1 **Title: Shifts in gut microbiome and metabolome are associated with risk of**
 2 **recurrent atrial fibrillation**

3 **Running title: Predictive model based on gut taxonomic signature**

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23 **Acknowledgements:** This work was supported by the National Natural Science
 24 Foundation of China (81670214, 81500383, 81870308, 81970271), the Beijing

25 Natural Science Foundation (7172080), the Beijing Municipal Administration of
 26 Hospitals' Youth Programme (QML20170303), and the 1351 personnel training plan
 27 (CYMY-2017-03).

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51 **ABSTRACT:** Specific alterations of gut microbiota (GM) in atrial fibrillation (AF)
52 patients, including elevated microbial diversity, particularly perturbed composition,
53 imbalanced microbial function, and associated metabolic pattern modifications have
54 been described in our previous report. The current work aimed to assess the
55 association of GM composition with AF recurrence (RAF) after ablation, and to
56 construct a GM-based predictive model for RAF. Gut microbial composition and
57 metabolic profiles were assessed based on metagenomic sequencing and metabolomic
58 analyses. Compared with non-AF controls (50 individuals), GM composition and
59 metabolomic profile were significantly altered between patients with recurrent AF (17
60 individuals) and the non-RAF group (23 individuals). Notably, discriminative taxa
61 between the non-RAF and RAF groups, including the families *Nitrosomonadaceae*
62 and *Lentisphaeraceae*, the genera *Marinitoga* and *Rufibacter*, and the species
63 *Faecalibacterium* sp. CAG:82, *Bacillus gobiensis*, and *Desulfobacteriales bacterium*
64 *PC51MH44*, were selected to construct a taxonomic scoring system based on LASSO
65 analysis. An elevated area under curve (0.954) and positive net reclassification index
66 (1.5601) for predicting RAF compared with traditional clinical scoring (AUC=0.6918)
67 were obtained. The GM-based taxonomic scoring system theoretically improves the
68 model performance. These data provide novel evidence that supports incorporating
69 the GM factor into future recurrent risk stratification.

70 **KEYWORDS:** Atrial fibrillation; Recurrence; Gut microbiota; Metabolism;
71 Predictive model.

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76 Introduction

77 Atrial fibrillation (AF), the commonest arrhythmia among human cardiac diseases, is
 78 considered to cause a heavy global burden and directly impair the patient's quality of
 79 life [1]. The morbidity rate of AF is becoming increasingly high worldwide, and the
 80 affected patients have approximately two-fold mortality rate increase compared with
 81 individuals with sinus rhythm due to cardiac and cerebrovascular events, such as
 82 cerebral stroke [2]. Medical management of AF with antiarrhythmic medications
 83 yields only partial effectiveness and is often associated with multiple adverse effects
 84 [3]. Hence, percutaneous radiofrequency catheter ablation represents an important
 85 treatment strategy and option for AF patients, especially individuals showing
 86 intolerance or symptomatic disease refractory to Class I or III antiarrhythmics [4].
 87 Another issue is therefore raised, as ablation still carries the possibility and risk of AF
 88 recurrence (RAF). Not all AF patients that undergo radiofrequency catheter ablation
 89 remain in stable sinus rhythm. The success rates of catheter ablation maintaining sinus
 90 rhythm and avoiding a recurrence of AF after ablation are hard to predict and control
 91 [5-7]. To date, multiple variables, such as left atrial diameter and N-terminal
 92 pro-B-type natriuretic peptide (NT-proBNP), are considered risk factors for the
 93 recurrence of AF upon catheter ablation; however, these biomarkers lack specificity,
 94 and their predictive powers are barely satisfactory [8, 9]. The clinical scoring system,
 95 including CAAP-AF (coronary artery disease [CAD], age, left atrial size, persistent
 96 AF, unsuccessful antiarrhythmics, and female gender), DR-FLASH (diabetes mellitus,
 97 abnormal renal function, persistent type of AF, LA diameter > 45 mm, age > 65 years,
 98 female gender, and hypertension), and APPLE (age > 65 years, persistent AF,
 99 abnormal estimated glomerular filtration rate [eGFR; < 60 ml/min/1.73 m²], as well as
 100 LA diameter above 43 mm and ejection fraction below 50%) scores, could provide a
 101 realistic AF ablation outcome expectation for individual patients [7, 10-13]. However,
 102 this scoring system is simple and requires further modifications for increased
 103 robustness via substitution of etiologic factors by surrogate variables. Consequently,

104 developing a novel and better predictive model, which would help physicians identify
105 which individual patients could really benefit from catheter ablation and choose the
106 best treatment option, is quite important.

107 The alteration and potential function of the gut microbiota in various pathologies,
108 either as diagnostic biomarkers or contributors to pathogenesis, have attracted
109 increasing attention [14, 15]. For example, the gut bacterium *Fusobacterium*
110 *nucleatum* has been identified as a potent biomarker for improving the diagnostic
111 performance of the fecal immunochemical test (FIT), helping detect tumors otherwise
112 missed by FIT. This could allow a step forward in designing a non-invasive,
113 potentially more accurate, and cost-effective diagnostic tool for advanced colorectal
114 neoplasia. Elevated amounts of species of the phylum *Bacteroidetes* are associated
115 with prevention of checkpoint-blockade-induced colitis. Therefore, the identification
116 of gut microbiota (GM)-based markers might help develop approaches for reducing
117 the risk of inflammatory complications upon administration of cancer
118 immunotherapeutic drugs [16, 17]. Recently, we have assessed the role of GM in AF.
119 Our team characterized the associations of GM alterations and metabolic patterns with
120 AF by revealing the altered GM and bacteria-associated metabolites identified in AF
121 cases in a previous research [18]. We further designed a random forest disease
122 classifier based on abundances of co-abundance gene groups as variables for building
123 a microbiota-dependent discrimination model for AF detection. The results showed
124 that the differential gut microbiome signature could help diagnose AF, and the
125 co-abundance gene groups originating from *Eubacterium*, *Prevotella*, *Ruminococcus*,
126 *Dorea*, *Blautia*, *Bacteroides*, and *Lachnospiraceae* were essential in separating AF
127 cases from control individuals. However, the correlation between altered GM and AF
128 recurrence post-ablation remains unclear. Given the significance of GM shifts in AF
129 patients as previously reported by our team, as well as the risk of AF recurrence
130 following radiofrequency catheter ablation, we wondered whether the GM factor
131 could be applied in predicting the risk of RAF, identifying patients who might benefit

132 more from catheter ablation. Here, we evaluated the profiles of GM and metabolic
133 patterns, assessed AF recurrence after radiofrequency ablation, and constructed a
134 GM-dependent signature to identify the risk of AF recurrence.

135 **Results**

136 *Characteristics of the study population and follow-up*

137 In the current study, we included 90 participants from our previous cohort [18], with
138 50 non-AF controls (CTRs) and 40 AF patients. All AF cases underwent
139 radiofrequency catheter ablation before feces collection, and the patients remained in
140 sinus rhythm (normal beating of the heart) until the end of the procedure, with
141 confirmed circumferential pulmonary vein isolation (CPVI) and ablation line
142 blockage [4]. The occurrence of RAF was regarded as the endpoint. RAF was defined
143 as any episode of non-sinus atrial tachyarrhythmia, as reported previously [19]. The
144 patients with recurrent AF after the ablation would be allocated to the RAF group.
145 And the patients without recurrence to the non-RAF group. To date, these AF
146 patients have been followed-up for 15.6 ± 12.57 months. RAF was documented in 17
147 AF patients, with a postoperative recurrence rate of 42.5%. The clinical features of
148 patients assessed in this study are summarized in Table 1. Briefly, compared with the
149 non-AF CTR group, AF patients showed older age, higher incidence of type 2
150 diabetes mellitus (T2DM), reduced serum total cholesterol amounts, and higher intake
151 frequency of drugs such as statins, dimethyl biguanide (DMBG), angiotensin receptor
152 blockers (ARB), angiotensin-converting enzyme inhibitors (ACEI), and amiodarone.
153 The baseline clinical characteristics of the non-RAF and RAF groups were
154 comparable in terms of age, gender, body mass index (BMI), type 2 diabetes mellitus,
155 hypertension and fasting blood glucose, serum creatinine, total cholesterol, and
156 bilirubin amounts. In addition, we determined the CAAP-AF, DR-FLASH, and
157 APPLE scores, which were significantly higher in the RAF group compared with the
158 non-RAF group (Table 1; $p=0.043$, $p=0.559$ and $p=0.564$ for the CAAP-AF,

DR-FLASH and APPLE scores, respectively). Furthermore, to assess the predictive values of the risk scores, receiver operating curve (ROC) analyses were performed. The results showed that the CAAP-AF score exhibited a higher area under curve (AUC) of 0.6981 than the DR-FLASH (AUC=0.5575) and APPLE (AUC = 0.4464) scores in predicting RAF. Therefore, the CAAP-AF score was selected to reflect clinical risk factors.

AF recurrence is associated with the dynamically advanced degree of GM dysbiosis

The diversity index indicates the variety and richness of microbial entities in the gut, and is known to be associated with different disease states [20-22]. To assess gut microbial diversity in RAF patients, total gene amounts (Fig. 1a); alpha (within the individual) diversity, comprising Shannon's index (Fig. 1b), Chao 1 richness (Fig. 1c), and Pielou's evenness (Fig. 1d); and beta (between individuals) diversity of principal component analysis (PCA) (Fig. 1e), principal coordinate analysis (PCoA) (Fig. 1 f), and non-metric dimensional scaling (NMDS) (Fig. 1g) plots were analyzed at the species level based on metagenomic sequencing data. Compared with the non-AF CTR group, the non-RAF and RAF groups had significantly altered alpha and beta GM diversity, except for the Chao 1 index in the non-RAF group. Although no dynamic discrepancy in microbial diversity was observed between the non-RAF and RAF groups, there was an increasing and aggravated tendency of shifts in the RAF group, suggesting that patients experiencing recurrence after ablation might possess a more advanced degree of GM dysbiosis than non-RAF individuals.

