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# Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer

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We aimed to characterize microbiota of the gastric mucosa as it progresses to intestinal type of cancer. Study included five patients each of non-atrophic gastritis (NAG), intestinal metaplasia (IM) and intestinal-type gastric cancer (GC). Gastric tissue was obtained and DNA extracted for microbiota analyses using the microarray G3 PhyloChip. Bacterial diversity ranged from 8 to 57, and steadily decreased from NAG to IM to GC ( $p = 0.004$ ). A significant microbiota difference was observed between NAG and GC based on Unifrac-presence/absence and weighted-Unifrac-abundance metrics of 283 taxa ( $p < 0.05$ ). HC-AN analyses based on presence/absence of 238 taxa revealed that GC and NAG grouped apart, whereas IM overlapped with both. An ordination analysis based on weighted-Unifrac distance given abundance of 44 taxa showing significance across categories revealed significant microbiota separation between NAG and GC. This study is the first to show a gradual shift in gastric microbiota profile from NAG to IM to GC.

*H. pylori* infection is recognized as the main risk factor for distal gastric cancer (GC), although just a fraction of those infected (<3%) ever develop GC, suggesting that other factors also play a role<sup>1</sup>. Pathological studies of GC show that this is an inflammation-driven disease, and all factors influencing the mucosal immune response may become involved in this multifactorial model<sup>2</sup>. Thus, host genetics, environment, and *H. pylori* genetics have a role to play. Polymorphisms in inflammation-related genes like IL-1 $\beta$  511T, Interferon- $\gamma$ R1 -56C/T, or in TLRs have been reported to be associated with GC<sup>3</sup>; reduced consumption of food rich in antioxidants (vegetables, fruits), smoking, alcohol or obesity also increase the risk<sup>4</sup>. Most of the above risk factors may have a role on the mucosal inflammatory and immune response, thus modulating the driving force for tissue damage and development of gastric cancer.

Distal GC may be of two types, the intestinal and the diffuse, each following different development routes. For the intestinal type, the development model predicted by Correa states that an initial gastric inflammation may go uncontrolled and lead to mucosal atrophy and hypochlorhydria, which in turn increases the risk for intestinal metaplasia, dysplasia and finally intestinal type GC<sup>5</sup>. Although little is known about the development of diffuse GC, it is accepted that *H. pylori* and inflammation may also play a role<sup>6</sup>.

The stomach microbiota may also modulate the intensity and type of inflammatory and immune responses in the gastric mucosa. Studies on the microbiota of the stomach are scarce, and one study found that the human stomach is colonized by a complex microbiota including mainly Proteobacteria, Firmicutes, Actinobacteria and Fusobacterium phyla, and showed clear differences with microbiota described in the mouth and esophagus<sup>7</sup>. The study also showed that patients positive for *H. pylori* culture showed significant increased colonization of Proteobacteria and decrease of Actinobacteria. Initial attempts to compare microbiota in GC vs dyspeptic patients reported no significant differences in bacterial communities, although the authors observed that *Streptococcus*, *Lactobacillus*, *Veillonella* and *Prevotella* dominated in GC patients<sup>8</sup>.

We know that *H. pylori* infection usually does not alter the acid barrier of the gastric mucosa, unless an unregulated inflammatory response in the corpus leads to atrophy and hypochlorhydria<sup>9</sup>. Alteration of acidity may result in a more permissive milieu for colonization with other bacteria<sup>8</sup>. We hypothesize that this shift in microbiota adds to the inflammatory response already in place and increases the risk for more atrophy and



