组测序可以更好地在细菌种水平和功能基因水平进行比较分析，本研究对中国人、丹麦人、德国人、瑞典人的肠道宏基因组与糖尿病关联研究进行了比较。由于临床样品受到的影响因素很多，本研究只对 T2D 疾病组与正常对照组之间的研究结果进行了总结 (表 2)。其中一项研究对阿拉伯联合酋长国的粪便样品进行了宏基因组测序分析，但由于只收集了 12 个 T2D 和 6 个正常对照，样品数较少，且没有发现与疾病相关的菌群差异[62]，所以没有列入表 2。尽管大部分研究认为，肠道产丁酸菌减少可能与 T2D 的发生发展密切相关，但从表 2 可以看出，各研究结果之间的一致性相对较差，甚至有相互矛盾的地方。如 Qin 等研究发现嗜黏蛋白阿克曼菌 *Akkermansia muciniphila* 在 T2D 患者中相对丰度较高[5]，但 Wang 等发现 *Akkermansia muciniphila* 在正常对照组中相对丰度较高。在基因功能水平，各研究结果之间的一致性相对更好[9]。Reitmeier 等筛选发现的区分 T2D 和正常对照的 26 个代谢通路中有 23 个与 Qin 等的研究结果一致[63]，且主要集中在与糖、脂、氨基酸和维生素代谢相关的信号通路。

1.3 高血压和动脉粥样硬化相关人肠道微生物宏基因组研究

心血管疾病 (Cardiovascular disease，CVD) 近年来一直是世界范围内的主要死亡原因。据世界卫生组织预测，到 2030 年，将有 2 300 多万人死于心血管疾病。CVD 是一类异质性复杂疾病，与 CVD 相关的因素包括基因、肠道微生物、生活环境、生活方式等。在过去 10 年中，微生物群与 CVD 的关联研究取得了许多重要进展。高血压和动脉粥样硬化是 CVD 发生发展的重要病理基础。本文重点对这 2 种疾病与肠道菌群的关联研究进行总结。

目前共统计到 2 项研究对中国高血压患者的肠道菌群使用宏基因组测序进行了分析。Li 等研究发现[41]，与健康对照组相比，高血压患者微生物丰富度和多样性显著降低，且与健康状况相关的细菌减少，如粪杆菌属 *Faecalibacterium*、颤螺旋菌属 *Oscillibacter*、罗氏菌属 *Roseburia*、双歧杆菌属 *Bifidobacterium*、粪球菌属 *Coprococcus* 和丁酸弧菌属 *Butyrivibrio*，但普氏杆菌 *Prevotella* 和克雷伯菌 *Klebsiella* 等细菌过度生长。在高血压患者中普雷沃菌肠型个体比例较高，而在健康对照组中拟杆菌肠型个体占比较高。此外，通过将高血压患者的粪便移植到无菌小鼠，观察到血压升高可通过微生物群传递，并证明肠道微生物群对宿主血压的直接影响。高血压患者中 39 个 KEGG (Kyoto encyclopedia of genes and genomes) 模块减少，涉及支链氨基酸生物合成和运输、酮体生物合成、双组分调节系统以及蛋氨酸和嘌呤的降解等，17 个模块在高血压患者中升高，包括 LPS 生物合成和输出、磷脂转运、磷酸转移酶系统、苯丙氨酸和磷脂酰乙醇胺的生物合成以及分泌。Yan 等研究发现克雷伯氏杆菌 *Klebsiella* spp.、链球菌 *Streptococcus* spp.和粪副拟杆菌 *Parabacteroides merdae* 等条件致病菌在高血压患者中较常见，

表 2　2 型糖尿病相关肠道微生物宏基因组分析结果比较

Table 2　Comparison of metagenomic analysis of gut microbiota associated with type 2 diabetes mellitus