Meanwhile, considering the differences in clinical features such as age, T2DM incidence, total cholesterol amounts, and glutamic-pyruvic transaminase levels as well as medications administered between the AF and non-AF CTR groups, PCA was performed to examine whether the above GM profile changes in the non-RAF and RAF groups were actually driven by these discrepant clinical factors [23, 24]. The results in Fig. S1 showed that the dots representing individuals of different ages,

186 T2DM diagnosis, total cholesterol, glutamic-pyruvic transaminase, or medication in
187 the various plots were mixed together and failed to cluster into separate groups,
188 indicating the low effects of these factors on the overall findings regarding the GM
189 (Additional files 1: Fig. S1).

190 ***Altered gut taxonomic profiles are associated with post-ablation RAF***

191 The elevated gut microbial diversity and increased degree of GM dysbiosis in RAF
192 indicate the possible overgrowth of some harmful microbes [25, 26]. Thus, the
193 phylogenetic signatures of the GM were analyzed with the aim of further examining
194 the changes in GM composition in RAF more specifically (Additional files 2-3: Table
195 S1-2). Overall, the non-RAF and RAF groups shared most microbes detected in the
196 non-AF CTR group, with 1219 genera (Additional files 3: Fig. S2a) and 5041 species
197 (Additional files 3: Fig. S2d). Interestingly, some abundant bacteria, such as the
198 genera *Faecalibacterium* and species *Faecalibacterium prausnitzii*, showed
199 dynamically decreasing tendencies from non-RAF to RAF. In addition, *Ruminococcus*
200 and *Eubacterium* exhibited progressively increasing trends from the non-RAF and
201 RAF groups (Additional files 3: Fig. S2b, c, e, f). These progressive GM shifts
202 associated with recurrent AF confirmed a dynamic and aggravating GM dysbiosis in
203 patients who would suffer from recurrent AF after ablation.

204 Next, we assessed the taxa that were dramatically altered in the gut of non-RAF
205 subjects and RAF patients at both the genus and species levels. Compared with the
206 non-AF CTR group, a total of 354 and 337 genera, 1735 and 1646 species were
207 significantly changed in the non-RAF and RAF groups, respectively (Additional files
208 5: Table S3). Generally speaking, the non-RAF and RAF groups shared 198 genera
209 and 1077 species that were simultaneously altered (Fig. 2a, d), with most of these
210 common bacteria exhibiting quite a similar tendency in the non-RAF and RAF groups
211 (Fig. 2b, e). Several genera (e.g., *Prevotella*) and species (e.g., *Prevotella copri* and
212 *Prevotella copri CAG:164*), which have been documented to be reduced in patients

with Parkinson's disease by a previous report [27], showed a decreased trend in the non-RAF group, and declined further in the RAF group. Consistently, genera such as *Ruminococcus*, *Blautia*, *Dorea*, and *Dialister*, as well as species including *Ruminococcus* sp. and *Dorea longicatena*, exhibited a progressively increased trend in patients experiencing recurrence post ablation (Fig. 2c, f). *Ruminococcus* exerts pro-inflammatory effects and contributes to inflammatory bowel disease (IBD) pathogenesis [28]. *Ruminococcus* transplantation in germ-free mice enhances interferon- γ , interleukin 17, and interleukin 22 amounts [29]. *Dialister* was shown to be associated with antepartum preeclampsia samples [30]. Thus, the balanced steady state in the gut is likely broken in AF subjects, especially in those who suffering from recurrence. The deficiency in health-promoting bacteria and the enrichment of disease-causing ones might be associated with RAF pathology after ablation.

Besides the common shifts in gut taxa between the non-RAF and RAF groups, distinct alterations of bacteria profiles were identified exclusively in non-RAF or RAF patients. A total of 8 families, 4 genera, and 28 species showed significant differences between the non-RAF and RAF groups (Fig. 3). Bacteria such as *Methanobrevibacter* sp., *Methanobrevibacter smithii*, and *Methanobrevibacter curvatus* were more abundant in the RAF group, while microbes such as *Candidatus* sp., *Phycomycetaceae* sp., *Bacteroidetes bacterium RBG_13_43_22*, and *Faecalibacterium* sp. CAG:82 were deficient in the non-RAF group. We speculated that the shared GM changes in the non-RAF and RAF groups might represent the core bacterial features of AF, and the unique shifts in GM composition in RAF patients might possibly account for the progression and recurrence of AF.

GM functional profiles associated with RAF

To depict gut microbial gene functions among different AF categories, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was applied as described previously by our team [15, 18]. Briefly, the non-RAF and RAF groups could not be

distinguished from each other, but were separated clearly when compared with non-AF CTRs by PCA, PCoA, and NMDS plots, which indicated drastic alterations in GM functions between the groups (Fig. 4a–c). Compared with non-AF CTRs, there were 201 KEGG modules in different enrichment shared between the non-RAF and RAF groups (adjusted $p < 0.05$, Wilcoxon rank-sum test, Fig. 4d; Additional files 6: Table S4). The majority of these functional modules also shared the same trends in the non-RAF and RAF groups (Fig. 4e). Some bacterial functions, including the citric acid cycle and amino acid biosynthesis, were deficient in the non-RAF and RAF groups, and are quite essential for human physiological health (Fig. 4e). Moreover, two KEGG modules, comprising the capsular polysaccharide transport system and xylitol transport system, were significantly elevated in the RAF group in compared with non-RAF individuals (Fig. 4f). However, the specific associations of these microbial functions with AF recurrence remain to be elucidated.

RAF is associated with disordered metabolomic profiles

The potential mechanisms mediating gut microbial function in human health rely on the interactions of gut microbe-derived metabolites with target organs [31, 32]. Therefore, metabolomic analyses based on LC-MS were performed to assess the metabolomic profiles of AF patients with or without the risk of AF recurrence following ablation. In this study, samples sufficient for metabonomic analysis were not obtained from all patients. Finally, a subset of 60 participants, comprising 36 non-AF CTRs, 13 non-RAF patients, and 11 RAF patients were included in serum metabolite analysis, and 52 individuals (17 non-AF CTRs, 20 non-RAF patients, and 15 RAF patients) were enrolled for metabolomic profile assessment in the feces. In serum, 2,500 and 1,733 features in the positive (ESI+) and negative (ESI–) ion modes were obtained, respectively. In fecal samples, 2,549 ESI+ and 1,894 ESI– features were observed. Overall, global metabolic changes in either serum or feces were revealed between the non-RAF and RAF groups by both partial least squares

discriminant analysis (PLS-DA) and orthogonal PLS-DA (OPLS-DA) plots, with significant separation detected between non-RAF and RAF patients in modes of ES+ and ES- (Additional files 6: Fig. S3).

Overall, 94 circulating and 52 fecal metabolites showed simultaneous alterations in both non-RAF and RAF patients compared with non-AF CTRs (Fig. 5a). Interestingly, all 17 metabolites showing overlap in serum and stool specimens (Fig. 5b, c, d) had similar variation trends in the non-RAF and RAF groups. Eight of them were synchronously altered in the serum and feces, and thus speculated to constitute the common features and core metabolites associated with AF development, which needs further investigation (Additional files 8: Table S5). In addition, two fecal metabolites, 7-methylguanine and palmitoleic acid, were found to be markedly reduced in cases with RAF in comparison with the non-RAF group. In addition, 7-methylguanine and palmitoleic acid showed no higher abundance in the CTR group compared with non-RAF and RAF cases (Fig. 5g).

For assessing the associations of altered metabolites with changed GM, Pearson's correlation analysis was carried out to evaluate the gut genera (Fig. 5e) and species (Fig. 5f) frequently changed in the non-RAF and RAF groups, in relation with the eight above-mentioned representative metabolites. Notably, the metabolites enriched in non-RAF and RAF patients, including lysophosphatidylethanolamine (LysoPE) (0:0/16:0), chenodeoxycholic acid (CDCA), and sebacic acid, were highly correlated with several AF-enriched genera (*Ruminococcus* and *Eubacterium*) and species, including *Eubacterium rectale*, *Roseburia inulinivorans*, and *Roseburia faecis*. Meanwhile, metabolites deficient in non-RAF and RAF patients, such as α -linolenic acid, were negatively correlated with AF-enriched genera, including *Eubacterium* and *Blautia*. Furthermore, correlation analyses between the two distinctive fecal metabolites and gut microbes showed that non-RAF enriched 7-methylguanine and palmitoleic acid were negatively associated with taxonomic (Tax) score decrease in

294 non-RAF patients (Fig. 5h, i. 7-methylguanine: $R^2 = 0.1048$, 95%CI: -0.593 to
295 0.01068, $p = 0.0578$; palmitoleic acid: $R^2 = 0.1902$, 95%CI: -0.6717 to -0.1203, $p =$
296 0.0088, respectively). Based on the significant associations of metabolites with gut
297 taxa, the possibility is raised that GM dysbiosis might cause a deficiency in select
298 hear-protective metabolic products and/or producing deleterious compounds, either
299 directly or indirectly, thereby contributing to the recurrence of AF post ablation. The
300 GM could be, therefore, considered a latent risk factor for RAF.

