At the same time, prevention and control of pandemic will be normalized. So we should be alert to the secondary harm in the new stage of COVID-19 pandemic.[8]

3.3 Promote the establishment of the combination of medical and health care model.

Combination of medical and health care mode can integrate medical treatment, rehabilitation, health care and elderly care, to combine the functions of traditional elderly care institutions and hospitals. [9]

At present, the elderly care services provided by common elderly care institutions are relatively simple, mainly focusing on the daily care. Health education and psychological health management are relatively poor. It cannot completely meet the diverse needs of the elderly.[10] So it is recommended that medical institutions and elderly care institutions should cooperate for a win-win mode, to form a health care service model with the goal of combination of medical and health care. Promoting the construction of a combination of medical and health care is conducive to enhancing the anti-pandemic capabilities to form a long-term mechanism.

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AKKERMANSIA MUCINIPHILA PREVENTS COLD-RELATED ATRIAL FIBRILLATION BY THE MODULATION OF TMA/TMAO PATHWAY

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Relevans of the brief summary: Cold exposure is one of the most important risk factors for atrial fibrillation (AF). Gut microbiota has been reported to be associated with AF. However, the role and pathogenesis of gut microbiota in cold-related AF remain poorly understood.

The research objective of the brief summary: To reveal the role and pathogenesis of gut microbiota in cold related atrial fibrillation.

Methods of the brief summary.Various techniques including 16S rRNA gene sequencing, fecal microbiota transplantation, and electrophysiological examination were used to determine whether gut microbiota dysbiosis promotes cold-related AF. Metabonomics were performed to investigate changes in fecal TMA and plasma TMAO during cold exposure. To identify the special microbiota responsible for cold-related AF, we evaluated the correlation between results of 16S rRNA and metabolomics. In addition, single-bacterial intervention experiments were conducted and clinical relevance was verificated among human subjects.

Results of the brief summary.We found that cold exposure induced AF by altering the composition and metabolism of microbiota, including the decrease of Akkermansia muciniphila(A. muciniphila) and increase in TMA and TMAO. TMAO promotes atrial remodeling and AF. Moreover, supplement with A. muciniphila virtually completely protected rats from cold-related AF by inhibiting the production of TMA. More importantly, the clinical relevance between A. muciniphila and cold-relatd AF has been confirmed in the population.

Conclusion of the brief summary. These findings demonstrate a novel causal role of aberrant gut microbiota and metabolites in contributing to the pathogenesis of cold-related AF, which raised the exciting possibility of selectively targeting microbiota and microbial metabolites as a potential therapeutic strategy for cold-related AF.

Key words : cold; atrial fibrillation; gut microbiota; Akkermansia muciniphila; TMAO

Relevance. Cold exposure is one of the most important risk factors for atrial fibrillation (AF). Gut microbiota has been reported to be associated with AF. However, the role and pathogenesis of gut microbiota in cold-related AF remain poorly understood.

The research objective. To reveal the role and pathogenesis of gut microbiota in cold related atrial fibrillation.

Methods.

Human stool samples：The study protocol was approved by Research Ethics Committees of The First Affiliated Hospital of Harbin Medical University (Harbin, Heilongjiang, China) and performed in accordance with the Declaration of Helsinki. Human stool sample from AF and healthy controls were obtained from First Affiliated Hospital of Harbin Medical University (Harbin, China) with patient informed consent under an approved institutional review board protocol. Exclusion criteria were predefined as follows: end-stage heart failure, cancer and inflammatory bowel diseases.

Establishment of Cold Exposed Rat Model ：Cold exposures were done at 6℃ for 2 weeks in a temperature-controlled chamber in SPF conditions using individually ventilated cages. The rats were housed under standard conditions and given access to food and water adlibitum.

Establishment of Rat Model of Fecal Bacteria Transplantation :Fecal bacteria transplantation was performed by gavage and omeprazole (50 mg/kg/day) was given three days before transplantation. Fasting was started 24 h before transplantation, and 1 ml citrate (0.16 mg/ml sodium picosulfate, 51.2 mg/ml magnesium oxide) was given at the same time. Another 2 ml citrate was given 12 h before transplantation. Bacterial flora transplantation (2 ml) was performed after fasting, and relevant indexes were detected 4 weeks later.

Statistical Analyses:The statistical analyses were performed with GraphPad Prism 8.0 software (GraphPad Software, Inc, La Jolla, CA) and R version 3.6.1 (https://www.r-project.org). Continuous variables are presented as the mean ± standard error of mean (SEM). Categorical variables are presented as numbers and percentages. Two-group comparisons were performed using Student's non-paired t-test or Wilcoxon (Mann-Whitney U) test, and Shapiro-Wilk was used for normality test. The chi-square test was used for categorical variables. And the statistics regarding microbiome analysis in the subsections on microbiome analysis was described in gut microbiota profiling of methods. Differences were considered as statistically significant when $P < 0.05$.