Population	T2D/ controls	Enriched in T2D group	Enriched in normal control group	References
Chinese	171/174	*A. muciniphila*, *Bacteroides intestinalis*, *Clostridium bolteae*, *Clostridium hatheway*, *ramosum ramosum*, *symbiosum symbiosum*, *Eggerthella lenta*, *E. coli* Transport of sugars, branched-chain amino acid transport, methane metabolism, xenobiotics degradation and metabolism, oxidative stress resistance, sulphate reduction	*Eubacterium rectale*, *Feacalibacterium prausnitzii*, *Haemophilus parainfluenzae*, *Roseburia intestinalis*, *Roseburia inulinivorans* Bacterial chemotaxis, flagellar assembly, butyrate biosynthesis, metabolism of cofactors and vitamins	[5]
Chinese	77/97	*Bacteroides caccae*, *Bacteroides finegoldii*, *Collinsella intestinalis*, *Megasphaera elsdenii* Sugar phosphotransferase systems (PTS), ATP-binding cassette transporters of amino acids, bacterial secretion systems, transport system for microcin C, transport system for autoinducer-2	*A. muciniphila*, *Clostridium bartlettii*, *Dialister invisus*, *Roseburia hominis* Modules of V-type ATPase, pyruvate: ferredoxin oxidoreductase, bacterial ribosomal proteins	[6]
Chinese	30/30	Paraprevotella unclassified, *Porphyromonas bennonis*, *Roseburia hominis* Fructose and mannose metabolism; starch and sucrose metabolism; amino sugar and nucleotide sugar metabolism, methane metabolism	*Bifidobacterium longum*, *Coprobacillus* unclassified, *Veillonella dispar* Amino acid biosynthesis, lipopolysaccharide biosynthesis, fatty acid metabolism, bacterial secretion system, ABC transporters, xenobiotics biodegradation and metabolism	[9]
Swedish	53/43	*Clostridium clostridioforme*, *Lactobacillus gasseri*, *Streptococcus mutans* Starch and glucose metabolism, fructose and mannose metabolism, ABC transporters for amino acids, ions and simple sugars, glycerolipid metabolism and fatty acid biosynthesis, cysteine and methionine metabolism	*B. intestinalis*, *Eubacterium eligens*, unknown *Clostridium* species Flagellar assembly, riboflavin metabolism	[64]
Germany	50/50	No microbiota difference analysis was done Bacterial invasion of epithelial cells, riboflavin metabolism, betaLactam resistance, chlorocyclohexane and chlorobenzene degradation, nitrotoluene degradation, phenylalanine metabolism, valine leucine and isoleucine degradation, retinol metabolism, drug metabolism cytochrome P450, metabolism of xenobiotics by cytochrome P450, fluorobenzoate degradation, penicillin and cephalosporin biosynthesis, alphaLinolenic acid metabolism, glycosaminoglycan degradation, nicotinate and nicotinamide metabolism, taurine and hypotaurine metabolism, toluene degradation, lipoic acid metabolism, ubiquinone and other terpenoidquinone biosynthesis	No microbiota difference analysis was done Photosynthesis, cysteine and methionine metabolism, RNA degradation, lysine biosynthesis, peptidoglycan biosynthesis, alanine aspartate and glutamate metabolism, novobiocin biosynthesis	[63]

而短链脂肪酸产生菌，如罗氏菌 *Roseburia* spp. 和普拉梭菌 *Feacalibacterium prausnitzii*，在对照组中含量较高[42]。在功能基因水平，高血压肠道微生物群表现出较高的膜转运、脂多糖生物合成和类固醇降解功能，而在对照组中，氨基酸、辅因子和维生素代谢活性较高[42]。