301 *Development and validation of a predictive model based on GM signature and* 302 *clinical scores for RAF*

303 Subsequently, we sought to establish and assess a predictive model to help make
304 individualized estimates of AF recurrence after ablation. Firstly, we selected the most
305 predictive taxa for RAF by performing least absolute shrinkage and selection operator
306 (LASSO) analyses [33, 34]. The results showed that seven bacterial strains consisting
307 of two families (*Nitrosomonadaceae* and *Lentisphaeraceae*), two genera (*Marinitoga*
308 and *Rufibacter*), and three species (*Faecalibacterium* sp. CAG:82, *Bacillus gobiensis*,
309 and *Desulfobacteriales bacterium PC51MH44*) among the candidate variables (taxon
310 differing between the non-RAF and RAF groups) remained statistically significant,
311 with nonzero coefficients based on 40 AF individuals (Fig. 6a, b). Then, we defined a
312 risk score as the Tax score based on a linear combination of these seven taxa-based
313 markers, and calculated the Tax score via weighting with their respective coefficients.
314 The model was constructed as follows: Tax score = $[-0.5104 \times (\text{Intercept})] +$
315 $[35,896.6613 \times \text{Nitrosomonadaceae}] + [564,576.2087 \times \text{Lentisphaeraceae}] +$
316 $[25.6052 \times \text{Marinitoga}] + [71,729.3882 \times \text{Rufibacter}] + [-236.5270 \times$
317 $\text{Faecalibacterium sp. CAG:82}] + [-6180.8888 \times \text{Bacillus gobiensis}] + [730,762.9872$
318 $\times \text{Desulfobacteriales bacterium PC51MH44}]$ (Fig. 6c, d). Patients in the RAF group
319 generally had significantly higher Tax scores ($p = 3.5973e-08$, Additional files 9:
320 Table S6).

321 In addition, to evaluate whether the gut microbiota (GM) signature could improve
322 the predictive value of conventional risk factors such as clinical characteristics and
323 drug usage, we performed univariate and multivariate Cox regression analyses,
324 determining hazard ratios (HRs) and respective 95% confidence intervals (CIs) for
325 parameters showing associations with AF recurrence upon ablation. The diagnostic
326 performance of the model was evaluated by the C index [35]: 0.9–1.0, outstanding;
327 0.8–0.9, excellent; 0.7–0.8, acceptable [36].

328 Because of the limitation of sample size, the CAAP-AF score was selected as the
329 synthetical reflection of numerous clinical characteristics (including age, gender, left
330 atrial size, AF persistence, antiarrhythmics failed and CAD). We found that the Tax
331 score and statin usage were significantly associated with RAF (Tax score, HR=2.5,
332 95%CI: 1.1–5.8, P=0.026; Statin usage, HR=4.8, 95%CI: 1.3–17, P=0.019).
333 Meanwhile, other medication factors, including ACEI, ARB, CCB, β -blocker,
334 propafenone and amiodarone administration were not significantly associated with
335 RAF (Additional files 10: Table S7).

336 We next carried out multivariate-adjusted Cox regression based on the
337 abovementioned indexes to assess whether GM could improve approaching utilizing
338 conventional clinical factors. Thus, a clinical model incorporating the CAAP-AF
339 score and statin usage, as well as a combined model including the CAAP-AF score,
340 statin usage and Tax score were built (Table S8). After incorporating the clinical
341 factors of RAF, Tax score retained a significant association with RAF incidence
342 (HR=2.647, 95%CI: 1.038–6.749, P=0.041). Notably, the combined model had
343 excellent (C index=0.8329, 95%CI: 0.7249-0.9410) and significant (p=0.0428)
344 improvement in performance, in comparison with the clinical model (C index=0.7261,
345 95%CI: 0.5813-0.8709).

346 Then, using the enter method, logistic regression analysis with the clinical
347 CAAP-AF score and the developed Tax score was carried out. The Tax score was
348 identified as an independent predictor (Tax score: Coef=14.4496, Odds ratio

[OR]=1,885,289.4390, 95%CI: 36.5170-97,333,313,606.5091; $p=0.0091$; CAAP-AF score, Coef=0.5445, OR=1.7237; 95%CI: 0.9477-3.1350; $p=0.0744$) (Fig. 6 c). Then, a combined predictive model containing two predictive scores, named CAAP-AF-Tax score, was constructed as follows: CAAP-AF-Tax score = $14.4496 \times \text{Tax score} + 0.5445 \times \text{CAAP-AF} + 2.2966$, with values ranging from -12.4340 to 18.0986 (Additional files 10: Table S6). Patients with a score of -12.4340 and 18.0986 had predicted recurrence risks of 3.98e-04% and 99.9999986%, respectively (Fig. 6c).

To assess the predictive value of the CAAP-AF-Tax model, the AUC based on the ROC curve was determined and compared with those of the CAAP-AF and Tax scores. Notably, compared with the AUC for the CAAP-AF score alone (AUC=0.6918, 95%CI: 0.525-0.85, $p=0.04$), AUC for either the Tax score model (AUC=0.954, 95%CI: 0.8974-1.000, $p=0.0055$) or CAAP-AF-Tax model (AUC=0.9668, 95%CI: 0.9216-1.000, $p=0.0011$) was significantly higher (Fig. 6e). The predictive model was subsequently validated using the net reclassification index (NRI). The NRI after adding the CAAP-AF score to the Tax score was 1.1509 ($p=0.0003$), while that after adding the Tax score to the CAAP-AF score was 1.5601 ($p=1.0735e-06$). Therefore, the Tax score theoretically improved the CAAP-AF model for performance.

Then, the Kaplan–Meier method and log-rank test were carried out for assessing the prognostic capacities of the developed CAAP-AF-Tax model, and AF cases were assigned to high- and low-CAAP-AF-Tax score groups according to the optimal cut-off of 0.633286. There was a significant difference in overall survival between the high- and low-score groups ($p < 0.0001$, Fig. 6f, g).

To provide a quantitative method for predicting individual risk and RAF probability, a nomogram of the CAAP-AF-Tax model was established (Fig. 6h) and submitted to internal validation as previously reported [37]. The results showed C-index=0.9668 (95%CI: 0.9155–1). The calibration curve in Fig. 6i demonstrated

376 good agreement with the probability of RAF. The Hosmer–Lemeshow test revealed
 377 non-statistical significance ($p=0.6318$) and suggested inconsiderable departure from
 378 the perfect fit [38]. Subsequently, in Fig. 6j, to determine the clinical value of the
 379 above Tax nomogram, we carried out decision curve analysis (DCA) via net benefit
 380 quantitation at various threshold probabilities [39]. We found that at a threshold
 381 probability (patient or doctor) of 1%, Tax score or CAAP-AF-Tax score nomogram
 382 use to predict RAF would provide more benefits compared with the treat-all- or
 383 treat-none schemes. Thus, the developed and validated predictive model might be a
 384 reliable method for RAF prediction, and help clinicians identify candidates who may
 385 benefit from future ablation therapy.

386 Discussion

387 In the current study, we have acquired a series of intriguing results describing the
 388 profiles of altered GM and metabolic patterns in AF patients more likely to
 389 experience recurrence following radiofrequency ablation. The predictive model based
 390 on the gut taxonomic signature was built for identifying patients at high risk of RAF.
 391 We identified a gradually increasing degree of gut dysbiosis from non-AF to RAF,
 392 and found multiple bacteria simultaneously enriched in non-RAF and RAF patients.
 393 Meanwhile, imbalanced GM functions and metabolic alterations were observed,
 394 which indicates the possible GM function in eliciting AF recurrence via interactions
 395 with metabolites. This feature of GM is therefore suggested to be a
 396 potent risk biomarker for the selection of patients who would benefit from
 397 radiofrequency catheter ablation. It is recommended to include an additional focus
 398 on GM profiling in the future development of ablation risk stratification and
 399 strategies.

400 Strikingly, this study demonstrated that disordered GM constituted an
 401 independent risk factor for AF recurrence. Catheter ablation is an efficient therapeutic
 402 option for AF, and has been widely used in clinical settings. CPVI of

extra-pulmonary vein (PV) AF substrate is the main strategy of ablation, which aims to suppress the arrhythmogenic substrate constituting the ablation target [4]. However, a high post-ablation recurrence rate calls for identifying novel markers that would enable an improved selection of patients who could benefit from ablation. PV reconnection and unrecognized or progressive extra-PV AF substrate constitute the immediate reflection of recurrence, but is neither necessary nor sufficient for RAF occurrence [40]. Therefore, based on the electro-physiological substrate behind AF persistence and progression, it remains difficult to identify beyond clinical AF parameters.

In our previous research, disordered GM was shown to be associated with the development of AF [18]. Interestingly, the present study indicated an incremental prognostic accuracy over clinical predictors of the novel predictive model based on GM taxonomic profiles. The newly defined Tax-score based on taxonomic profiles in the current work independently predicted AF recurrence, and findings from nomogram and decision curve analyses further confirmed its clinical value. Therefore, GM should be considered a potent predictive model for selecting patients for ablation, and additional focus on disordered GM profiles is strongly recommended in future ablation risk stratification. Although the number of samples included in the current research was small—and hence the robustness of the novel predictive model may not be strong enough—it provides preliminary results and offers a novel concept. Further validation studies with increased sample sizes could improve the overall universality.

Besides the distinctive taxa contained in the predictive score, two distinctive metabolites identified between non-RAF and RAF were significantly correlated with the Tax score. Although studies describing the direct protective effects of 7-methylguanine and palmitoleic acid against AF are scarce, investigators have identified the potential roles of 7-methylguanine and palmitoleic acid in the

430 pathophysiological process related to AF. 7-methylguanine, a nucleotide contributing
 431 to the metabolic pathways of guanine-containing purines, is linked to cognition
 432 phenotype [41]. A recent study showed that 7-methylguanine was increased in
 433 incident type 2 diabetes mellitus [42]. Meanwhile, palmitoleic acid has been suggested
 434 to enhance insulin sensitivity, stimulate insulin secretion, increase liver oxidation of
 435 fatty acids, improve blood lipid profile, alter the differentiation of macrophages [43],
 436 and improves metabolic functions in fatty liver tissue through peroxisome
 437 proliferator-activated receptor- α (PPAR α)-dependent 5' AMP-activated protein kinase
 438 (AMPK) activation [44]. Emerging evidence suggests that metabolic impairment is
 439 important for AF pathophysiology [45]. Notably, PPAR α -dependent AMPK
 440 activation could result in suppressed inflammation [46]. Therefore, we speculate that
 441 reduction of fecal metabolites such as palmitoleic acid in RAF patients might
 442 contribute to excessive inflammation and facilitate AF recurrence. These GM-related
 443 metabolic changes may contribute to the progress of atrial tissue's arrhythmogenic
 444 substrate aggravation following catheter ablation.