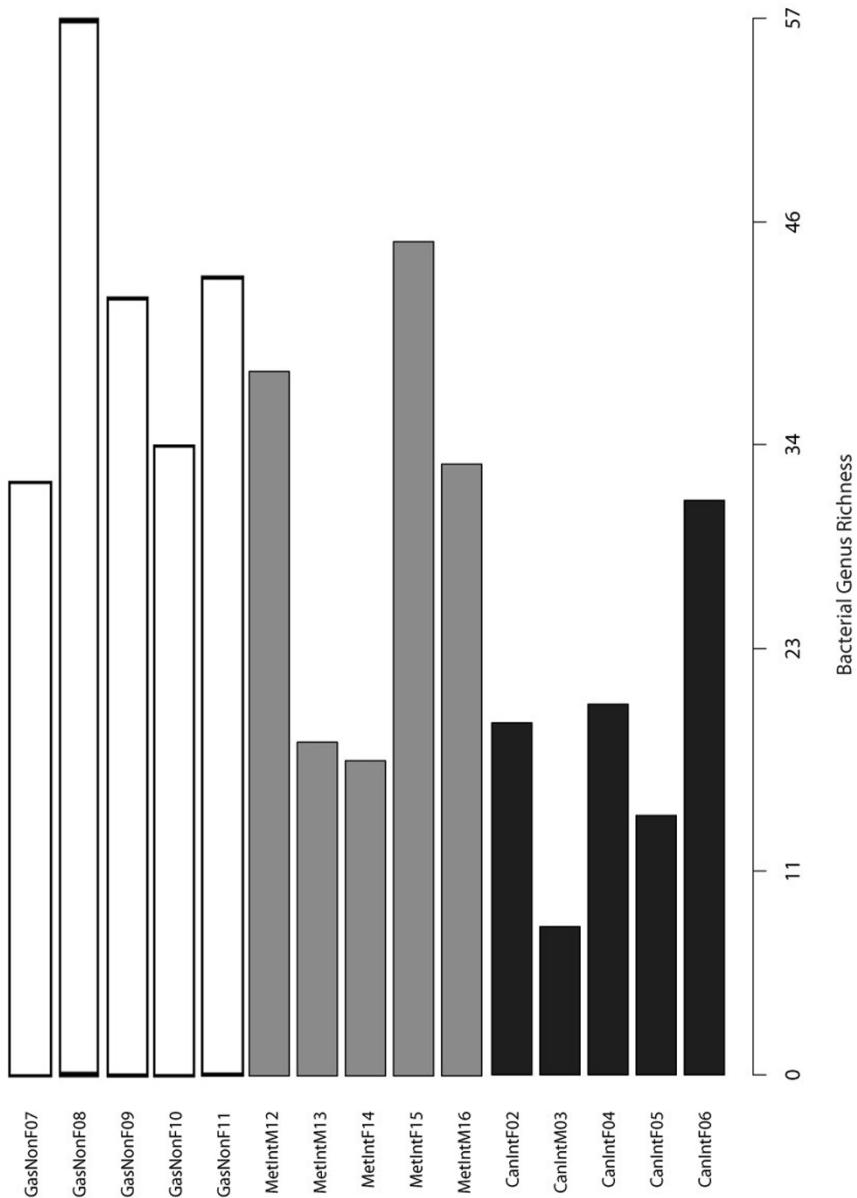
intestinal metaplasia, thus increasing the risk to develop GC. In this work we aimed to study changes in stomach microbiota in gastric tissue of patients with progressive histologic stages leading to gastric cancer, from non-atrophic gastritis (NAG), to intestinal metaplasia (IM) and to GC.