国外也有 2 项研究分别对日本人和芬兰人的肠道菌群与高血压的关系进行了微生物宏基因组测序分析。Stevens 等研究发现，在日本高血压患者中，嗜黏蛋白阿克曼菌 *Akkermansia muciniphila*、*Alistipes senegalensis*、马赛拟杆菌 *Bacteroides massiliensis*、沃氏嗜胆菌 *Bilophila wadsworthia*、内脏臭气杆菌 *Odoribacter splanchnicus* 丰度较高，在正常对照组中长双歧杆菌 *Bifidobacterium longum*、灵巧粪球菌 *Coprococcus catus*、大肠杆菌 *Escherichia coli*、肠道罗斯拜瑞氏菌 *Roseburia intestinalis* 丰度较高[65]。Palmu 等研究发现在芬兰人中高压指数与 45 个微生物属之间存在显著的、主要为正的相关性，其中 27 个属于厚壁菌门。有趣的是，他们发现 19 种不同的乳酸杆菌 *Lactobacillus* 与血压指数之间大多呈负相关。其中，公认的益生菌副干酪乳杆菌 *Lactobacillus paracasei* 的丰度越高，平均动脉压越低[66]。

动脉粥样硬化与肠道菌群的关联研究堪称研究疾病与肠道菌群的经典。肠道菌群依赖性代谢产物氧化三甲胺和短链脂肪酸以相反的模式调节动脉粥样硬化相关代谢过程，从而影响动脉粥样硬化的发生发展[67]。有 2 项研究分别对中国人和瑞典人使用宏基因组测序方法研究了肠道菌群与动脉粥样硬化的相关性[43,68]。在瑞典患者和对照组样本之间共有 17 个物种的丰度存在显著差异，在中国患者和对照组样本之间共有 162 个物种的丰度存在显著差异。其中 5 个常见物种的丰度在 2 项研究的对照样本中均显著高于患者组。这些细菌包括木糖降解拟杆菌 *Bacteroides xylanisolvens*、内脏臭气杆菌 *Odoribacter splanchnicus*、挑剔真杆菌 *Eubacterium eligens*、食葡糖罗斯拜瑞氏菌 *Roseburia inulinivorans*、肠道罗斯拜瑞氏菌 *Roseburia intestinalis*。由于瑞典总共只有 25 个样品，中国收集了 385 份样品，所以相关的比较研究还有待进一步加强。

随着高通量测序等生命科学技术的进步，产生了大量的科学数据，如基因组数据、蛋白质组数据、转录组数据和微生物组数据等，从原始数据到科学知识引导的个性化应用，标准化数据的共享和集成至关重要。在心血管疾病研究过程中总结发展的以数据驱动指导疾病相关的微生物群生物标记物发现方法，将对其他疾病的相关研究具有很强的借鉴和指导作用 (表 3)。

表 3　数据驱动的心血管疾病生物标志物筛选数据库和生物信息学工具[69]

Table 3　Data-driven cardiovascular biomarker screening database and bioinformatics tools[69]

Classifications	Names	Descriptions	Websites
Microbial reference genome	SILVA	Ribosomal RNA sequence data	https://www.arb-silva.de
	RDP	16S rRNA sequences, and Fugal 28S rRNA sequences	http://rdp.cme.msu.edu
	NCBI-Refseq	RefSeq microbial genomes database	http://www.ncbi.nlm.nih.gov/genome
	MG-RAST	Metagenomics database and portal	http://metagenomics.anl.gov
	Mgnify	Comprehensive microbial analysis, archiving platform	http://www.ebi.ac.uk/metagenomics
	IGC	Human gut microbiome reference genes that includes 9 879 796 genes	https://db.cngb.org/microbiome
	UHGG	The most comprehensive reference genome for human gastrointestinal microbes to date	http://ftp.ebi.ac.uk/pub/databases/metagenomics/mgnify_genomes/

(待续)

(续表 3)

