Salivary microbiota associated with Immunoglobulin A nephropathy (IgAN)

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Introduction and objectives

The human oral cavity contains ca. 700 bacterial species, which reach numbers of 1011/g of wet weight dental plaque and 108-109 CFU/g of saliva. Oral bacteria have a pivotal role on the genesis of oral human diseases, mainly dental caries and periodontitis. Non-oral diseases such as chronic kidney disease (CKD) are also somewhat related to the dysbiosis of oral bacteria. Immunoglobulin A nephropathy (IgAN) is one of the most common forms of primary glomerular disease. Among structural IgA abnormalities, hyperproduction of poorly galactosylated IgA1 is thought to play a role in the pathogenesis of primary IgAN. The downstream effector mechanisms triggered by mesangial IgA1 deposition and its etiology are poorly understood. Recently, a probable role of the enteric microbiota in educating the immune system and disease development was shown. Aim of our study was to compare the composition of the salivary microbiota between twenty eight IgAN pts and fourteen Healthy Controls (HC). The total salivary microbiota was characterized through an integrated approach of culture-dependent and -independent methods.

Materials and Methods

Two groups of caucasian volunteers aged between 35 and 50 were enrolled in the study: (i) twenty eight IgAN (11 female and 17 male) patients (subjects numbered: 1 - 28 IgAN) and (ii) fourteen healthy control (HC) (6 female and 8 male) subjects, without known diseases (subjects numbered: 1 - 14 HC) (able 1. Saliva specimens were collected from each volunteer and employed for:

- Enumeration of cultivable bacteria
- DNA extraction from fecal samples
- Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) and data analyses
- Taxonomic identification

Results

Enumeration of salivary cultivable bacteria

No significant (P>0.05) differences were found between IgAN and HC for the main microbial groups. The only exception was found for Bifidobacterium that was found at the lowest level (P<0.05) in the salivary samples of IgAN patients **(Fig. 1)**.

Richness and Diversity of the Salivary Microbiota Based on 16S rRNA Gene Sequencing Data Analysis

Pyrosequencing analysis yielded an average of 4881 bacterial reads sequences (average length 529.0 bp) per sample. A highest level of microbial diversity was found in the saliva samples of HC, with a median number of estimated OTUs of 362 versus 292 of IgAN samples **(Fig. 2)**. Overall, ten phyla (Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria, Spirochaetes, Tenericutes, Deferribacteres, Synergistetes, and Aquificae) and one candidate division (TM7) were identified **(Fig. 3)**. Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria represented more than 98 % of all 16S rRNA sequences. No significant (P>0.05) differences were found for Firmicutes between IgAN and HC (median value 39.28 and 44.68 %, respectively). Compared to HC, Bacteroidetes (P=0.048) decreased in IgAN patients (median value 17.01 and 25.30 % for IgAN and HC, respectively). An opposite trend (P=0.044) was found for Proteobacteria, which showed the lowest value in HC (28.03 and 16.49 % for IgAN and HC, respectively). No significant (P>0.05) differences were found for the other bacterial phyla. The difference for the community structure was further analyzed, using three phylogeny-based beta-diversity measures. The salivary microbiota of HC and IgAN were differentiated based on the bacterial principal coordinate analysis, with unweighted Unifrac distance matrix **(Fig. 4)**.

Distinctive Salivary Microbiome Associated with IgAN

The differences (P<0.05) for the relative abundance of OTU, which were associated with the salivary samples of IgAN or HC subjects are shown in **Table 2**.

Conclusion

The salivary microbiota of IgAN patients differed from that of HC. Nevertheless, such differences could be related/involved in the IgAN pathogenesis or could be the consequence of the IgAN therapy. The limitation of this study was to not having assessed the patients at the time of renal biopsy, but with a cross-sectional approach. Further studies, using a higher number of IgAN patients and including subjects with other glomerular diseases, could provide further insights to discover noninvasive salivary biomarkers specific to IgAN, and/or therapy and dietary interventions.