445 Given the associations of 7-methylguanine and palmitoleic acid with GM
 446 identified in the current work, these two metabolites are speculated to be potential
 447 players mediating the impact of GM dysbiosis on RAF progression, at least in part.
 448 Although it has not been assessed whether these two metabolites are directly produced
 449 by the GM, evidence demonstrating a link between 7-methylguanine, palmitoleic acid
 450 and GM is increasing. Palmitoleic acid belongs to the class of organic compounds
 451 known as long-chain fatty acids. Recent findings suggest that the metabolic activities
 452 of enteric microbiota may affect the levels of long-chain fatty acids [47]. Oral gavage
 453 of *Enterococcus faecalis* has been reported to affect a variety of long-chain fatty acids,
 454 including palmitoleic [48]. In addition, increased palmitoleic acid was previously
 455 observed in mice fed gut *Bifidobacterium breve* [49] and *Lactobacillus rhamnosus*
 456 LA68 [50]. Moreover, palmitoleic acid is considered a metabolic phenotype biomarker
 457 in acute anterior uveitis patients due to its positive correlation with gut *Roseburia* [51].

458 7-methylguanine belongs to the category of DNA adduct inducers [52]. The level of
459 DNA adducts including 7-methylguanine and O⁶-methylguanine in the rat colon
460 following administration of normal gut bacterial organisms has been previously
461 investigated [52]; a slight reduction in the half-life of 7-methylguanine was observed
462 after administration of *Lactobacillus acidophilus*. Based on the associations of fecal
463 7-methylguanine, palmitoleic acid and Tax score, the possibility was raised that GM
464 dysbiosis might lead to deficiency in 7-methylguanine and palmitoleic acid in RAF
465 patients, either directly or indirectly.

466 Inflammation is associated with multiple pathological events, including oxidative
467 stress, apoptosis and fibrosis, which induce AF substrate generation. Therefore,
468 low-grade inflammation is considered a potential mechanism contributing to AF. We
469 found the species *Faecalibacterium sp. CAG:82* exhibited a decreased trend in the
470 RAF group compared with non-RAFs. A previous study has demonstrated an
471 anti-inflammatory effect of gut *Faecalibacterium* through inhibition of interleukin-6
472 and transcription 3/interleukin-17 pathway activation [53]. Therefore, it is speculated
473 that reduced *Faecalibacterium* abundance in the intestine might increase various
474 inflammatory cytokines, elicit low-grade inflammation, and thus lead to RAF. These
475 interconnected microbial and metabolic changes suggest the involved microbes might
476 contribute to AF recurrence through interactions with specific metabolites in the host.

477 In addition to the disparity between the non-RAF and RAF groups, similarities
478 shared by these groups were also revealed in the current study, which may be more
479 important and constitute key events in the onset—but not development—of AF.
480 Furthermore, bacterial organisms and metabolites commonly altered in the non-RAF
481 and RAF groups were significantly associated. CDCA and sebacic acid were found to
482 be significantly correlated with several taxa. Elevated serum CDCA in the metabolic
483 patterns of non-RAF and RAF patients has been indicated to have a critical function
484 in the progress of structural remodeling in AF. CDCA is positively correlated with the
485 left atrial low voltage area (LVA) and promotes apoptosis in atrial myocytes [54].

486 Furthermore, sebacic acid belonging to medium-chain fatty acids is significantly less
487 abundant in both ulcerative colitis or Crohn disease patients [55]. Therefore, the
488 shared GM and metabolic profile demonstrated above may be associated with or even
489 contribute to AF onset.

490 These findings provided opportunities to take advantage of the GM for clinical
491 application for improving GM-related AF pathogenesis [56, 57]. For example,
492 utilizing fecal markers for identifying patients at high risk of RAF. Modulating
493 microorganisms using antibiotics to inhibit disease-enriched bacteria [58],
494 supplementing commensals [59, 60] or performing fecal microbiota transplantation to
495 replenish disease-decreased bacteria are also recommended [61, 62]. Meanwhile, with
496 the host–microbe crosstalk being in part mediated by bacterial metabolites, chemical
497 approaches could be regarded as a promising therapeutic strategy. Dietary
498 interventions, food compounds that can modify the GM (prebiotics) [59, 63] or
499 metabolites generated from gut bacteria (postbiotics) [64, 65] might be beneficial.
500 Engineering approaches like bacteriophages [66-68] specifically modifying gut
501 bacteria are also suggested. These extensive findings will pave the way to translate
502 GM use for clinical intervention, and more studies are imperative to evaluate its
503 clinical value in the context of AF.

504 In conclusion, the present findings provide a comprehensive description of
505 disordered GM profiles as well as its possible functions in AF patients with a high
506 risk of recurrence following radiofrequency ablation. The importance and clinical
507 value of bacterial taxonomic markers in patient selection for ablation are
508 highlighted. More attention should be paid to disordered GM profiles while
509 developing future ablation risk stratification strategies.

510 **Methods**

511 *Study cohort*

Forty non-valvular persistent AF (psAF) patients who underwent radiofrequency catheter ablation and 50 non-AF CTRs were included from our previous study [18]. Fecal samples were collected before radiofrequency ablation; the gut microbiome obtained before the ablation procedures was therefore utilized for predicting AF recurrence risk. Metagenomic sequencing results of 50 non-AF controls' fecal specimens previously assessed by our team were employed as controls. For the 50 non-AF controls, exclusion criteria were: previous heart failure; CAD; structural heart disease; concurrent pathologies such as IBD, autoimmune disorders, liver disease, kidney diseases or malignancy; antibiotic or probiotic use within a month prior to enrolment. Patient baseline features were collected by face-to-face interviews and from hospital records.

Catheter ablation and follow-up

In general, indications for AF ablation are symptomatic AF refractory or intolerant to one or more Class I or III antiarrhythmics, as well as symptom-free AF before administration of Class I or III antiarrhythmics [69]. A decision to perform ablation is taken upon careful consideration of complication risks, success rate, substitute options and patient preference.

Upon double-transseptal puncture under guidance of a 3D-electroanatomic mapping system (CARTO 3; Biosense Webster, Inc., Diamond Bar, CA, USA), a 3D-reconstructed image of left atrium was generated with a circular mapping catheter (NaviStarThermocool, Biosense Webster, Inc., Diamond Bar, CA, USA) followed by merging to 3D VR cardiac CT scan. Following circumferential pulmonary vein isolation (CPVI), linear ablation and complex fractionated atrial electrogram ablations were appended [4]. All catheter ablations were carried out by a single surgical team.

Following catheter ablation, patients underwent systematic follow-up and 12-lead electrocardiography at 3, 6, 12, and 18 months; respectively; an

538 electrocardiogram would be recorded in case a patient complained of discomfort.
 539 Holter monitoring was performed at three- and six- months post-ablation and at
 540 six-month intervals afterwards. Antiarrhythmic medications were discontinued in the
 541 third month. AF recurrence was defined as any episode of non-sinus atrial
 542 tachyarrhythmia (atrial tachycardia, atrial flutter, or AF) lasting more than 30 s and
 543 occurring after the three-month post ablation blanking period [19]. The patients with
 544 recurrent AF after ablation would be allocated to the RAF group. And those without
 545 recurrence would be classified as the nonrecurrence (non-RAF) group.

546 In our electrophysiological team, antiarrhythmic medications would be
 547 discontinued at five half-lives or more prior to ablation. After ablation, except for
 548 cases with contraindications (e.g., sinus bradycardia, second degree atrial-ventricular
 549 block, systolic blood pressure < 100 mmHg, hepatic dysfunction and dysthyroidism),
 550 all cases with persistent AF and some with paroxysmal AF would receive oral
 551 antiarrhythmic drugs for a 3-month period. In the current work, amiodarone and
 552 propafenone were administered to 30 (75%) and 7 (17.5%) individuals, respectively.
 553 Antiarrhythmic drugs were stopped in case of RAF recurrence following
 554 discontinuation for a period of time. During follow-up, the patients who experienced
 555 RAF would resume with anti-arrhythmic medication; in case of persistent recurrent
 556 episodes in spite of drug administration, a second ablative surgery would be offered.

557 Furthermore, according to current guidelines [1], the risk of stroke in AF patients
 558 should be assessed by the CHA2DS2-VASc score. Generally, cases with no clinically
 559 proven risk factors for stroke do not require antithrombotic treatment. Meanwhile,
 560 those showing stroke's risk factors (CHA2DS2-VASc scores ≥ 1 and ≥ 2 in males and
 561 females, respectively) without contraindications are recommended for oral
 562 anticoagulant (OAC) drugs. In our center, patients would take warfarin, dabigatran or
 563 rivaroxaban for ≥ 4 weeks before ablation. We carried out transesophageal

564 echocardiography within 3 days prior to ablation. During the ablative surgery, heparin
565 was administered before or right after transseptal puncture, adjusting the dose for
566 achieving and maintaining an ACT ≥ 300 seconds. OACs should be administered for
567 ≥ 3 months post-ablation. The continuation of OACs for more than 3 months
568 post-ablation depends on stroke's risk profile.