Classifications	Names	Descriptions	Websites
Microbial and disease relationships	HMDAD	Data sets on microbe and human disease associations	http://www.cuilab.cn/hmdad
	Disbiome	Database for microbiot a disease information	https://disbiome.ugent.be/home
	gutMDisorder	Database for gut microbiota in disorders and interventions	http://bio-annotation.cn/gutMDisorder
Cardiovascular disease related databases	MorCVD	A database for host-pathogen PPIs involved in CVD	http://morcvd.sblab-nsit.net/About
	CVDHD	A herbal database in CVD	http://pkuxxj.pku.edu.cn/CVDHD
	MIRKB	A myocardial infarction risk knowledge-base	http://www.sysbio.org.cn/mirkb
	CARDIO-LNCRNAS	The database of the lncRNA transcriptome in human cardiovascular system	http://bio-bigdata.hrbmu.edu.cn/CARDIO-LNCRNAS
	CVDncR	Non-coding RNA related to cardiovascular diseases	http://sysbio.org.cn/cvdncr
	CardioGenBase	Multi-omics database for major CVD	http://www.CardioGenBase.com
	C/VDdb	Multi-omic studies of cardiovascular-related traits	http://www.padb.org/cvd
	CADgene	Database for coronary artery disease genes	http://www.bioguo.org/CADgene
	In-Cardiome	Knowledgebase for coronary artery disease	http://www.tri-incardiome.org
	CHD@ZJU	Knowledgebase research platform on CHD	http://tcm.zju.edu.cn/chd
Microbe-disease associations prediction	KATZHMDA	A computational model of KATZ measure for predict the human microbe-disease association	http://dwz.cn/4oX5mS
	BMCMDA	Prediction microbe-disease association based on binary matrix completion	NA
	LGRSH	Learning graph representations and a modified scoring mechanism on the heterogeneous network	NA
Risk-prediction model in CVD	The Framingham Heart Study	Multi-omics and multivariable risk-prediction algorithms	NA
	SPoRT	A stroke risk prediction model based on health behaviors	NA
	QRISK3	A comprehensive cardiovascular disease prediction algorithm that includes physical signs, lifestyle, other diseases, drugs, etc.	NA
	HHS	A lifestyle-based tool that estimates ASCVD events	https://healthyheartscore.sph.harvard.edu/.
	PREDICT	Cardiovascular disease cohort in New Zealand	NA
	CVDPoRT	A cardiovascular disease risk-prediction model using population health surveys	https://github.com/Ottawa-mHealth/predictive-algorithms.
Human microbe-drug associations prediction	MDAD	Database for microbe-drug association	http://chengroup.cumt.edu.cn/MDAD
	RapidAIM	Microbiome responded to drugs based on culture and metaproteomics	NA
	GCNMDA	Predicting human microbe-drug associations via graph convolutional network	https://github.com/longyahui/GCNMDA

1.4 慢性肠炎和肠癌相关人肠道微生物宏基因组研究

2021 年发布的中国人健康大数据显示，不管是发病率还是死亡率，肠癌均名列中国常见癌症的前 5 位，对人类健康造成严重威胁。炎性肠病 (Inflammatory bowel disease，IBD) 是一种病因不明的慢性肠道炎症性疾病，因发病率逐渐升高，已成为我国常见的消化系统疾病，临床上分为溃疡性结肠炎 (Ulcerative colitis，UC) 和克罗恩病 (Crohn's disease，CD)。众所周知，肠道微生物群组成的改变与 IBD 的发生相关。此外，IBD 患者在晚年发生大肠癌的风险增加，对渗透性共生微生物的异常免疫反应可能在促进疾病进展中发挥关键作用。