Basic characteristics	HC	IgAN
Age (years)	40±9	39±10
Male (%)	57	67
Serum creatinine (mg/dl)	0.87±0.25[b]	2.28±1.04[a]
Proteinuria (g/day)	0.06±0.03[b]	1.53±0.86[a]
MDRD GFR (ml/min/1.73 m²)	96±5[a]	38±19[b]
Body mass index (kg/m²)	24±4	26±2
Frequency of pathologic features (percentages) in 28 biopsies scored according to Oxford Classification (MEST)		
Mesangial hypercellularity (M)	M0=48	M1=52
Endocapillary proliferation (E)	E0=78	E1=22
Segmental glomerulosclerosis (S)	S0=20	S1=80
Tubular atrophy/interstitial fibrosis (T)	T0=48	T1=34
		T2=17
Therapy		
	HC	IgAN
ACE inhibitors (%)	0[b]	100[a]
Dietary habits		

a, b Values within a row with different superscript letters are significantly different (P < 0.05)

Table 1 Basic characteristics of studied HC and IgAN pts

Fig. 1 Cultivable cells (log CFU/g) found in the salivary samples IgAN pts HC. Data are the means of three independent experiments (n=3). Group Student's t test p values were shown.

Phylum	Family	Avg (%) HC	Avg (%) IgAN (R)	Avg (%) IgAN (P)	P value HCR	P value HCP
Firmicutes	Bacillales (family)	1.569	2.182	0.830	0.391	0.056
	Carnobacteriaceae	2.304	1.729	1.251	0.267	0.020
	Clostridiales (family)	0.129	0.036	0.032	0.060	0.049
	Lachnospiraceae	0.561	0.246	0.294	0.033	0.075
	Peptostreptococcaceae	0.440	0.146	0.236	0.005	0.129
Proteobacteria	Pasteurellaceae	4.791	9.293	5.135	0.018	0.781
Fusobacteria	Fusobacteriaceae	6.001	5.375	3.580	0.716	0.048
Phylum/family	Genus					
Firmicutes/Bacillales (family)	Gemella	1.568	2.182	0.830	0.391	0.035
Firmicutes/Carnobacteriaceae	Granulicatella	2.304	1.728	1.251	0.266	0.019
Firmicutes/Peptostreptococcaceae	Filifactor	0.164	0.027	0.032	0.002	0.003
	Peptostreptococcus	0.275	0.419	0.203	0.026	0.511
Proteobacteria/Neisseriaceae	Neisseria	20.576	20.779	12.060	0.046	0.049
Proteobacteria/Pasteurellaceae	Haemophilus	3.530	6.929	4.330	0.015	0.464
	Terrahaemophilus	0.807	1.782	0.136	0.176	0.158
Fusobacteria/Fusobacteriaceae	Fusobacterium	6.00	5.375	3.579	0.715	0.059
Phylum/family	Species					
Firmicutes/Bacillales (family)	Gemella haemolysans	0.525	0.251	0.110	0.124	0.017
Firmicutes/Carnobacteriaceae	Granulicatella adiacens	0.893	0.663	0.524	0.234	0.018
Firmicutes/Peptostreptococcaceae	Filifactor sp.	0.165	0.027	0.032	0.003	0.004
Firmicutes/Veillonellaceae	Veillonella parvula	0.374	0.065	0.046	0.057	0.042
Bacteroidetes/Prevotellaceae	Prevotella aurantiaca	0.003	0.887	0.160	0.009	0.070
	Prevotella intermedia	1.039	0.170	0.589	0.049	0.371
	Prevotella nigrescens	0.251	0.052	0.077	0.026	0.056
	Prevotella pallens	1.579	0.715	1.219	0.049	0.498
	Prevotella salivae	0.400	0.300	0.049	0.049	0.412
Proteobacteria/Neisseriaceae	Neisseria sp.	11.283	19.559	19.570	0.049	0.047
Proteobacteria/Pasteurellaceae	Haemophilus parainfluenzae	1.687	3.515	2.584	0.038	0.265
Fusobacteria/Fusobacteriaceae	Fusobacterium nucleatum	1.319	0.686	0.313	0.171	0.012
Actinobacteria/Corynebacteriaceae	Corynebacterium sp.	0.151	0.032	0.053	0.029	0.045

R IgAN patients with the lowest proteinuria (<1 g/24 h), P IgAN patients with persistent proteinuria (>1 g/24 h)