569 ***GM assessment by Metagenomics***

570 Whole-metagenome sequencing data of 90 fecal specimens assessed in the current
571 study were obtained from a previous report by our team [18]. Bacterial DNA
572 extraction utilized a TIANGEN kit (TIANGEN BIOTECH, China). Then, paired-end
573 sequencing was carried out on an Illumina Novaseq 6000 (Illumina, USA), by
574 Novogene Bioinformatics Technology (China), with an insert size of 300 bp and a
575 read length of 150 bp. Metagenomic analyses followed the procedures described by
576 our group [14, 15, 18]. Genes were predicted from various contigs with
577 MetaGeneMark v12 (GeneMark, USA). Gene abundance was determined by
578 numbering reads after normalization to gene length. Then, DIAMOND v0.7.9.58 was
579 utilized for taxonomic assignments (default settings with the exception of $-k$ 50
580 $-sensitive$ $-e$ 0.00001). Matches showing statistical significance for various genes
581 with the first hit $e \leq 10 \times e\text{-value}$ were utilized for distinguishing taxonomic groups.
582 The taxonomic levels of different genes were assessed with the MEGAN software
583 (MEtaGenomeANalyzer); the abundance levels of different taxonomic groups were
584 evaluated by summing up those of all included genes. The KEGG database (Release
585 73.1) was employed for gene alignment with DIAMOND (as described above) to
586 evaluate the function of gut microbe. Proteins were assigned to the KEGG based on
587 respective highest-scoring hits with ≥ 1 high-scoring segment pair comprising more
588 than 60 hits. The abundance levels of KEGG modules were determined by summing
589 up those of all genes included, respectively.

590 ***GM assessment by metabolomics***

591 Of the above 90 cases, metabolomic data of 60 serum and 52 fecal specimens were
592 available [18]. Liquid chromatography-mass spectrometry (LC/MS) was carried out
593 with a Hypercarb C18 column (Thermo Fisher, USA; 3 μ m internal diameter, 4.6
594 mm $\times$ 100 mm) on an UltiMate 3000 chromatography system (Thermo Fisher).
595 Acetonitrile (Merck, Germany), methanol (Merck), formic acid (CNW, China), and
596 DL-o-Chlorophenylalanine (GL Biochem, China) were used during the process. Data
597 analysis was carried out as described in our previous reports [14, 18]. Partial
598 least-squares discriminant analysis (PLS-DA) as well as orthogonal partial
599 least-squares discriminant analysis (OPLS-DA) utilized the SIMCA-P software for
600 clustering specimen plots across groups. Compounds with significant between-group
601 changes were determined based on variable effect on projection > 1 and $P < 0.05$
602 according to peak areas.

603 *Construction and validation of the predictive model for RAF*

604 The LASSO method was employed for selecting the most efficient predictive indexes
605 from distinctive taxa between the non-RAF and RAF groups [33, 34]. A taxonomic
606 score (Tax-score) was determined for individual patients by linearly combining the
607 retained taxa weighted by the corresponding coefficients. Predictive model
608 performance was assessed using the AUC. Internal validation followed a reported
609 protocol [37]. Meanwhile, the mean of 500 bootstrapped estimates of optimism was
610 subtracted from the initial (full cohort model) estimate of the AUC and Nagelkerke
611 R² to obtain the bootstrap optimism-corrected estimates of performance.
612 Lasso-logistic regression was carried out with the “glmnet” package in R. The “rms”
613 package was utilized for multivariate binary logistic regression, nomogram
614 construction and calibration graphs. C-index calculation was performed the “Hmisc”
615 package. DCA was carried out with the “rmda” package. The optimal cut-off value
616 for Tax score and Kaplan-Meier survival curves (with log-rank test) were obtained by

the “survminer” and “survival” packages in R [70]. AUC, comparison, and NRI were obtained and calculated by the StataSE software.

Statistical analysis

Normally distributed quantitative variables are mean±standard deviation (SD), and assessed by the t-test. Those with non-normal distribution were shown as median and quartiles, and compared by the Wilcoxon rank sum test. Qualitative data were shown as percentage, with the χ^2 test for comparisons. Shannon index, Chao richness, and Pielou evenness were calculated with R version 3.3.3 in vegan package. PCA was carried out utilizing the FactoMineR package. PCoA used the vegan and ape packages. NMDS was carried out with the vegan package. Plot visualization utilized the ggplot2 package in R. Abundance differences at the gene, genus, species and KEGG module, respectively, were assessed by the Wilcoxon rank sum test, with Benjamini and Hochberg correction. Pearson correlation analysis was carried out for identifying microbiome-metabolome associations. Two-sided $P < 0.05$ indicated statistical significance.

Data Availability

The data supporting the results of this article has been deposited in the EMBL European Nucleotide Archive (ENA) under the BioProject accession code PRJEB28384 [<http://www.ebi.ac.uk/ena/data/view/PRJEB28384>]. And the raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2017) in BIG Data Center (Nucleic Acids Res 2019), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession numbers CRA002277, that are publicly accessible at <https://bigd.big.ac.cn/gsa>. Metabolomics data can be found on the NIH’s Common Fund’s Data Repository and Coordinating Center website (Metabolomics Work-

642 bench; Study IDs ST001168 and ST001169 for fecal and serum metabolomics,
643 respectively).

644 **Competing interests**

645 The authors declared no competing interests to this work.

646 **Author contributions**

647 XCY, KBL, KZ, and JL carried out study conception, design, and supervision, as well
648 as data interpretation and manuscript writing. JZ, PW, JJ, ZL, XDY and XQL were
649 involved in patient enrolment, diagnosis, and data collection. KZ and JL performed
650 data analysis. XCY, JZ and KBL carried out manuscript revision. The submitted
651 manuscript had approval from all authors after reading.

652 **Acknowledgments**

653 The present study was funded by the National Natural Science Foundation of China
654 (81670214, 81500383, 81870308, and 81970271), the Beijing Natural Science
655 Foundation (7172080), the Beijing Municipal Administration of Hospitals' Youth
656 Programme (QML20170303), and the 1351 personnel training plan
657 (CYMY-2017-03).

658 **Ethics**

659 The study had approval from the ethics committee of Beijing Chaoyang Hospital and
660 Kailuan General Hospital and the signed informed consent was provided by each
661 participant.

662 **References**

663 [1] Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC
664 Guidelines for the management of atrial fibrillation developed in collaboration with
665 EACTS. *Europace* 2016;18:1609-78.

666 [2] Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D.
667 Impact of atrial fibrillation on the risk of death: the Framingham Heart Study.
668 *Circulation* 1998;98:946-52.

669 [3] Dan GA, Martinez-Rubio A, Agewall S, Boriani G, Borggrefe M, Gaita F, et al.
670 Antiarrhythmic drugs-clinical use and clinical decision making: a consensus
671 document from the European Heart Rhythm Association (EHRA) and European
672 Society of Cardiology (ESC) Working Group on Cardiovascular Pharmacology,
673 endorsed by the Heart Rhythm Society (HRS), Asia-Pacific Heart Rhythm Society
674 (APHRS) and International Society of Cardiovascular Pharmacotherapy (ISCP).
675 *Europace* 2018;20:731-2an.

676 [4] Calkins H, Hindricks G, Cappato R, Kim YH, Saad EB, Aguinaga L, et al. 2017
677 HRS/EHRA/ECAS/APHRS/SOLAECE expert consensus statement on catheter and
678 surgical ablation of atrial fibrillation: executive summary. *J Interv Card*
679 *Electrophysiol* 2017;50:1-55.

680 [5] Mujovic N, Marinkovic M, Lenarczyk R, Tilz R, Potpara TS. Catheter Ablation of
681 Atrial Fibrillation: An Overview for Clinicians. *Adv Ther* 2017;34:1897-917.

682 [6] Vizzardi E, Curnis A, Latini MG, Salghetti F, Rocco E, Lupi L, et al. Risk factors
683 for atrial fibrillation recurrence: a literature review. *J Cardiovasc Med (Hagerstown)*
684 2014;15:235-53.

685 [7] Black-Maier E, Parish A, Steinberg BA, Green CL, Loring Z, Barnett AS, et al.
686 Predicting atrial fibrillation recurrence after ablation in patients with heart failure:
687 Validity of the APPLE and CAAP-AF risk scoring systems. *Pacing Clin*
688 *Electrophysiol* 2019.

689 [8] Carballo D, Noble S, Carballo S, Stirnemann J, Muller H, Burri H, et al.
690 Biomarkers and arrhythmia recurrence following radiofrequency ablation of atrial
691 fibrillation. *J Int Med Res* 2018;46:5183-94.

692 [9] Shen XB, Zhang SH, Li HY, Chi XD, Jiang L, Huang QL, et al. Rs12976445
693 Polymorphism Is Associated with Post-Ablation Recurrence of Atrial Fibrillation by
694 Modulating the Expression of MicroRNA-125a and Interleukin-6R. *Med Sci Monit*
695 2018;24:6349-58.

696 [10] Winkle RA, Jarman JW, Mead RH, Engel G, Kong MH, Fleming W, et al.
697 Predicting atrial fibrillation ablation outcome: The CAAP-AF score. *Heart Rhythm*
698 2016;13:2119-25.

699 [11] Liu CF, Lerman BB. The search for links between atrial fibrillation pathogenesis
700 and ablation outcomes. *Heart Rhythm* 2016;13:2126-7.

701 [12] Kosich F, Schumacher K, Potpara T, Lip GY, Hindricks G, Kornej J. Clinical
702 scores used for the prediction of negative events in patients undergoing catheter
703 ablation for atrial fibrillation. *Clin Cardiol* 2019;42:320-9.

704 [13] Kornej J, Hindricks G, Shoemaker MB, Husser D, Arya A, Sommer P, et al. The
705 APPLE score: a novel and simple score for the prediction of rhythm outcomes after
706 catheter ablation of atrial fibrillation. *Clin Res Cardiol* 2015;104:871-6.

707 [14] Zuo K, Li J, Xu Q, Hu C, Gao Y, Chen M, et al. Dysbiotic gut microbes may
708 contribute to hypertension by limiting vitamin D production. *Clin Cardiol* 2019.

709 [15] Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, et al. Gut microbiota dysbiosis
710 contributes to the development of hypertension. *Microbiome* 2017;5:14.