## Results

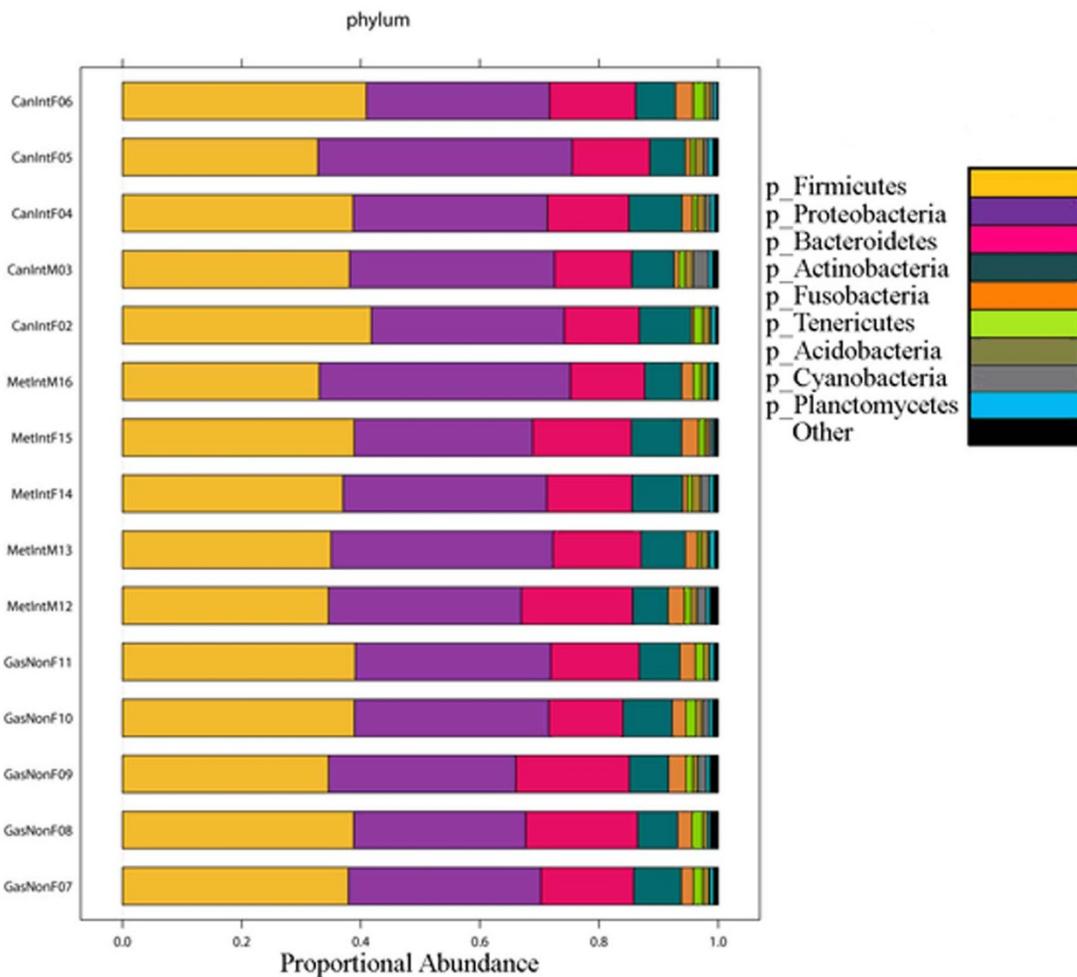
**Gastric microbiota diversity is low, with 9 families representing >50% of all OTUs.** Bacterial genus diversity was low in all samples and ranged from 8 in GC patient M03, to 57 in NAG patient F08 (Figure 1). Diversity was significantly different between patients with NAG and patients with GC ( $p = 0.004$ , two-tailed heteroscedastic t-test), but not between NAG and IM; still, we observed a trend of diversity that diminished from NAG to IM to GC (Figure 1). The proportion of OTUs for each phylum is depicted in Figure 2, which shows that two phyla, Firmicutes and Proteobacteria represented almost 70% of phyla in all samples. We also found that the top 9

families represented on average as much as 55.6% of each sample's OTUs, with *Lachnospiraceae* and *Streptococcaceae* representing over 20% of families in patients from all three disease groups.

**A whole microbiota profile showed separation between GC and NAG, but not between IM and GC or IM and NAG.** We first analyzed the possible effect of sex and age on microbiota composition given presence/absence of 283 taxa present in at least one sample. A Principal Coordinate Analysis (PCoA) including all 15 patients, and based on unweighted Unifrac distance showed no significant microbiota differences based on either age or sex ( $p$ -value  $> 0.50$ , Adonis test). A similar PCoA analyses comparing NAG vs IM vs GC, given presence/absence of 283 taxa present in at least one sample was done (Figure 3). The Adonis test yielded a  $p$ -value of 0.026, indicating a significant microbiota difference between at least one of the disease groups from the others. In addition, a binary metric analysis revealed separation of GC microbiota from



**Figure 1 | Microbial diversity.** Bacterial taxon diversity at the genus level in the gastric microbiota of patients with non-atrophic gastritis (GasNon), intestinal metaplasia (MetInt) and gastric cancer of the intestinal type (CanInt). Bacterial diversity was significantly higher in non-atrophic gastritis than in gastric cancer patients ( $p = 0.004$ , heteroscedastic t-test).