Table 2 Relative abundance (%) of predominant bacterial taxa, showing significant (P<0.05) differences between salivary samples of IgAN HC
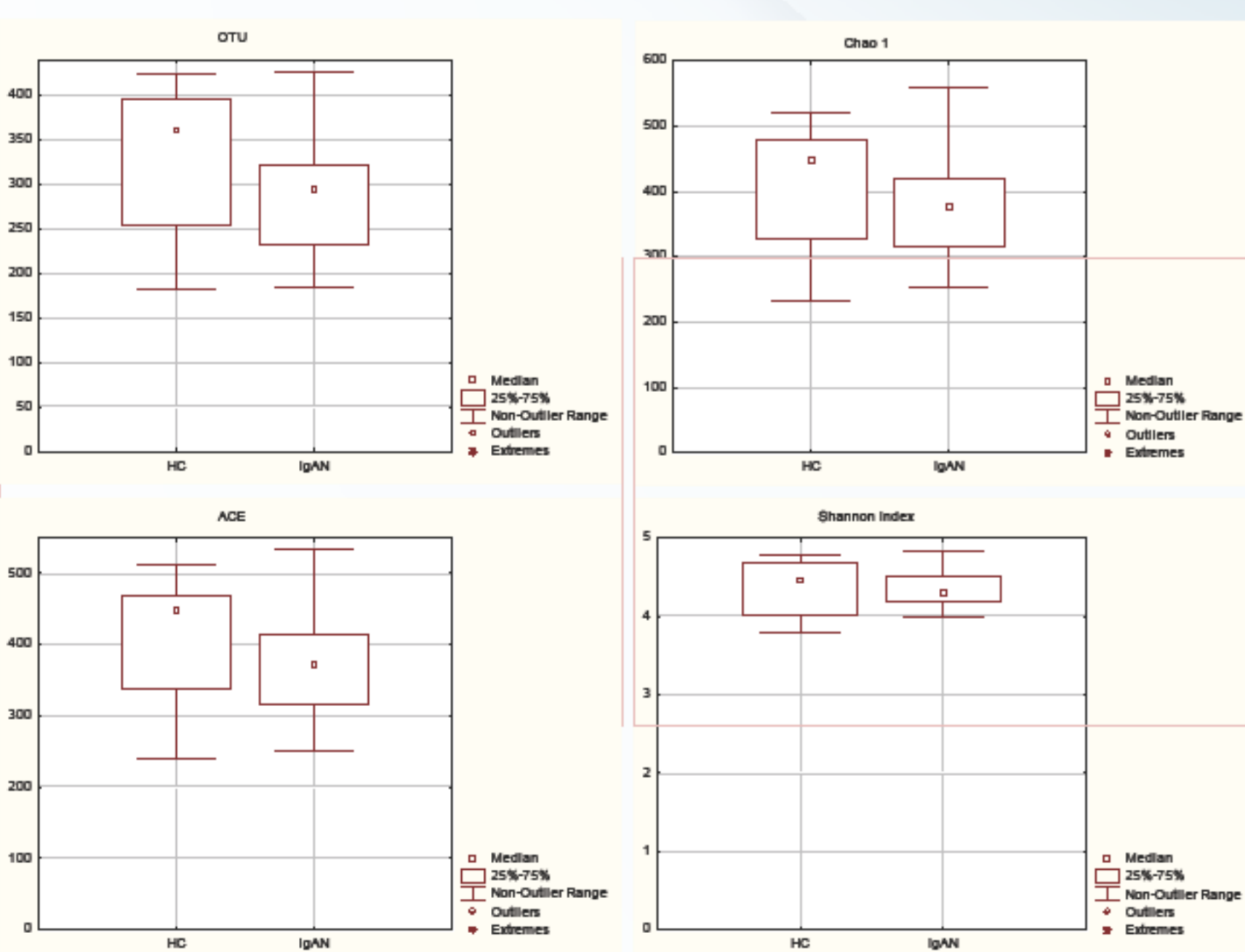