711 [16] Wong SH, Kwong TNY, Chow TC, Luk AKC, Dai RZW, Nakatsu G, et al.
712 Quantitation of faecal *Fusobacterium* improves faecal immunochemical test in
713 detecting advanced colorectal neoplasia. *Gut* 2017;66:1441-8.

714 [17] Dai Z, Coker OO, Nakatsu G, Wu WKK, Zhao L, Chen Z, et al. Multi-cohort
715 analysis of colorectal cancer metagenome identified altered bacteria across
716 populations and universal bacterial markers. *Microbiome* 2018;6:70.

717 [18] Zuo K, Li J, Li K, Hu C, Gao Y, Chen M, et al. Disordered gut microbiota and
718 alterations in metabolic patterns are associated with atrial fibrillation. *Gigascience*
719 2019;8.

720 [19] Calkins H, Kuck KH, Cappato R, Brugada J, Camm AJ, Chen SA, et al. 2012
721 HRS/EHRA/ECAS expert consensus statement on catheter and surgical ablation of
722 atrial fibrillation: recommendations for patient selection, procedural techniques,
723 patient management and follow-up, definitions, endpoints, and research trial design: a
724 report of the Heart Rhythm Society (HRS) Task Force on Catheter and Surgical
725 Ablation of Atrial Fibrillation. Developed in partnership with the European Heart
726 Rhythm Association (EHRA), a registered branch of the European Society of
727 Cardiology (ESC) and the European Cardiac Arrhythmia Society (ECAS); and in
728 collaboration with the American College of Cardiology (ACC), American Heart
729 Association (AHA), the Asia Pacific Heart Rhythm Society (APHRS), and the
730 Society of Thoracic Surgeons (STS). Endorsed by the governing bodies of the
731 American College of Cardiology Foundation, the American Heart Association, the
732 European Cardiac Arrhythmia Society, the European Heart Rhythm Association, the
733 Society of Thoracic Surgeons, the Asia Pacific Heart Rhythm Society, and the Heart
734 Rhythm Society. *Heart Rhythm* 2012;9:632-96 e21.

735 [20] Menni C, Lin C, Cecelja M, Mangino M, Matey-Hernandez ML, Keehn L, et al.
736 Gut microbial diversity is associated with lower arterial stiffness in women. *Eur Heart*
737 *J* 2018;39:2390-7.

738 [21] Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, et al. Gut
739 dysbiosis is linked to hypertension. *Hypertension* 2015;65:1331-40.

740 [22] Menni C, Jackson MA, Pallister T, Steves CJ, Spector TD, Valdes AM. Gut
741 microbiome diversity and high-fibre intake are related to lower long-term weight gain.
742 *Int J Obes (Lond)* 2017;41:1099-105.

743 [23] Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A metagenome-wide association
744 study of gut microbiota in type 2 diabetes. *Nature* 2012;490:55-60.

745 [24] Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen
746 T, et al. Population-based metagenomics analysis reveals markers for gut microbiome
747 composition and diversity. *Science* 2016;352:565-9.

748 [25] Ou Y, Zhang C, Yao M, Wang L. Gut Flora: Novel Therapeutic Target of
749 Chinese Medicine for the Treatment of Cardiovascular Diseases. *Evid Based*
750 *Complement Alternat Med* 2019;2019:3719596.

751 [26] Ahmadmehrabi S, Tang WHW. Gut microbiome and its role in cardiovascular
752 diseases. *Curr Opin Cardiol* 2017;32:761-6.

753 [27] Petrov VA, Saltykova IV, Zhukova IA, Alifirova VM, Zhukova NG, Dorofeeva
754 YB, et al. Analysis of Gut Microbiota in Patients with Parkinson's Disease. *Bull Exp*
755 *Biol Med* 2017;162:734-7.

756 [28] Hall AB, Yassour M, Sauk J, Garner A, Jiang X, Arthur T, et al. A novel
757 *Ruminococcus gnavus* clade enriched in inflammatory bowel disease patients.
758 *Genome Med* 2017;9:103.

759 [29] Hoffmann TW, Pham HP, Bridonneau C, Aubry C, Lamas B, Martin-Gallausiaux
760 C, et al. Microorganisms linked to inflammatory bowel disease-associated dysbiosis
761 differentially impact host physiology in gnotobiotic mice. *ISME J* 2016;10:460-77.

762 [30] Lv LJ, Li SH, Li SC, Zhong ZC, Duan HL, Tian C, et al. Early-Onset
763 Preeclampsia Is Associated With Gut Microbial Alterations in Antepartum and
764 Postpartum Women. *Front Cell Infect Microbiol* 2019;9:224.

765 [31] Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat*
766 *Rev Immunol* 2016;16:341-52.

767 [32] Lin L, Zhang J. Role of intestinal microbiota and metabolites on gut homeostasis
768 and human diseases. *BMC Immunol* 2017;18:2.

769 [33] Sauerbrei W, Royston P, Binder H. Selection of important variables and
770 determination of functional form for continuous predictors in multivariable model
771 building. *Stat Med* 2007;26:5512-28.

772 [34] Huang YQ, Liang CH, He L, Tian J, Liang CS, Chen X, et al. Development and
773 Validation of a Radiomics Nomogram for Preoperative Prediction of Lymph Node
774 Metastasis in Colorectal Cancer. *J Clin Oncol* 2016;34:2157-64.

775 [35] Royston P, Altman DG. External validation of a Cox prognostic model:
776 principles and methods. *BMC Med Res Methodol* 2013;13:33.

777 [36] Cortaredona S, Pambrun E, Verdoux H, Verger P. Comparison of
778 pharmacy-based and diagnosis-based comorbidity measures from medical
779 administrative data. *Pharmacoepidemiol Drug Saf* 2017;26:402-11.

780 [37] Keyhani S, Madden E, Cheng EM, Bravata DM, Halm E, Austin PC, et al. Risk
781 Prediction Tools to Improve Patient Selection for Carotid Endarterectomy Among
782 Patients With Asymptomatic Carotid Stenosis. *JAMA Surg* 2019;154:336-44.

783 [38] Kramer AA, Zimmerman JE. Assessing the calibration of mortality benchmarks
784 in critical care: The Hosmer-Lemeshow test revisited. *Crit Care Med* 2007;35:2052-6.

785 [39] Vickers AJ, Cronin AM, Elkin EB, Gonen M. Extensions to decision curve
786 analysis, a novel method for evaluating diagnostic tests, prediction models and
787 molecular markers. *BMC Med Inform Decis Mak* 2008;8:53.

788 [40] Johnner N, Shah DC, Giannakopoulos G, Girardet A, Namdar M. Evolution of
789 post-pulmonary vein isolation atrial fibrillation inducibility at redo ablation:
790 Electrophysiological evidence of extra-pulmonary vein substrate progression. *Heart*
791 *Rhythm* 2019;16:1160-6.

792 [41] Shi M, Bazzano LA, He J, Gu X, Li C, Li S, et al. Novel serum metabolites
793 associate with cognition phenotypes among Bogalusa Heart Study participants. *Aging*
794 (Albany NY) 2019;11:5124-39.

795 [42] Ottosson F, Smith E, Gallo W, Fernandez C, Melander O. Purine Metabolites and
796 Carnitine Biosynthesis Intermediates Are Biomarkers for Incident Type 2 Diabetes. *J*
797 *Clin Endocrinol Metab*. 2019;104:4921-4930. [43] de Souza CO, Vannice GK, Rosa
798 Neto JC, Calder PC. Is Palmitoleic Acid a Plausible Nonpharmacological Strategy to

799 Prevent or Control Chronic Metabolic and Inflammatory Disorders? *Mol Nutr Food*
800 *Res* 2018;62.

801 [44] de Souza CO, Teixeira AAS, Biondo LA, Lima Junior EA, Batatinha HAP, Rosa
802 Neto JC. Palmitoleic Acid Improves Metabolic Functions in Fatty Liver by
803 PPARalpha-Dependent AMPK Activation. *J Cell Physiol* 2017;232:2168-77.

804 [45] Bai F, Liu Y, Tu T, Li B, Xiao Y, Ma Y, et al. Metformin regulates lipid
805 metabolism in a canine model of atrial fibrillation through AMPK/PPAR- α /VLCAD
806 pathway. *Lipids Health Dis* 2019;18:109.

807 [46] Jung TW, Kim HC, Abd El-Aty AM, Jeong JH. Protectin DX ameliorates
808 palmitate- or high-fat diet-induced insulin resistance and inflammation through an
809 AMPK-PPARalpha-dependent pathway in mice. *Sci Rep* 2017;7:1397.

810 [47] Zhao L, Huang Y, Lu L, Yang W, Huang T, Lin Z, et al. Saturated long-chain
811 fatty acid-producing bacteria contribute to enhanced colonic motility in rats.
812 *Microbiome* 2018;6:107.

813 [48] Quan LH, Zhang C, Dong M, Jiang J, Xu H, Yan C, et al. Myristoleic acid
814 produced by enterococci reduces obesity through brown adipose tissue activation. *Gut*
815 2019.

816 [49] Barrett E, Fitzgerald P, Dinan TG, Cryan JF, Ross RP, Quigley EM, et al.
817 *Bifidobacterium breve* with alpha-linolenic acid and linoleic acid alters fatty acid
818 metabolism in the maternal separation model of irritable bowel syndrome. *PLoS One*
819 2012;7:e48159.

820 [50] Ivanovic N, Minic R, Djuricic I, Dimitrijevic L, Sobajic S, Zivkovic I, et al.
821 Brain and liver fatty acid composition changes upon consumption of *Lactobacillus*
822 *rhamnosus* LA68. *Int J Food Sci Nutr* 2015;66:93-7.

823 [51] Huang X, Ye Z, Cao Q, Su G, Wang Q, Deng J, et al. Gut Microbiota
824 Composition and Fecal Metabolic Phenotype in Patients With Acute Anterior Uveitis.
825 *Invest Ophthalmol Vis Sci* 2018;59:1523-31.

