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Gut microbiota and plasma amino acids profile connections in patients with coronary artery disease and atrial fibrillation

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Abstract: *The aim of our work to check the connections between gut microbiota composition and plasma amino acids profile in patients with coronary artery disease (CAD) and atrial fibrillation (AF). Materials and methods: 300 patients were divided into 3 groups: first (CAD) – 149 patients with CAD but without arrhythmias, second (CAD+AF) – 124 patients with CAD and AF paroxysm and control group – 27 patients without CAD and arrhythmias. Plasma AA level was detected by ion exchange liquid column chromatography method. 16-S rRNA sequencing checked gut microbiota composition. Results: The II group patients are characterized by a significant increase in Actinobacter Spp. and a decrease in Blautia Spp., Bacteroides Thetaiotaomicron;a significant increase in glutamate, branched-chain amino acids, Fishers' ratio, and a decrease in glycine levels (P<0.05). Pathogenic species have such significant correlations: Streptococcus Spp. with tyrosine (r=0.301), phenylalanine (r=0.340), aromatic amino acids (r=0.345), Fishers' ratio (r=0.319); Escherichiacoli with arginine (r=-0.331), serine (r=-0.337), valine (r=-0.332);(P<0.05). Probiotic species have such significant correlations: Bifidobacterium Spp. with glycine (r=0.505), Fishers' ratio (r=-0.371); Blautia Spp. with ornithine (r=0.329), glycine (r=0.414), alanine (r=0.316); Eubacteriumrectale. with ornithine (r=0.379), glycine (r=0.467), alanine (r=0.384), Fishers' ratio (r=0.418);(P<0.05).Conclusions: Plasma amino acids and gut microbiota are closely connected.*

Keywords– *coronary artery disease, atrial fibrillation, amino acids, gut microbiota composition, gut microbiota metabolites.*

I. INTRODUCTION:

Atrial fibrillation (AF) is the most common arrhythmia in the world. Coronary artery disease (CAD) is an important risk factor in AF development. Near the half of all AF paroxysm occurred in patients with CAD. Moreover, CAD and AF have the majority of mutual risk factors, such as obesity, dyslipidemia, arterial hypertension, chronic inflammation, atherosclerosis, diabetes mellitus, heart failure, etc. [1, 2], which are closely linked with gut microbiota composition [3] and amino acids (AA) exchange [4, 5, 6]. Nowadays the pathological mechanism of AF paroxysm formation is still unclear [1], so the search of new AF risk factors and their correction methods is an important up-to-date scientific problem.

Undoubtedly, that gut microbiota condition plays an important role in human health. It influences at intestinal barrier permeability and synthetized the variety of metabolites. According to the several data, one of

the gut microbiota metabolites are plasma AA, especially branched-chain AA (BCAAs) and aromatic AA (AAAs) [7, 8]. In turn, AAAs increase endothelial dysfunction, platelets responsiveness and thrombosis potential. Their metabolism is closely linked with anaerobic, pathogenic bacteria, such as *Clostridium spp.*, etc. and generated proatherogenic metabolites: phenylacetylglutamine, indoxyl sulfate and p-cresol sulfate[7].According to the experimental studies, BCAAs exchange is depend on *Bifidobacterium, Akkermansia, Eubacterium, Prevotella* speciesmetabolism. BCAAs catabolism is essential for lipids and carbohydrates exchange. They take part in thermogenesis regulation, visceral fat tissue production, increase insulin resistance, directly connected with obesity and diabetes mellitus. On the other hand, BCAAs are crucial for hepatocyte proliferation, protein synthesis and immunity [8]. So, to check the connection between plasma amino acids profile and gut microbiota composition is an important pathogenetic question for deeper understanding mechanism of development cardiovascular disorders.

II.   THE AIM:

To check the connections between gut microbiota composition and plasma amino acids profile in patients with coronary artery disease and atrial fibrillation.

III.   MATERIALS AND METHODS:

300 patients were divided into 3 groups: first (CAD) – 149 patients with CAD but without arrhythmias, second (CAD+AF) – 124 patients with CAD and AF paroxysm and control group (CG) – 27 patients without CAD and arrhythmias. CAD and AF diagnosis were based on the latest ESC guidelines [1, 2]. All patients were treated in the Kiev City Clinical Hospital No. 12, cardiological and therapeutic departments during 2018-2023 years. AF paroxysm was checked by resting 12 leads electrocardiography. Diagnosis CAD was confirmed by history of coronary arteries stenotic changes during invasive coronarography. Exclusion criteria were: reported malignancies, chronic kidney disease (Glomerular Filtration Rate, GFR <60 mL/min), valvular AF, heart failure Class III to IV (by New York Heart Association), thyroid pathology, inflammatory bowel disease, irritable bowel syndrome,vegetarians and vegans, pregnancy, taking probiotics and antibiotics for a month before the study.No significant difference in risk factors at baseline were seen between investigated groups. The study was conducted at the base and was approved by the ethical commission of the Kyiv City Clinical Hospital No. 12 (protocol # 8 from 22/08/2018). Informed consent was obtained from all subjects in accordance with the Declaration of Helsinki.