我国学者分别针对中国 UC、CD、结肠癌和直肠癌患者各开展了 1 项肠道微生物宏基因组测序研究 (表 1)，并通过生物信息学与欧美多国的肠道微生物宏基因组进行了大数据比较分析[36,70-72]。比较研究发现，普拉梭菌 *Faecalibacterium prausnitzii*、直肠真杆菌 *Eubacterium rectale*、布氏瘤胃球菌 *Ruminococcus Bromii*、青春双歧杆菌 *Bifidobacterium adolescentis*、长双歧杆菌 *Bifidobacterium Longum* 和产气柯林斯菌 *Collinsella aerofaciens* 在健康人群中相对丰度较高，单形拟杆菌 *Bacteroides uniformis*、普通拟杆菌 *Bacteroides vulgatus*、*Blautia stercoris*、肠道罗斯拜瑞氏菌 *Roseburia intestinalis*、脆弱类杆菌群 *Bacteroides fragilis*、卵形拟杆菌 *Bacteroides ovatus* 和粪拟杆菌 *Bacteroides caccae* 在 IBD 患者中相对丰度较高，嗜黏蛋白阿克曼菌 *Akkermansia muciniphila*、大肠杆菌 *Escherichia coli*、*Prevotella copri*、*Alistipes putredinis* 和扭链瘤胃球菌 *Ruminococcus torques* 在结直肠癌 (Colorectal cancer，CRC) 患者中相对丰度较高。尽管不同人群队列研究结果存在一定的差异，但基本公认具核梭杆菌 *Fusobacterium nucleatum* 在 CRC 发病过程中发挥重要作用。

1.5 强直性脊柱炎 (Ankylosing spondylitis，AS) 和类风湿性关节炎 (Rheumatoid arthritis，RA) 相关人肠道微生物宏基因组研究

AS 和 RA 是 2 种临床上较常见的由于慢性炎症引起的关节功能障碍性疾病。目前有 5 项研究采用宏基因测序方法分析了肠道菌群与 AS 发生的相关性，其中 4 项针对中国人群，1 项针对美国人群。有 2 项研究采用宏基因测序方法分析了肠道菌群与 RA 发生的相关性，其中针对中国人群和日本人群的研究各 1 项。从表 4 可以看出，各项研究结果之间差异较大，不管是在细菌种水平，还是在基因功能水平，国内研究之间及国内与国外研究之间均没有相对一致的研究发现。

2 总结与展望

除了以上详细总结的疾病外，如表 1 所示，其他疾病与中国人肠道微生物宏基因组之间的关联还有零散少数报道，如肥胖、多囊卵巢综合征、肾衰竭、更年期综合征、冠心病、心房颤动、自闭症、注意缺陷多动障碍、精神分裂症、系统性红斑狼疮、幽门螺杆菌感染、HIV 感染、肺结核感染、肝硬化和肺癌等。另外，也有学者对肠道菌群在发育与长寿、民族特性和城镇化等方面的作用进行了研究。

尽管新一代测序技术的发展使得研究人员能够从更广泛、更深入的角度探索和了解肠道微生物群。然而，即使在同一种疾病中，肠道菌群的不同研究结果也存在较大差异，难以直接指导临

表 4　强直性脊柱炎和类风湿性关节炎相关肠道微生物宏基因组分析结果比较

Table 4　Comparison of metagenomic analysis of gut microbiota associated with ankylosing spondylitis and rheumatoid arthritis