826 [52] Arimochi H, Kinouchi T, Kataoka K, Kuwahara T, Ohnishi Y. Effect of
827 intestinal bacteria on formation of azoxymethane-induced aberrant crypt foci in the rat
828 colon. *Biochem Biophys Res Commun* 1997;238:753-7.

829 [53] Zhou L, Zhang M, Wang Y, Dorfman RG, Liu H, Yu T, et al. *Faecalibacterium*
830 *prausnitzii* Produces Butyrate to Maintain Th17/Treg Balance and to Ameliorate
831 Colorectal Colitis by Inhibiting Histone Deacetylase 1. *Inflamm Bowel Dis* 2018.

832 [54] Wang XH, Li Z, Zang MH, Yao TB, Mao JL, Pu J. Circulating primary bile acid
833 is correlated with structural remodeling in atrial fibrillation. *J Interv Card*
834 *Electrophysiol* 2019.

835 [55] Weng YJ, Gan HY, Li X, Huang Y, Li ZC, Deng HM, et al. Correlation of diet,
836 microbiota and metabolite networks in inflammatory bowel disease. *J Dig Dis*
837 2019;20:447-59.

838 [56] Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and
839 clinical applications. *Nat Rev Gastroenterol Hepatol* 2019;16:690-704.

840 [57] Jia Q, Li H, Zhou H, Zhang X, Zhang A, Xie Y, et al. Role and Effective
841 Therapeutic Target of Gut Microbiota in Heart Failure. *Cardiovasc Ther*
842 2019;2019:5164298.

843 [58] Bullman S, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, et al.
844 Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer.
845 *Science* 2017;358:1443-8.

846 [59] Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al. Gut
847 microbiome influences efficacy of PD-1-based immunotherapy against epithelial
848 tumors. *Science* 2018;359:91-7.

849 [60] Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, et al.
850 Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota.
851 *Science* 2015;350:1079-84.

852 [61] Kang Y, Cai Y. Gut microbiota and obesity: implications for fecal microbiota
853 transplantation therapy. *Hormones (Athens)* 2017;16:223-34.

- 854 [62] Weingarden AR, Vaughn BP. Intestinal microbiota, fecal microbiota
855 transplantation, and inflammatory bowel disease. *Gut Microbes* 2017;8:238-52.
- 856 [63] Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et
857 al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates
858 anti-PD-L1 efficacy. *Science* 2015;350:1084-9.
- 859 [64] Kaiko GE, Ryu SH, Koues OI, Collins PL, Solnica-Krezel L, Pearce EJ, et al.
860 The Colonic Crypt Protects Stem Cells from Microbiota-Derived Metabolites. *Cell*
861 2016;167:1137.
- 862 [65] Bultman SJ, Jobin C. Microbial-derived butyrate: an oncometabolite or
863 tumor-suppressive metabolite? *Cell Host Microbe* 2014;16:143-5.
- 864 [66] Reardon S. Phage therapy gets revitalized. *Nature* 2014;510:15-6.
- 865 [67] Kingwell K. Bacteriophage therapies re-enter clinical trials. *Nat Rev Drug*
866 *Discov* 2015;14:515-6.
- 867 [68] Duan Y, Llorente C, Lang S, Brandl K, Chu H, Jiang L, et al. Bacteriophage
868 targeting of gut bacterium attenuates alcoholic liver disease. *Nature* 2019;575:505-11.
- 869 [69] Calkins H, Hindricks G, Cappato R, Kim YH, Saad EB, Aguinaga L, et al. 2017
870 HRS/EHRA/ECAS/APHRS/SOLAECE expert consensus statement on catheter and
871 surgical ablation of atrial fibrillation: Executive summary. *J Arrhythm*
872 2017;33:369-409.
- 873 [70] Peng D, Wang L, Li H, Cai C, Tan Y, Xu B, et al. An immune infiltration
874 signature to predict the overall survival of patients with colon cancer. *IUBMB Life*
875 2019.

876 **Figure Legends**

877 **Figure 1. AF recurrence is associated with the dynamically advanced degree of**
878 **dysbiosis in the GM**

879 Gene number (a) and within individuals (alpha) diversity comprising Shannon index
880 (b), Chao richness (c), and Pielou evenness (d) according to the species profile in

881 non-AF CTR, non-RAF and RAF patients. Boxes are interquartile ranges, with lines
882 denoting medians and circles being outliers. Between individuals (beta) diversity
883 comprising PCA (e), PCoA (f), and NMDS (g) according to species abundances. The
884 results depicted a dynamically increasing tendency of diversity among control,
885 non-RAF and RAF cases. Blue squares represent non-AF CTR, pink triangles refer to
886 non-RAF, and red circles denote RAF.

887 **Figure 2. Common taxa in the non-RAF and RAF groups**

888 a. Venn diagram showing the count of altered genera common to the non-recurrence
889 of atrial fibrillation (AF) (non-RAF) (pink) and RAF (red) groups when compared to
890 the non-AF control (CTR) group. The overlap revealed 198 genera simultaneously
891 detected in AF patients with or without recurrence.

892 b. Heat-map revealing 198 commonly altered genera in the non-RAF and RAF groups
893 when compared to the non-AF CTR ($q < 0.05$ from Wilcoxon rank-sum test) and
894 phylogenetic associations. Abundance profile is reflected by the z-score, with genera
895 grouped according to the Bray–Curtis distance. Negative (blue) and positive (pink)
896 Z-scores reflect lower and higher abundance levels compared with the mean value,
897 respectively. The colors of the lines inside denote the phyla of given genera.

898 c. Heat-map of the first 10 shared genera ($q < 0.05$; Wilcoxon rank-sum test).
899 Abundance profiles underwent transformation into Z-scores via average abundance
900 subtraction and division by the standard deviation. Negative (blue) and positive (red)
901 Z-scores reflected row abundance levels lower and higher compared with the mean,
902 respectively.

903 d. Venn diagram depicting the count of differential species common to the non-RAF
904 (pink) and RAF (red) groups when compared with the non-AF CTR group. The

905 overlap revealed 1077 species simultaneously detected in AF patients with or without
906 recurrence.

907 e. Heat-map depicting 1077 genera differentially present in the non-RAF and RAF
908 groups when compared with non-AF CTR ($q < 0.05$ from Wilcoxon rank-sum test),
909 and the corresponding phylogenetic associations. Abundance profiles were plotted as
910 z-scores, with genera grouped according to Bray–Curtis distance. Negative (blue) and
911 positive (pink) Z-scores reflected row abundance levels lower and higher than the
912 average, respectively. The colors of the lines inside denote the phyla of given genera.

913 f. Heat-map of the first 10 shared species ($q < 0.05$; Wilcoxon rank-sum test). The
914 abundance profiles were analyzed as in c. Negative (blue) and positive (red) Z-scores
915 reflected row abundance levels lower and higher compared with the mean,
916 respectively.

917 **Figure 3. Distinctive taxa between non-RAF and RAF**

918 Box plots of relative abundance (log transformed) of the distinctive taxa between
919 individuals from non-RAF and RAF, including 5 families (a), 4 genera (b) and 22
920 species (c) at the criteria of q value < 0.05 ; Wilcoxon rank sum test after filtering out
921 in less than 10% of the samples detected and medians of zero. Boxes represent the
922 interquartile ranges, lines inside the boxes mean medians and circles are outliers.

923 **Figure 4. GM functional profiles associated with RAF**

924 a, b, c. PCA (a), PCoA (b), NMDS (c) according abundance levels of KEGG modules
925 showing disordered GM functional profiles in non-RAF and RAF cases. Blue squares
926 represent non-AF CTR, pink triangles refer to non-RAF, and red circles denote RAF.

927 d. Venn diagram depicting the count of differentially represented KEGG modules
 928 common in non-RAF (pink) and RAF (red) versus non-AF CTR. The overlap
 929 revealed 201 KEGG modules shared by non-RAF and RAF.

930 e. Heat-map of 38 shared functional modules ($q < 0.0001$; Wilcoxon rank sum test).
 931 Abundance profiles underwent transformation into Z-scores via average abundance
 932 subtraction and division by the standard deviation. Negative (blue) and positive (red)
 933 Z-scores reflected row abundance levels lower and higher compared with the mean,
 934 respectively.

935 f. Box plots of 2 differential KEGG modules between non-RAF (pink) and RAF (red)
 936 at the criteria of annotation in more than 20% of the samples. Box, interquartile range;
 937 line inside a box, median; circle, outlier.

938 **Figure 5. Abnormal metabolic patterns associated with recurrent AF.**

939 a. Venn diagram showing the amount of common differential metabolites in the
 940 non-RAF (pink) and RAF (red) groups when compared with the non-AF control
 941 (CTR). The overlap revealed 94 serum and 52 fecal metabolites simultaneously
 942 detected in the non-RAF and RAF groups, while 17 endogenous substances were
 943 simultaneously found in fecal and serum samples.

944 b, c. Heat-map of 17 serum (b) and fecal (c) shared metabolites. Abundance profiles
 945 underwent transformation into Z-scores via average abundance subtraction and
 946 division by the standard deviation. Negative (yellow) and positive (pink) Z-scores
 947 reflected row abundance levels lower and higher compared with the mean,
 948 respectively.

949 d. Heat-map depicting fold changes (AF/CTR) of 17 molecules with alterations in
 950 both serum and fecal specimens from AF cases. Fold changes underwent
 951 transformation into t -scores. Negative (blue) t -scores reflect compounds showing a

952 decreasing trend in the non-RAF or RAF groups. Substances increasing or decreasing
953 in both groups (n=8) or in a single group (n = 9) in fecal and serum specimens are
954 depicted in pink and green, respectively.

955 e, f. Relationship between eight simultaneously altered metabolites and the first 10
956 commonly detected genera (e) and species (f). Since the abundance levels of fecal
957 metabolites mirrored those of GM-produced substances, fecal metabolomics data
958 underwent Spearman's correlation analysis. Blue, negative correlation; yellow,
959 positive correlation, *p < 0.05, +p < 0.01.