**Figure 2 | Abundance of OTUs.** Proportion of OTUs at the phylum level in the gastric microbiota of patients with non-atrophic gastritis (GasNon), intestinal metaplasia (MetInt) and gastric cancer of the intestinal type (CanInt). Firmicutes and Proteobacteria represented almost 70% of phyla in each sample.

NAG, whereas microbiota of IM was not separated from either GC or NAG groups. To further confirm these findings, a PCoA comparing NAG vs GC was done, and the Adonis test yielded a p-value of 0.005, indicating a significant microbiota difference between these two groups. In addition, comparison between IM and NAG ( $p = 0.42$ ) and between IM and GC ( $p = 0.129$ ) showed no difference between groups. Hierarchical clustering analysis (HC-AN) based on presence/absence of 283 taxa revealed two clusters, one containing only GC and IM and the other NAG and IM, with one GC possible outlier (case CanIntF06).

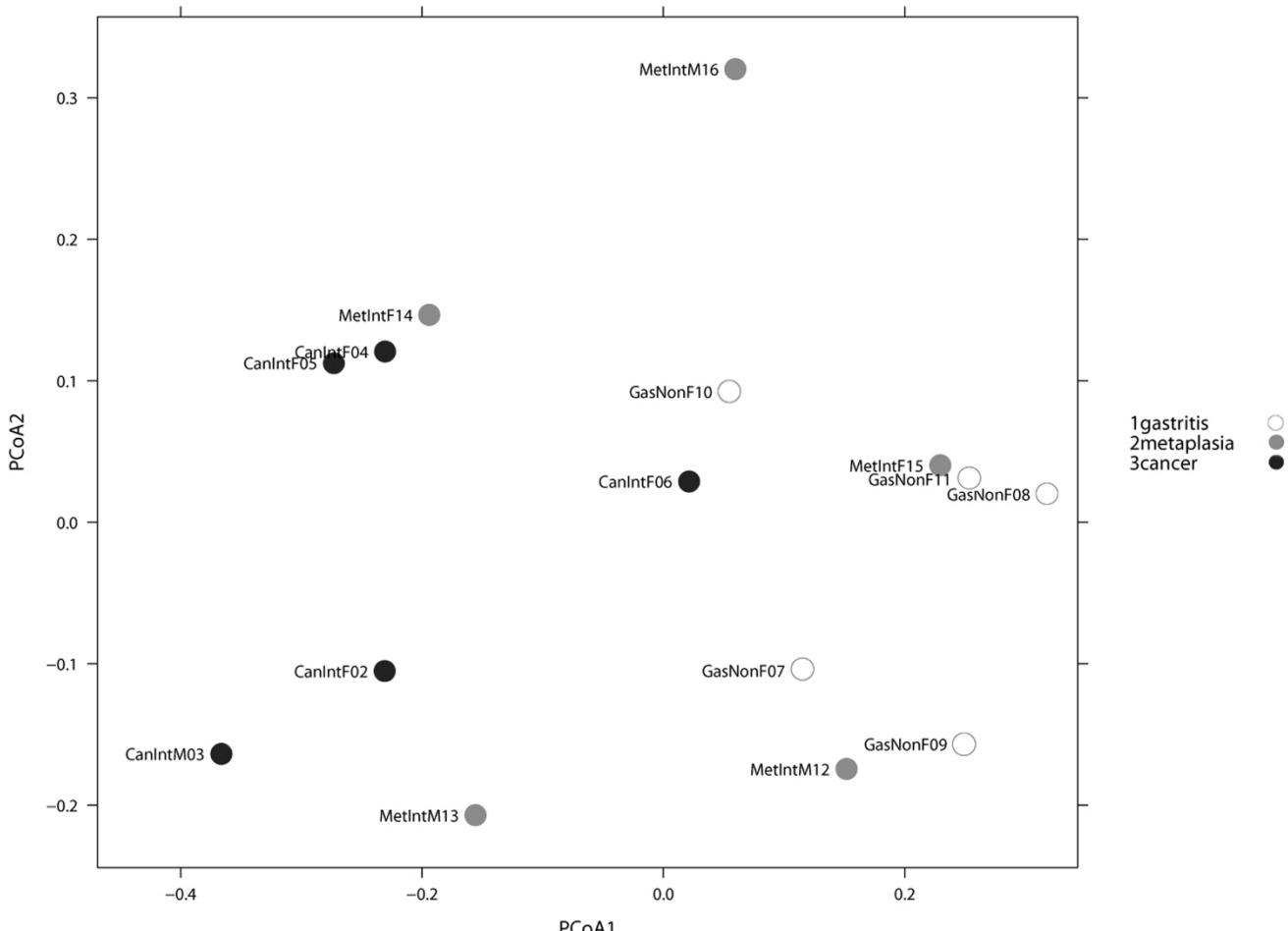
We next did PCoA analyses based on wUnifrac distance between samples given abundance of 283 taxa present in at least one sample. Whereas abundance metric analysis showed no significant separation of microbiota according to disease group, the presence/absence metric analyses showed a significant difference between NAG and GC ( $p\text{-value} = 0.03$ , Adonis test). The same analyses revealed no significant differences between NAG and IM and between GC and IM; although together GC and IM showed significant microbiota difference when compared with the NAG group ( $p = 0.048$ , Adonis test).