Diseases	Patients/ Controls	Enriched in patients	Enriched in normal controls	References
Ankylosing spondylitis	127/123 Chinese	*Clostridiales bacterium 1747FAA*, *C. bolteae*, *C. hatheway*	*Bifidobacterium adolescentis*, *Coprococcus comes*, *Lachnospiraceae bacterium* 5163FAA, *Roseburia inulinivorans*	[20]
		Superoxide proteinase	ATP-dependent serine phosphatase, ATP phosphoribosyltransferase, histidinol-phosphate transaminase, polyphosphate kinase, pyridoxal 5′-phosphate synthase	
	97/114 Chinese	*Prevotella melaninogenica*, *Prevotella copri*, *Prevotella* sp. *C561*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*	*Bacteroides* spp.	[21]
		Cell motility, membrane transport, metabolism of cofactors and vitamins, proteasome functions, signal transduction	Glycosaminoglycan metabolism, secondary metabolites biosynthesis, and symbiosis	
	113/37 Chinese		*Bacteroides nordii*, *Flavonifractor plautii*, Oscillibacter unclassified, *Parabacteroides distasonis*	[22]
		Carbohydrate metabolism, glycan biosynthesis and metabolism, glycosaminoglycan degradation, starch and sucrose metabolism	Degrade dioxin, energy metabolism, folding, replication and repair, sorting and degradation, translation, vitamin B12 transport system, xenobiotics biodegradation and metabolism	
	85/62 Chinese	*Acidaminococcus fermentans*, *Bacteroides coprophilus*, *Eubacterium siraeum*, *Prevotella copri*, *Parabacteroides distasonis*		[23]
		Glycosaminoglycan degradation, lipopolysaccharide (LPS) biosynthesis, oxidative phosphorylation	ATP-binding cassette (ABC) transporters, butanoate metabolism, gluconeogenesis, glycolysis, phosphoenolpyruvate-dependent phosphotransferase (PTS) system	
	21/24 American	*B. adolescentis*, *P. bennonis*	*Bacteroides dorei*, *Streptococcus anginosus*	[73]
		Tryptophan synthesis		
Rheumatoid arthritis	77/17 Chinese	*Bifidobacterium dentium*, *Clostridium asparagiforme*, *E. lenta*, *Gordonibacter pamelaeae*, *Lactobacillus* sp., *Ruminococcus lactaris*	*B. bifidum*, *Klebsiella pneumoniae*, *Megamonas hypermegale*, *Sutterella wadsworthensis*	[24]
		Converting acetate to methane, reductive acetyl-CoA	Lipopolysaccharide biosynthesis, lipopolysaccharide transport, secretion systems (type Ⅱ, type Ⅳ and type Ⅵ)	
	82/42 Japanese	*Bacteroides sartorii*, *Gardnerella vaginalis*, *Prevotella amnii*, *Prevotella corporis*, *Prevotella denticola*, *Prevotella disiens*, *Prevotella marshii*, *Prevotella somerae*		[74]
		Adipocytokine signalling pathway, fatty acid biosynthesis, folate biosynthesis, glycosaminoglycan degradation, MAPK signalling pathwayplant, nitrotoluene degradation, ubiquinone and other terpenoid-quinone biosynthesis	Transport and catabolism	

床诊断和治疗[75]。这可能是由于：(1) 肠道菌群会受到地理位置、年龄、性别、饮食、抗菌药物、心理状况、运动等因素的影响[76-77]，健康微生物组仍难以定义。如中国人群研究发现的阿克曼菌 *Akkermansia* 可以用于 T2D 的鉴别诊断，不适用于德国人群，德国人群研究发现可以用于 T2D 鉴别诊断的乳杆菌 *Lactobacillus* 不适用于中国人群[63]。(2) 临床疾病非常复杂，同一种疾病分为不同的亚型和不同的疾病发展阶段。如 Wang 等研究发现单纯收缩型高血压和单纯舒张压其菌群变化存在一定的差异[78]。同时，不同疾病之间存在着临床症状相似和菌群变化交叉重叠等现象，增加了利用菌群进行鉴别诊断和靶向治疗的难度。如 IBD 与 CRC 等疾病，在与正常对照组相比后存在一些相似的菌群变化特征[70]。(3) 微生物组样品收集与储存、测序平台和分析流程等特别容易出现重大差异[79-80]，导致研究结果的高度可变性[81]。(4) 目前大部分研究使用粪便样本替代肠道微生物群，但小肠和大肠的长度不同，包括化学和营养梯度的生理变化以及宿主免疫活性的划分也不同，所有这些都会影响微生物的组成。另外，粪便样本采集前的均质化会扰乱粪便的生物结构，但如果不均质化处理，样本的代表性又可能不够。另外，有些细菌虽然能被测序检测到，但可能是死细菌[82]，因而影响了测序结果的可靠性。