Baseline characteristics of study patients includes age, body mass index (BMI), uric acid, total bilirubin, GFR, total cholesterol (TC) levels. Uric acid, total bilirubin, creatinine and TC were checked by Kyiv City Clinical Hospital No. 12 laboratory (certificate # ПТ – 257/21). GFR was calculated by CKD-EPI creatinine equation. Advanced age, obesity, hypercholesterolemia, high stages of chronic kidney disease, gout and hyperbilirubinemia are known risk factors of AF paroxysm development [1]. That's why this baseline characteristics were analyzed and compared, because it can help us to exclude their influence on obtained results.

Plasma AA profile was checked by ion exchange liquid column chromatography method – such AA were identified: lysine, histidine, arginine, ornithine, taurine, asparagine acid, threonine, serine, glutamine acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, glutamine. Automatic amino acids analyzer T 339 (Mikrotehna, Czech Republic) were used in our study. Study was done in Bogomolets National Medical University, Kyiv, Ukraine. Blood sampling from patients was performed on an empty stomach from the cubital vein on the first day of hospitalization, before treatment.

Determination of the gut microbiota composition was carried out using quantitative PCR qRT-PCR using primers for the 16S rRNA gene and taxon-specific primers. Such domains were checked: bacteria – Firmicutes (Lactobacillus spp., Faecalibacterium prausnitzii, Enterococcus spp., Blautia spp., Streptococcus spp., Eubacterium rectale, Roseburia inulinivorans, Ruminococcus spp.), Bacteroides (Bacteroides spp.,

Bacteroides thetaiotaomicron, Prevotella spp.), Actinomycetota (Bifidobacterium spp.), Verrucomicrobiota (Akkermansia muciniphila), Pseudomonadota (Escherichia coli, Acinetobacter spp.) and Archaea (Methanobrevibacter smithii and Methanosphaera stadmanae). Also, Firmicutes/Bacteroides (F/B) ratio was compared [3].

Results were presented as mean ± standard error or [95% confidence interval (CI)] for continuous variables or as a number for categorical variables. Data were compared using Wilcoxon signed-rank test or Student t-test with two critical regions by the type of distribution; Spearman's rank correlation coefficient [9]. All calculations were done in MATLAB R2014a (License number 271828).

IV.   RESULTS:

At first, we compared baseline characteristics of investigated groups. All investigated groups were similar by age, BMI and total bilirubin values. I and II groups patients were characterized by increase of TC (by 32.64% and 43.06% respectively), uric acid (by 22.66% and 30.53% respectively) and decrease of GFR (by 26.16% and 19.38% respectively) in comparison with CG. Data are shown in table 1.

Table 1. Baseline characteristics of study sample, mean ± standard error

Characteristic /group	I group	II group	CG	P1-2	P2-CG	P1-CG
Age (years)	67.71±3.90	67.96±0.94	56.25±2.18	P>0.05	P>0.05	P>0.05
BMI (kg/m^2)	27,02±0,33	26,93±0,43	28,12±2,10	P>0,05	P>0,05	P>0,05
Total bilirubin (mmol/l)	11,3±0,09	12,4±0,08	11,7±0,11	P>0,05	P>0,05	P>0,05
Uric acid (mmol/l)	380,5±28,16	404,9±36,11	310,2±29,12	P>0,05	P<0,05	P<0,05
GFR (ml/min)	62,03±2,31	67,73±1,98	84,01±5,48	P>0,05	P<0,05	P<0,05
TC (mmol/l)	5,73±0,37	6,18±0,31	4,32±0,21	P>0,05	P<0,05	P<0,05