Fig. 2 Average number of species (OTU), richness (Chao 1), abundance-based coverage estimator (ACE), and diversity (Shannon index) values in the salivary samples of IgAN patients and HC
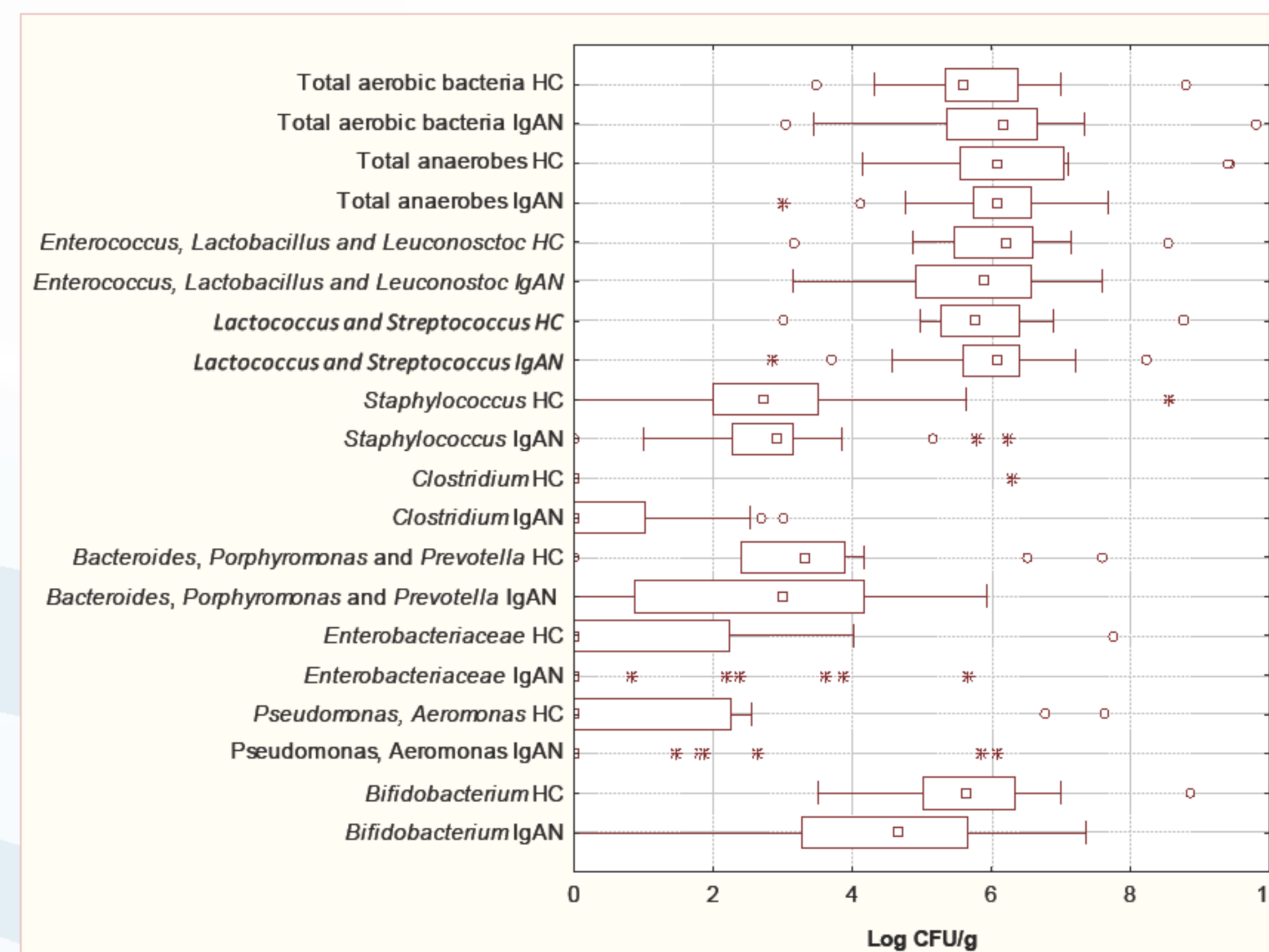