目前，大多数研究仍集中在基因组水平，很少涉及转录组、蛋白质组或代谢组。即使在基因组水平，shotgun 宏基因组测序由于成本较高、数据分析门槛较高，使得基于标记的扩增子测序如 16S rRNA 基因测序盛行。尽管 16S rRNA 基因测序已经成为揭示微生物群变化与疾病相关性的有力工具，但在揭示微生物群如何改变哺乳动物生理学的机制研究方面仍受到很多限制[83]：(1) 许多疾病关联是在细菌门、纲、目或属等比较高级的细菌分类水平上分析。考虑到同一物种不同菌株的功能不同，如 Gálvez 等研究发现 *Prevotella copri* 不同菌株对多糖的代谢利用能力存在较大差异[84]，鉴定与疾病相关的微生物菌株是一个挑战。(2) 由于 90%以上肠道细菌属于难培养或未培养微生物，即使鉴定到了疾病相关菌株，要成功获得靶标菌株仍然存在较大困难。(3) 基于 PCR 扩增的测序在 DNA 扩增过程中会存在一定偏倚。(4) 忽视了真菌[85]和病毒[86]等在维护人类健康中发挥的重要作用。此外，尽管人体肠道被成百上千种不同细菌所占据，但它们生物合成产生的代谢物可能存在较多冗余[87]。鉴于人体肠道菌群是一个动态的受到多种因素影响、微生物群落内部以及微生物群落与宿主之间相互作用关系非常复杂的生态系统，采用多组学整合策略，研发新的实验模型和数据算法等仍然是更好认识肠道菌群组成和生理功能的必要条件。

鉴于肠道菌群的重要生理功能及其与疾病发生发展的密切关系，肠道菌群已经成为很多药物研发的新靶标。尤其在肿瘤免疫治疗中，肠道菌群具有影响宿主免疫反应的功能，已经被列为肿瘤免疫治疗药物药效评价的重要指标之一[88]。同时越来越多研究表明肠道菌群广泛参与药物代谢，从而影响药物的有效利用度和疗效[89-90]。系统地理解药物-微生物-宿主三者之间的复杂关系将有利于新药的研发和新型治疗方法的选择，从而提高药物疗效，减少不良反应。我国传统中医药已经有几千年的临床疾病治疗经验积累，然而，由于缺乏系统的科学验证，缺乏有关中药作用机制的详细阐述限制了其应用。中药以口服给药为主，中药成分往往不被宿主直接吸收，而是进入肠道被肠道菌群转化。近年来的研究表明，肠道微生物群参与了食物和营养物质的代谢，在中药成分转化为功能性代谢产物过程中发挥着重要作用，这可能影响了中药的治疗活性。在各种中草药与肠道菌群相互作用研究不断增加的同时，善于使用高通量测序和代谢组学平台等前沿多组学研究工具以及先进的生物信息学分析、数据库和

算法，将为新的功能代谢物识别和未来中医药的研究奠定基础[91]。

近年来，尽管关于肠道微生物的研究开展得如火如荼，但关于肠道微生物与疾病的关系仍有许多未解之谜。(1) 关于什么是“好的”肠道微生物群仍没有形成共识。如何定义临床上肠道微生物紊乱和发展可靠准确的微生物诊断方法仍然具有困难。(2) 很多疾病与肠道微生物紊乱之间的因果关系仍不清楚。其发病原因是由于某一种细菌或菌株数量的改变引起，还是整个菌群紊乱导致仍有待进一步研究。肠道微生物在疾病发生发展中的作用及其作用机制仍不清楚。(3) 大数据分析建立了一套 204 938 个基因组和 1.71 亿个蛋白质序列的人体肠道微生物组数据集[92]。但在 4 644 个物种中，71%的细菌仍属于未培养菌，微生物的分离培养和功能研究仍有待进一步加强。(4) 除粪微生态移植外，是否可以使用单一或少数几种菌株组合用于疾病的有效防治仍需不断探索。(5) 疾病不仅是复杂的，而且是高度动态的，在整个疾病治疗过程中可能需要采用不同的靶向肠道菌群的治疗策略。

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