Secondary, the plasma AA spectrum was analyzed. In the I group patients a significant decrease in glycine, valine, and alanine levels was found in comparison with the CG (P<0,05). In the II group patients a significant increase in glutamate, and a significant decrease in glycine, valine, alanine, serine, and glutamine levels were checked in comparison with the CG (P<0,05). In the II group, a significant rise in glutamate and a significant depletion in glycine levels were detected in comparison with the I group (P<0,05).The data are shown in Fig. 1.
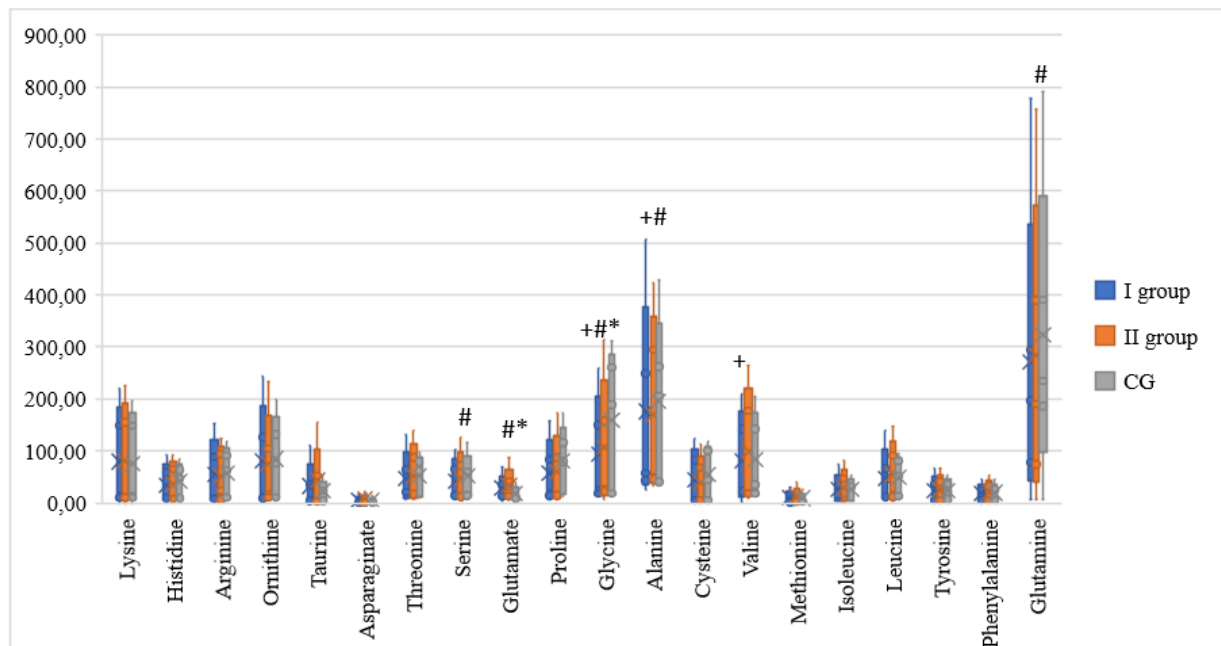
Figure1. Plasma amino acids spectrum in investigated groups, mμmol/l.
 Notes: +-P<0.05 I group – CG; #-P<0.05 II group – CG; *-P<0.05 I-II groups.