Fig. 3 Relative abundance (%) of total bacteria found at the phylum level in the salivary samples of immunoglobulin A nephropathy (IgAN) patients and healthy controls (HC). A % of presence of each phylum; b proportion of each phylum within IgAN and HC groups
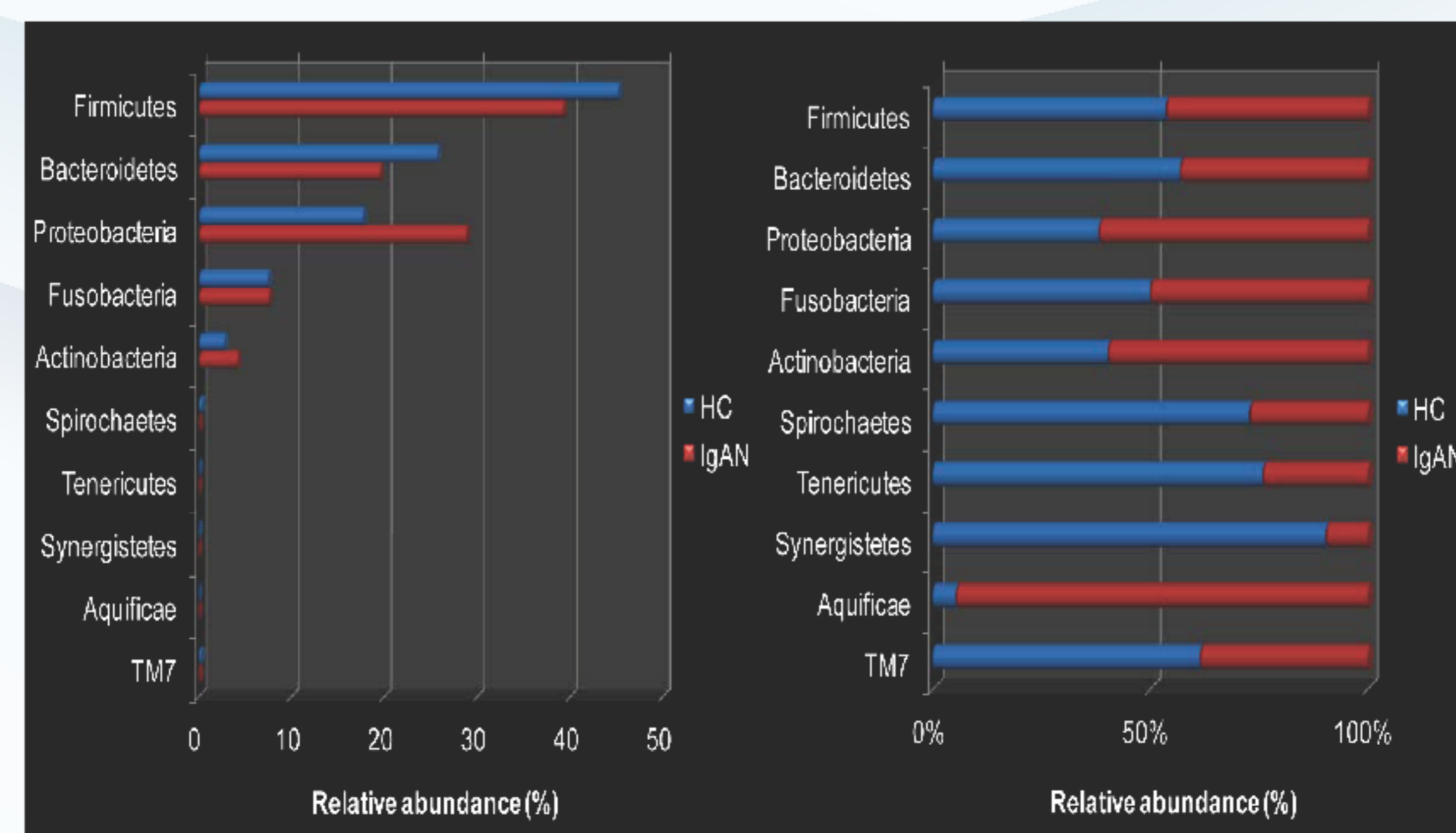

Fig. 4 Score plot of the three principal components (PC) after principal component analysis (PCA) of all 16S rRNA gene sequences found in the salivary samples of IgAN pts and HC

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