The results and discussion.

To study the effects of cold exposure on the susceptibility to AF in rats, atrial electrophysiological testing in RT (26℃；2 weeks) and cold rats (6℃；2 weeks) were conducted. Compared with RT rats, the AF inducibility and AF duration were significantly increased, which is consistent with the clinical study[1]. Mounting studies have highlighted the crucial role of gut microbiota in various CVD. Meanwhile, gut microbiota has been reported to be influenced by cold exposure. Therefore, we collected fecal samples on the 14th day of cold exposure for 16S rRNA analysis. Principal coordinates analysis(PCoA) based on unweighted UniFrac distance revealed distinct clustering between microbiota from RT and cold rats. We measured the α-diversity at the OTU level, whereas no statistical difference was observed between two groups. Notably, the top 10 differential microbiotas in genus level showed significant shifts in proportions between RT and cold group. In particular, the abundance of A. muciniphila in cold group was clearly lower than that in RT group.

To investigate the importance of the microbiota changes in cold-related AF, we transplanted the microbiota from cold or RT rats to wild type normal rats for 4 weeks. Strikingly, rats transplanted with cold microbota showed a significant increase in the induction rate and duration of AF, while transplantion with RT microbota did not lead to detectable changes in AF susceptibility. Taking together, these data suggested that the cold microbiota causally increased AF susceptibility.

The gut microbiota produce bioactive metabolites that can impact host physiology. We conducted the untargeted metabolomics of plasma from RT and cold rats by UHPLC-MS/MS.

Strikingly, the levels of certain metabolites in plasma were dramatically changed, of which the choline and carnitine-related metabolites markly increased. As we know, choline and carnitine are the key sources of TMAO synthesis. Furthermore, TMAO has been identified as a important contributor promoting the occurrence and development of AF [2]. Thus, we further examined TMAO-targeted metabolomics of the plasma from RT and cold rats. As expected, elevated levels of TMAO, as well as choline and carnitine, were seen in cold rats when compared to that in RT rats. Intriguingly, the elevated TMAO was also obtained from the rats receiving cold flora transplantation. We further examined the fecal trimethylamine (TMA), a precursor of TMAO, by targeted metabolomics. The concentration of TMA is significantly higher in cold rats than that in RT rats.

3,3-dimethyl-1-butanol (DMB), a structural analogue of choline, has been proved to non-lethally inhibit TMA production, the first step in TMAO generation[3]. To determine whether cold-related AF is mediated by TMAO, we administered cold rats with DMB (1.0%, v/v. in the drinking water) for 2 weeks. Noticeably, DMB treatment reduced the AF inducibility and AF duration in cold rats, as compared with cold rats treated with vehicle. Taking together, those results indicated that cold-related AF was mediated by TMAO and elevated circulating TMAO of cold phenotype may due to the enhancement in TMA synthetic capacity of gut microbiota.

To identify the specific microbial species responsible for TMA metabolism in cold rats, we evaluated the correlation between changes in the 16S rRNA sequencing and metabolomics. The levels of TMA in feces were negatively correlated with the abundence of A. muciniphila. More importantly, a significant decrease in A. muciniphila was also found in cold rats. A. Muciniphila, as a gram-negative anaerobic bacterium, has been considered to be a promising probiotics which improve metabolic disorders and gut barrier function[4]. Since A. muciniphila showed obvious changes under cold exposure and exhabited negative relation with TMA, we investigated whether this strain alone could prevent cold-related AF. Strikingly, compared with cold rats, the AF inducibility and AF duration significantly reduced in cold rats supplemented with A. Muciniphila. Moreover, the level of TMAO was also decreased after treatment with A. Muciniphila.

To translate our findings to clinical relevance, human stool sample from AF and healthy subjects during winter and summer were collected. We must emphasize that it was of considerable difficulty to conduct this experiment in human subjects, because many individuals were unwilling to collect their own feces according to our request. Compared with individuals in winter, there was significantly increased abundance of A. Muciniphila in stool sample of people in summer. Then further subgroup analysis was performed. We found that patients with AF had lower abundence of A. Muciniphila than sinus rhythm in winter. More importantly, ROC curve analysis revealed that the reduction of A. Muciniphila was an independent risk factor for cold-related AF.

Conclusion. In summary, our work has demonstrated that gut microbiota dysbiosis is associated with cold-related AF. The function of cold flora and the pathogenesis of AF are extremely sophisticated. Cold exposure reduces the abundances of A. muciniphila which subsequently increases fecal TMA and plasma TMAO levels, which promoted AF. This study also suggests that the gut microbiota may be a potential target for the prevention and treatment of cold-related AF.

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RESEARCH PROGRESS ON SEPARATION AND PURIFICATION OF EXTRACELLULAR VESICLES

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