Also, we combined plasma AA according to their biochemical properties and exchange and compared these results in investigated groups. In the II group, a significant rise in BCAA and Fishers' ratio was found in comparison with the I group (P<0,05). In the II group, a significant rise in BCAA and Fishers' ratio and a decrease in Glycine + Serine sum was found in comparison with the CG (P<0,05). In the I group, a significant rise in Fishers' ratio and a decrease in Glycine + Serine sum was checked in comparison with the CG (P<0,05). The data are shown in Fig. 2.
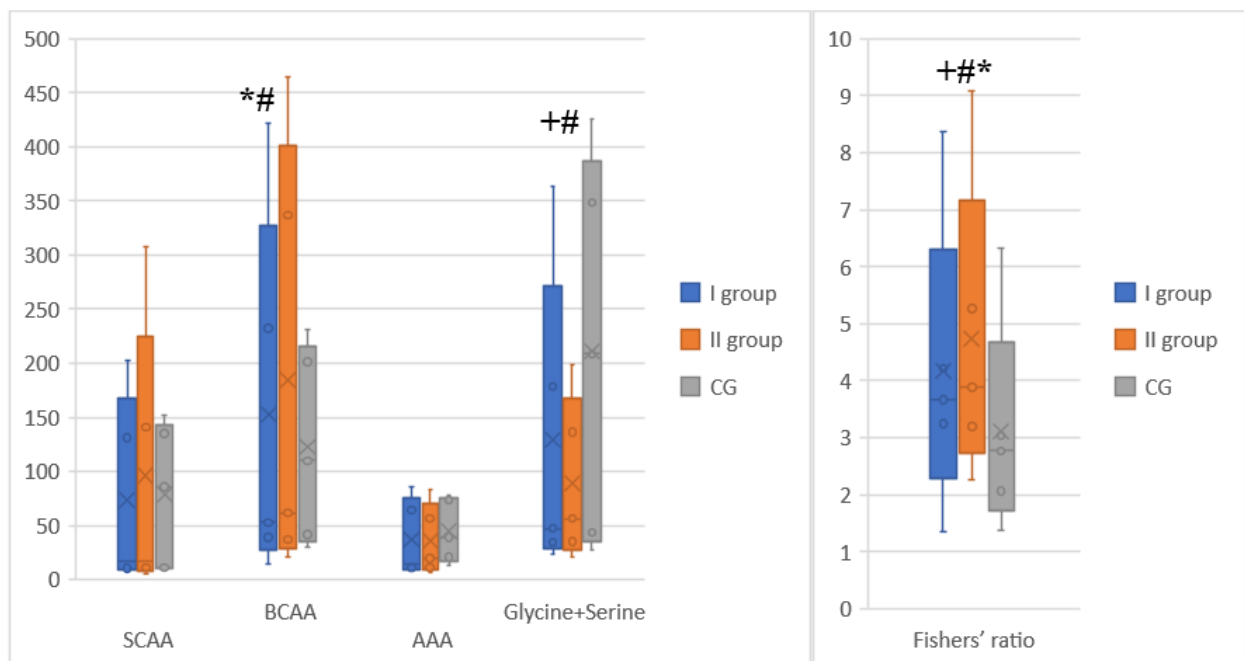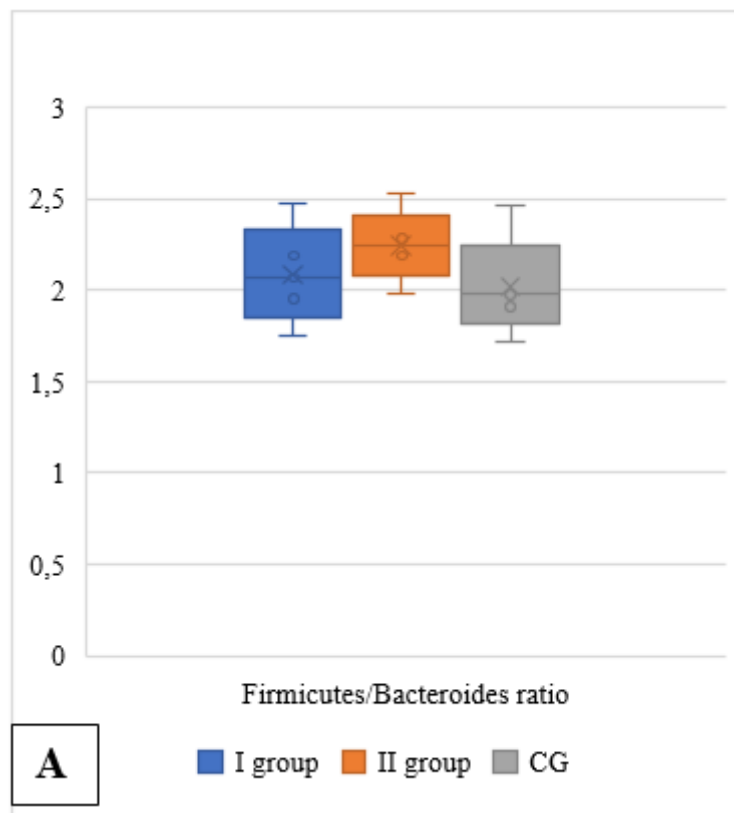

Figure2. Plasma amino acid combinations in investigated groups, mμmol/l.

Notes: +-P<0.05 I group – CG; #-P<0.05 II group – CG; *-P<0.05 I-II groups.