960 g. Box plots of two fecal distinctive metabolites between the non-RAF (pink) and
961 RAF (red) groups. Box, interquartile range; line inside a box, median; circle, outlier.

962 h, i. Correlation between taxonomic (Tax) score and two taxa distinctive between the
963 non-RAF and RAF groups ($R^2=0.181$, $p=0.0023$ for 7-methylguanine; $R^2=0.1217$,
964 $p=0.014$ for palmitoleic acid. Pearson linear correlations).

965 **Figure 6. Taxonomic signature to predict recurrence following AF ablation.**

966 a. The tuning index (lamda) was selected in the LASSO model. Receiver operating
967 characteristic curve generation was carried out, and its AUC was plotted against log
968 (lamda). Dotted vertical lines depict the optimal values employing the minimum
969 criteria and 1 standard error of the minimum criteria (-SE criteria). A lamda of
970 0.1267904, with log (lamda) of -0.8969136 was selected (1-SE criteria) based on the
971 five-fold cross-validation method.

972 b. LASSO coefficients of 37 taxonomic features. After excluding highly correlated ($|r|$
973 ≥ 0.9) taxonomic features and linear combinations, 37 taxonomic features were
974 retained. Coefficients were plotted versus log (lamda). A vertical line is shown at the
975 value determined by five-fold cross-validation; optimal lamda yielded eight non-zero
976 coefficients.

c. The taxonomic (Tax) score was based on a linear combination of seven taxa-based markers, and calculated via weighting with their respective coefficients. Logistic regression analysis with the clinical CAAP-AF score and the developed Tax score was carried out using the enter method. A combined CAAP-AF-Tax score formula was constructed by weighting with the respective coefficients.

d. Box plots of seven distinctive taxa between the non-RAF (pink) and RAF (red) groups. Box, interquartile range; line inside a box, median; circle, outlier.

e. RAF is identifiable based on the Tax score or CAAP-AF score. Receiver operating curves for the CAAP-AF score, Tax score, and CAAP-AF-Tax score. The areas under the receiver operating curves (AUC values) were: CAAP-AF score, 0.6918 (95% confidence interval [CI]: 0.525–0.85, $p=0.04$); Tax score, 0.954 (95%CI: 0.8974–1.000, $p=0.0055$); CAAP-AF-Tax score, 0.9668 (95%CI: 0.9216–1.000, $p=0.0011$).

f. Prognostic information provided by the CAAP-AF-Tax score model. Patients were ranked according to increased CAAP-AF-Tax score, and maximum difference in overall survival was obtained with a CAAP-AF-Tax score = 0.633286, splitting patients into high- and low-risk groups.

g. Kaplan–Meier curves for overall survival prediction by the CAAP-AF-Tax score model. Cases were assigned to the high (red)- and low (green)-CAAP-AF-Tax score groups according to the corresponding cut-off CAAP-AF-Tax score value of 0.633286. There was a significant difference in overall survival between the high- and low-Tax score groups ($p < 0.0001$).

h. Nonogram for recurrence risk prediction upon catheter ablation based on the Tax score. In the nomogram, each Tax score has a corresponding score on the score scale.

1001 A vertical line drawn down the score scale corresponding to the Tax score allows the
1002 risk of recurrence in a given patient to be easily and accurately read.

1003 i. Calibration curves of the Tax nomogram. Plots show calibrations for various models
1004 in terms of agreement between predicted and actual outcomes. Model performance is
1005 depicted by the apparent plot, and bias correction denotes the corrected value of the
1006 deviation, versus the 45-degree line representing the ideal prediction.

1007 j. Decision curve analysis of the Tax score-nomogram. The y-axis reflects the net
1008 benefit, with the red line representing the Tax score-nomogram; the grey and black
1009 lines denote the hypothetical cases with all and no cases exhibiting AF recurrence,
1010 respectively. At a threshold probability (patient or doctor) $> 1\%$, employing the Tax
1011 score-nomogram for AF recurrence prediction shows elevated efficacy compared with
1012 the treat-all- or treat-none schemes. For instance, with an individualized threshold
1013 probability of 60% (a patient would be ineligible for therapy with a probability above
1014 60%), a net benefit of 0.3125 is achieved in deciding whether to perform catheter
1015 ablation therapy.

1016 **Supplementary material**

1017 **Additional files 1: Fig. S1, Effects of baseline features (age, T2DM, total** 1018 **cholesterol, and drug utilization) on GM shift**

1019 a. PCA evaluating age and microbial abundance at the genus level. A total of 90
1020 specimens were assigned to 3 groups based in age (< 55 years, yellow; 55-65, light
1021 pink; > 65 , violet). Squares represent non-AF CTR, triangles mean non-RAF and
1022 circles denote RAF.

1023 b. PCA evaluating type 2 diabetes mellitus and microbial abundance at the genus level.
1024 A total of 90 specimens were assigned to 2 groups based on previous T2DM (no

1025 T2DM, grey; T2DM, dark purple). Squares represent non-AF CTR, triangles mean
1026 non-RAF and circles denote RAF.

1027 c. PCA assessing TC and microbial abundance at the genus level. A total of 90
1028 specimens were assigned to 2 groups based on TC levels (TC < 5.18, green; TC ≥
1029 5.18, purple). Squares represent non-AF CTR, triangles mean non-RAF and circles
1030 denote RAF.

1031 d. PCA assessing drug and microbial abundance at the genus level. A total of 40 AF
1032 specimens were assigned to 11 groups based on used drugs: ACEIs, yellow triangles;
1033 ARBs, inverted green triangles; amiodarone, blue rhombi; statins, green asterisks;
1034 DMBG, red circles; an ACEI or ARB with amiodarone, brown hexagons; ACEI and
1035 statins, red hexagons; amiodarone and DMBG, dark red hexagons; ARB, amiodarone
1036 and statin, dark brown circles with forks; no medication, grey squares.

1037 **Additional files 2: Table S1, Relative abundance levels of annotated genera**

1038 **Additional files 3: Table S2, Relative abundance levels of annotated species**

1039 **Additional files 4: Fig. S2, Altered taxonomic profiles from non-RAF to RAF**

1040 a. Venn diagram depicting the count of annotated genera common to non-AF CTR
1041 (blue), non-RAF (pink) and RAF (red) cases. The overlap reveals 1219 genera
1042 simultaneously detected in non-AF CTR and AF with or without recurrence.

1043 b. Venn diagram depicting the count of annotated species common in non-AF CTR
1044 (blue), non-RAF (pink) and RAF (red) cases. The overlap reveals 5041 species
1045 simultaneously detected in non-AF CTR and AF with or without recurrence.

1046 c. Bar plots of relative abundance levels of the first 10 genera detected in the non-AF
1047 CTR (blue), non-RAF (pink) and RAF (red) groups. Distinct genera are depicted by
1048 different colors.

d. Bar plots of relative abundance levels of the first 10 species detected in the non-AF CTR (blue), non-RAF (pink) and RAF (red) groups. Distinct species are depicted by different colors.

e. Bar plots of relative abundance levels of the first 10 genera detected in non-AF CTR (blue), non-RAF (pink) and RAF (red) cases. Distinct genera are depicted by different colors.

f. Bar plots of relative abundance levels of the first 10 species detected in non-AF CTR (blue), non-RAF (pink) and RAF (red) cases. Distinct genera are depicted by different colors.

Additional files 5: Table S3, Differential genera and species

Additional files 6: Table S4, Differential KEGG modules

Additional files 7: Fig. S3, Serum and fecal metabolic patterns in the control, non-RAF and RAF groups

a, b. PLS-DA scores according to serum metabolic profiles in the non-RAF and RAF groups in ESI+ (a) and ESI- (b) modes. Pink triangles represent non-RAF and red circles denote RAF. A clear separation between non-RAF and RAF patients was obtained under both the ESI+ and ESI- modes.

c, d. OPLS-SA scores according to serum metabolic profiles in the non-RAF and RAF groups in the ESI+ (c) and ESI- (d) modes. Pink triangles and red circles represent non-RAF and RAF, respectively. The non-RAF and RAF groups were overtly separated under both the ESI+ and ESI- modes.

e, f. PLS-DA scores according to fecal metabolic profiles in the non-RAF and RAF groups in the ESI+ (e) and ESI- (f) modes. Pink triangles and red circles represent

1072 non-RAF and RAF, respectively. The non-RAF and RAF groups were overtly
1073 separated under both the ESI+ and ESI- modes.

1074 g, h. Orthogonal PLS-DA (OPLS-SA) scores according to fecal metabolic profiles in
1075 the non-RAF and RAF groups in the ESI+ (e) and ESI- (f) modes. Pink triangles and
1076 red circles represent non-RAF and RAF, respectively. The non-RAF and RAF groups
1077 were overtly separated under both the ESI+ and ESI- modes.

1078 **Additional files 8: Table S5, Data of 8 metabolites with differential enrichment**
1079 **across groups**

1080 **Additional files 9: Table S6, Taxonomic scores and CAAP-AF-Tax scores from**
1081 **non-RAF to RAF**

1082 **Additional files 10: Table S7, Univariate and multivariable Cox regression**
1083 **analyses of factors potentially predicting AF recurrence**

a

b

198 non-RAF & RAF common genera

c

d

e

1077 non-RAF & RAF common species

f

a

b

c

d

e

f

g

h

7-Methylguanine	p value	VIP	Fold change
non-RAF vs. CTR	0.0002	1.18175	5.1409
RAF vs. CTR	0.0008	1.6870	3.8956
non-RAF vs. RAF	0.0391	2.1251	-1.2453

palmitoleic acid	p value	VIP	Fold change
non-RAF vs. CTR	not significant		
RAF vs. CTR	0.0196	1.2319	-1.7298
non-RAF vs. RAF	0.0395	2.1210	-1.7680