Gut microbiota composition was estimate in investigated groups. The F/B ratio was not significantly different in the investigated groups ($P>0.05$). By the taxonomic analysis in the I and II groups was a significant increase in *Pseudomonadota* and a decrease in *Actinomycetota* and *Verrucomicrobiota* compared with CG; in the II group compared with the I group was a significant rise in*Pseudomonadota* ($P<0.05$). By the species analysis in the I and II groups comparing with CG was a significant rise in *Bacteroides Spp., FaecalibacteriumPrausnitzii, Actinobacter Spp., Streptococcus Spp.* and adecrease in *Lactobacillus Spp., Bifidobacterium Spp., AkkermansiaMuciniphila, EubacteriumRectale;* in the I group in comparison with CG was a significant rise in *Ruminococcus Spp.;* in the II group in comparison with CG was a significant decrease in *RoseburiaInulinivorans;* in the II group in comparison with I group was a significant rise in *Actinobacter Spp.* and a decrease in *Blautia Spp., Bacteroides Thetaiotaomicron* ($P<0.05$). The data are shown in Fig. 3.
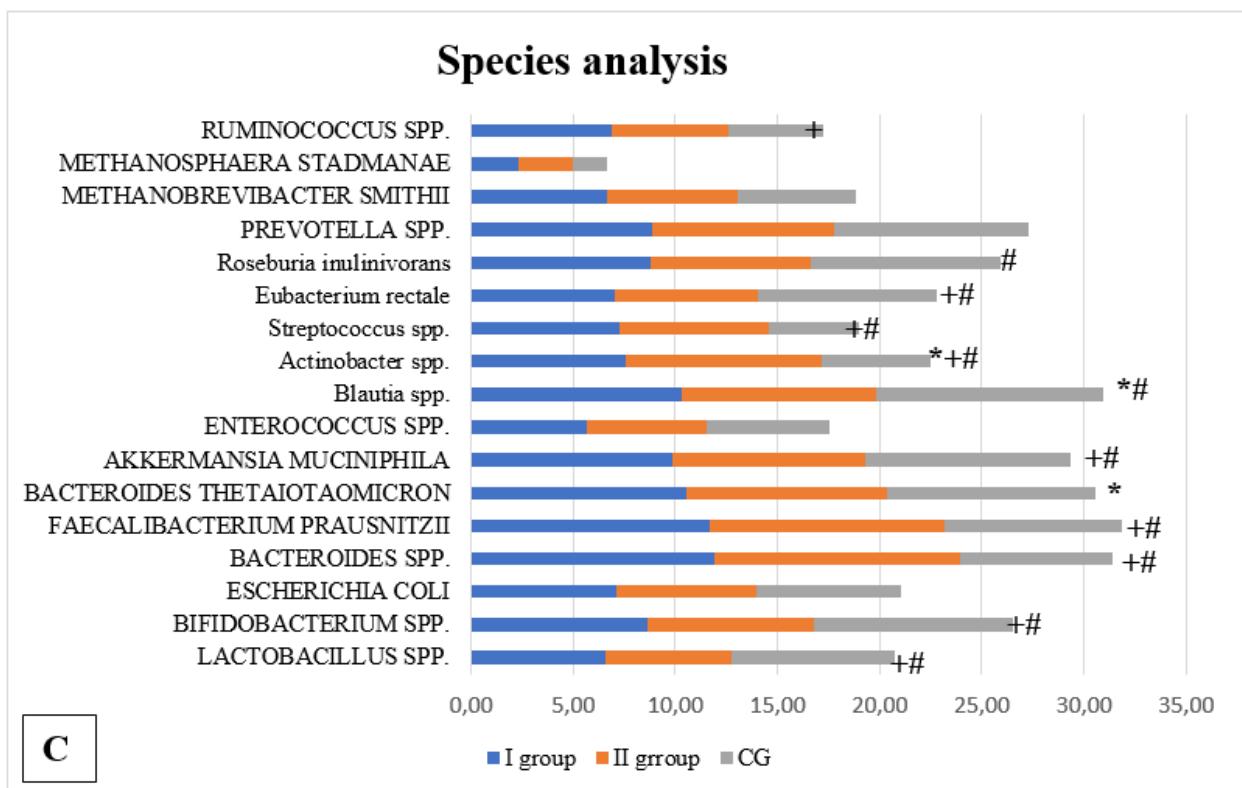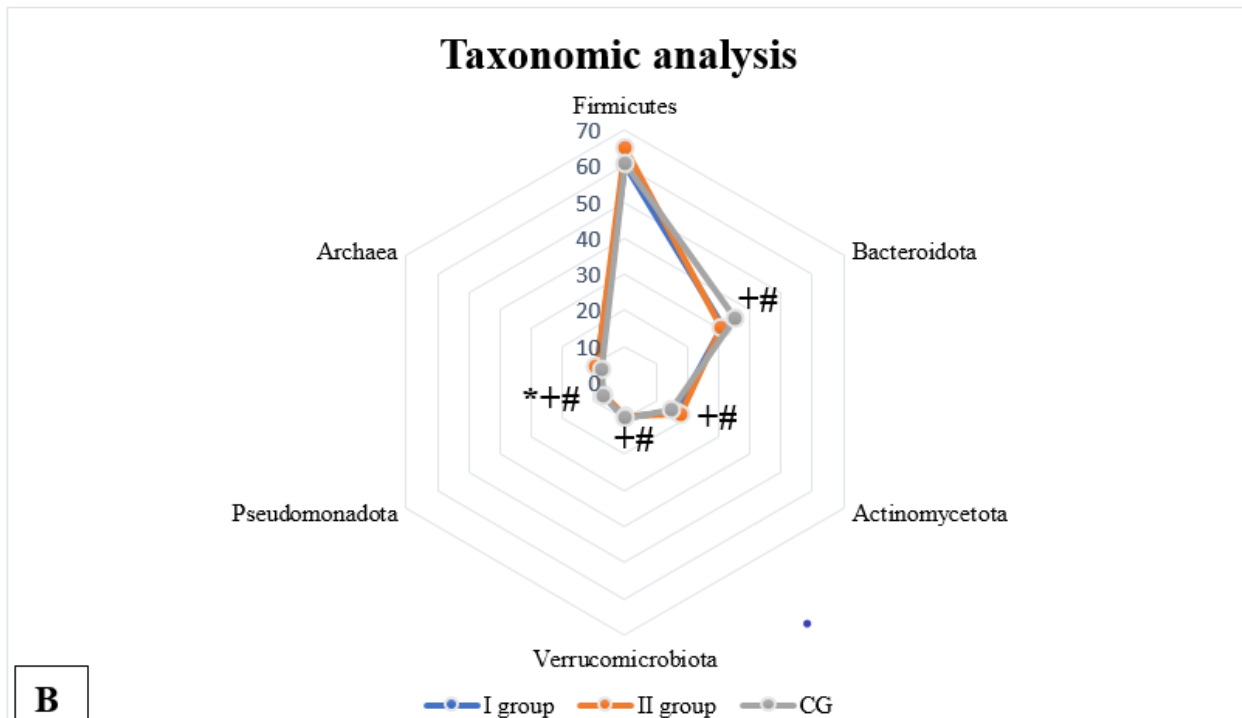
Figure 3. Gut microbiota composition in investigated groups: *a* – F/B ratio; *b* – taxonomic analysis, mean [95% CI], lg/CFU/ml; *c* – species analysis, mean [95% CI], lg/CFU/ml; *-P<0.05 I-II groups; +-P<0.05 I group – CG; #-P<0.05 II group – CG.

The correlation analysis between the gut microbiota composition and plasma amino acids profile was performedin the examined groups. The largest amount of correlations was between plasma amino acids profile and such phyla as *Bacteroidota* (total number = 6), andF/B ratio (total number = 5). The largest amount of correlations was between gut microbiota composition and such AA as glycine (total number = 3) and Fishers' ratio (total number = 3). Data are shown in table 2.

Table 2. Correlation matrices between plasma amino acids profile and gut microbiota composition

Plasma AA/ Gut microbiota species	Lysine	Histidine	Arginine	Ornithine	Taurine	Asparagine acid	Threonine	Serine	Glutaminic acid	Proline	Glycine	Alanine	Cysteine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Glutamine	SCAA	AAA	BCAA	Fishers ratio	Glycine+Serine
Firmicutes	0,083	-0,061	-0,010	0,128	-0,014	-0,063	-0,129	-0,102	-0,144	0,057	0,182	0,342*	0,03	-0,171	-0,332*	-0,023	-0,166	0,072	-0,263	0,086	0,032	-0,044	-0,149	-0,122	-0,127
Bacteroidota	-0,244	-0,238	-0,331*	-0,197	-0,340*	-0,220	-0,428*	-0,443*	-0,115	-0,355*	-0,278	-0,138	-0,278	-0,219	-0,473*	-0,214	-0,282	-0,210	-0,295	-0,204	-0,261	-0,283	-0,209	0,085	-0,165
Actinomycetota	0,066	0,020	0,143	0,094	-0,034	-0,041	0,058	0,061	-0,293	0,142	0,505*	0,202	-0,012	-0,085	-0,023	0,029	0,092	0,201	0,039	0,297	-0,056	0,193	-0,098	-0,371*	-0,200
Verrucomicrobiota	0,266	0,192	0,201	0,206	0,168	0,133	0,194	0,045	0,052	0,180	0,030	0,004	0,178	-0,064	0,040	0,116	0,083	0,213	0,079	0,029	0,179	0,300	0,002	-0,328*	0,104
Pseudomonadota	-0,194	-0,050	-0,216	-0,154	-0,013	-0,032	-0,116	-0,118	0,287	-0,287	-0,356*	-0,194	-0,138	0,070	0,052	-0,121	-0,099	-0,302	0,018	-0,106	-0,062	-0,245	0,054	0,248	0,183
Archaea	0,065	0,054	-0,022	0,026	0,054	0,176	-0,024	0,001	0,175	-0,147	-0,123	-0,021	-0,083	-0,001	0,063	-0,042	-0,158	0,120	-0,061	-0,010	0,021	0,021	-0,023	-0,055	0,151
F/B ratio	0,285	0,270	0,234	0,327*	0,244	0,179	0,156	0,175	-0,137	0,359*	0,581*	0,423*	0,233	0,008	0,097	0,206	0,119	0,245	0,130	0,259	0,211	0,299	0,029	-0,328*	-0,061

Notes: *- correlations with moderate or strong force (r>0.3 or r<-0.3), P<0.05

The correlation analysis between the gut microbiota composition and plasma amino acids profile was performedin the examined groups. The largest amount of correlations was between plasma amino acids profile and such species as *BlautiaSpp* (total number = 4), *Streptococcus Spp* (total number = 4), and *Eubacterium Rectale* (total number = 4).The largest amount of correlations was between gut microbiota composition and such AA as glycine (total number = 7)and Fishers' ratio (total number = 6). Data are shown in table 3.

Table 3. Correlation matrices between plasma amino acids profile and gut microbiota composition

Plasma AA/ Gut microbiota species	Lysine	Histidine	Arginine	Ornithine	Taurine	Asparagine acid	Threonine	Serine	Glutaminic acid	Proline	Glycine	Alanine	Cysteine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Glutamine	SCAA	AAA	BCAA	Fishers ratio	Glycine+Serin
Lactobacillusspp.	-0,064	-0,186	-0,143	-0,049	-0,283	0,088	-0,168	-0,200	-0,250	-0,091	0,212	0,042	-0,211	-0,357	-0,275	-0,194	-0,221	0,093	-0,252	-0,024	-0,259	0,005	-0,265	-0,511*	-0,147
Bifidobacteriumspp.	0,066	0,020	0,143	0,094	-0,034	-0,041	0,058	0,061	-0,293	0,142	0,505*	0,202	-0,012	-0,085	-0,023	0,029	0,092	0,201	0,039	0,297	-0,056	0,193	-0,098	-0,371*	-0,200
Escherichiacoli	-0,157	-0,176	-0,331*	-0,193	-0,217	-0,196	-0,187	-0,337*	-0,109	-0,221	-0,143	-0,217	-0,221	-0,332*	-0,120	-0,180	-0,242	-0,199	-0,215	-0,221	-0,232	-0,173	-0,287	-0,218	-0,105
Bacteroidesspp.	-0,117	-0,122	-0,192	-0,238	-0,076	-0,186	-0,076	-0,226	0,066	-0,162	-0,392*	-0,225	-0,017	-0,043	-0,136	-0,107	-0,232	-0,225	-0,143	-0,144	0,010	-0,233	-0,048	0,278	-0,020
Faecalibacteriumprausnitzii	-0,194	-0,236	-0,199	-0,133	-0,205	-0,176	-0,336*	-0,237	0,209	-0,249	-0,199	-0,096	-0,110	-0,108	-0,275	-0,203	-0,227	-0,224	-0,246	-0,281	-0,173	-0,238	-0,102	0,211	0,115
Bacteroidesthetaiotaomicron	-0,183	-0,239	-0,291	-0,249	-0,304	-0,334*	-0,220	-0,260	-0,294	-0,213	-0,143	-0,129	-0,298	-0,258	-0,361*	-0,188	-0,177	-0,163	-0,222	-0,18	-0,366*	-0,229	-0,245	-0,107	-0,270
Akkermansiamuciniphila	0,266	0,192	0,201	0,206	0,168	0,133	0,194	0,045	0,052	0,180	0,030	0,004	0,178	-0,064	0,040	0,116	0,083	0,213	0,079	0,029	0,179	0,300	0,002	-0,328*	0,104
Enterococcusspp.	0,005	-0,028	-0,094	0,136	0,138	-0,079	-0,068	-0,311*	-0,069	-0,068	-0,320*	-0,057	0,115	-0,121	-0,065	-0,019	-0,092	0,031	0,016	0,154	0,144	0,110	-0,090	-0,174	-0,084
Blautiaspp.	0,249	0,234	0,265	0,329*	0,145	-0,022	0,086	0,125	-0,292	0,298	0,414*	0,316*	0,219	0,020	-0,041	0,159	0,077	0,220	0,002	0,131	0,196	0,250	0,022	-0,142	-0,334*
Actinobacterspp.	-0,106	0,043	-0,054	-0,065	0,093	0,043	-0,036	0,010	0,335*	-0,198	-0,372*	-0,142	-0,027	0,165	0,123	-0,057	-0,014	-0,205	0,120	-0,014	0,060	-0,135	0,142	0,265	0,228
Streptococcusspp.	0,055	-0,068	-0,072	0,027	0,028	0,003	-0,163	-0,165	0,188	-0,035	-0,130	-0,052	0,016	0,020	-0,219	0,058	-0,078	0,301*	0,340*	-0,112	0,035	0,345*	0,057	0,319*	0,120

Eubacteriumrectale	0,169	-0,019	0,102	0,379*	0,035	0,090	-0,030	-0,015	-0,161	0,205	0,467*	0,384*	0,028	-0,250	-0,094	0,081	-0,022	0,223	-0,100	0,227	0,064	0,164	-0,186	-0,418*	-0,086
Roseburiainulinivorans	0,078	-0,091	0,069	0,223	-0,030	-0,008	-0,033	0,021	-0,232	0,176	0,318*	0,271	0,067	-0,153	-0,173	0,066	0,010	0,162	-0,116	0,157	0,021	0,095	-0,117	-0,173	-0,172
Prevotellaspp.	-0,093	-0,244	-0,091	0,047	-0,127	0,036	-0,218	-0,144	-0,099	-0,041	0,212	0,178	-0,219	-0,278	-0,241	-0,094	-0,093	-0,059	-0,340*	-0,086	-0,166	-0,094	-0,333*	-0,376*	-0,043
Methanobrevibactersmithii	-0,055	-0,102	-0,120	-0,222	-0,245	0,107	-0,188	-0,076	0,048	-0,192	0,019	-0,080	-0,241	-0,139	-0,069	-0,136	-0,157	-0,073	-0,256	-0,170	-0,206	-0,188	-0,140	-0,078	0,041
Methanosphaerastadmanae	0,138	0,134	0,034	0,231	0,168	0,229	0,101	0,080	0,257	-0,052	-0,143	0,077	0,057	0,102	0,094	0,014	-0,094	0,325*	0,047	0,021	0,131	0,137	0,061	0,010	0,244
Ruminococcusspp.	0,159	0,082	0,042	0,163	0,098	-0,058	0,031	-0,037	-0,126	0,091	0,252	0,323*	0,146	0,092	-0,232	0,020	-0,048	0,105	-0,067	0,321*	0,127	-0,007	0,044	0,075	-0,127

Notes: *- correlations with moderate or strong force ($r > 0.3$ or $r < -0.3$), $P < 0.05$

V.   DISCUSSION:

AA are crucial for intestinal integrity and rebuilding the microvilli of the gut's epithelial cells, restoring gut homeostasis. Threonine, serine, and glycine are important for gut mucosa production [10]. Also, glycine and taurine take part in the bile acids exchange, which are known gut microbiota metabolites. Glycine have antiatherogenic properties by supporting cholesterol incorporation in the cellular wall that reduced cholesterol level [11]. In the animal experiment, glycine supplementation has strong anti-inflammatory effect and directly connect with rise of Lactobacillus and Bifidobacterium species [12].AAAs exchange violations are associated with dyslipidemia, atherosclerosis, diabetes mellitus, liver pathology, and chronic inflammation [13]. According to the experimental data, *Streptococcus Spp.*can regulate biosynthesis and transport of AAAs [14].

Lactobacillus and *Bifidobacterium Spp.* are the known probiotics, which characterized by strong anti-inflammatory properties, takes part in normalization carbohydrates and lipids exchange, normalize intestinal barrier permeability. In the same time, the new promising probiotics strains are *Akkermansiamuciniphila, Blautiaspp., Eubacteriumrectale, Roseburiainulinivorans,* and *Faecalibacteriumprausnitzii.*They are takes part in the host metabolomic and have anti-inflammatory properties [15].

So, gut microbiota quotative and qualitative disturbances are closely associated with plasma amino acids profile violations.

VI.   CONCLUSIONS:

Patients with coronary artery disease and atrial fibrillation are characterized by plasma amino acids and gut microbiota composition changes:

1. A significant increasein *Actinobacter Spp.* and a decrease in *Blautia Spp., Bacteroides Thetaiotaomicron*in patients with coronary artery disease and atrial fibrillation was determined in comparison with patients with coronary artery disease and without atrial fibrillation (P<0.05);

2. A significant increase in glutamate, branched-chain amino acids, Fishers' ratio, and a decrease in glycine levels in patients with coronary artery disease and atrial fibrillation was determined in comparison with patients with coronary artery disease and without atrial fibrillation (P<0.05);

3. The highest amount of significant correlations between gut microbiota indexes and plasma glycine and Fishers' ratio was found (P<0.05);

4. Pathogenic species have such significant correlations:*Streptococcus Spp.*with tyrosine (r=0.301), phenylalanine (r=0.340), aromatic amino acids (r=0.345), Fishers' ratio (r=0.319); *Escherichiacoli*with arginine (r=-0.331), serine (r=-0.337), valine (r=-0.332); *Enterococcusspp.* with serine (r=-0.311), glycine (r=-0.320) (P<0.05);

5. Probiotic species have such significant correlations: *Bifidobacterium Spp.* with glycine (r=0.505), Fishers' ratio (r=-0.371);*Blautia Spp.* with ornithine (r=0.329), glycine (r=0.414), alanine (r=0.316);*Eubacteriumrectale.* with ornithine (r=0.379), glycine (r=0.467), alanine (r=0.384), Fishers' ratio (r=0.418)(P<0.05).

Perspectives of subsequent scientific research: Nowadays several amino acids can be used as postbiotics for gut microbiota compositioncorrection.

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