**Analysis based on taxa with significant abundance differences across groups confirmed microbiota separation between GC and NAG, and overlapping of IM with both.** We performed a parametric Welch test to identify OTUs that were significantly

increased or decreased across at least one disease group, and found 27 taxa with significant abundance difference across groups. A PCoA weighted Unifrac distance between groups based on the 27 taxa, showed no significant separation of microbiota between all three groups (NAG vs IM vs GC). However, when considering only NAG and GC patients, an entire separation of the microbiota along PCoA1 axis was observed (Figure 4). HC-AN analysis based on the same 27 taxa showed that NAG and GC clustered apart, whereas IM cases overlapped with the two NAG and GC groups (Figure 5).

From the 27 taxa with significant abundance differences across groups, we selected the 12 with the more significant p-values (Table 1) and plotted their abundance in each disease group (Figure 6). The results showed that 5 of these top 12 displayed a trend to decrease from NAG to IM to GC (2 *TM7*, 2 *Porphyromonas* and 1 *Neisseria*), whereas 2 significantly increased from NAG to IM to GC (*Lactobacillus coleohominis* and *Lachnospiraceae*).

**Analysis based on taxa with significant abundance differences between NAG and GC confirmed significant microbiota separation of these groups.** To further document the observed differences between NAG and GC, a parametric Welch test was done to look for OTUs that significantly increased or decreased between these two groups. The test identified 44 OTUs with a p-value  $< 0.05$  and with these a PCoA weighted Unifrac distance analyses showed a significant



**Figure 3 | PCoA analyses Unweighted Unifrac.** PCoA analyses of the gastric microbiota in non-atrophic gastritis vs intestinal metaplasia vs gastric cancer, based on Unweighted Unifrac distance between samples given presence/absence of 283 taxa present in at least one sample. Axis 1 explained 30% of variation and axis 2, 13%. Adonis test yielded a p-value of 0.026, indicating significant microbiota difference between at least one of the sample categories from the others.

separation of the microbiota of NAG vs GC (Figure 7). A HC-AN with the above 44 OTUs also showed separate clusters of NAG and GC (results not shown). A profile of the top 12 with the lowest p-values showed that 9 of the 12 displayed a significant increase in NAG patients, whereas *Lactobacillus coleohominis* and *Lachnospiraceae* were significantly more abundant in GC patients (Table 2).

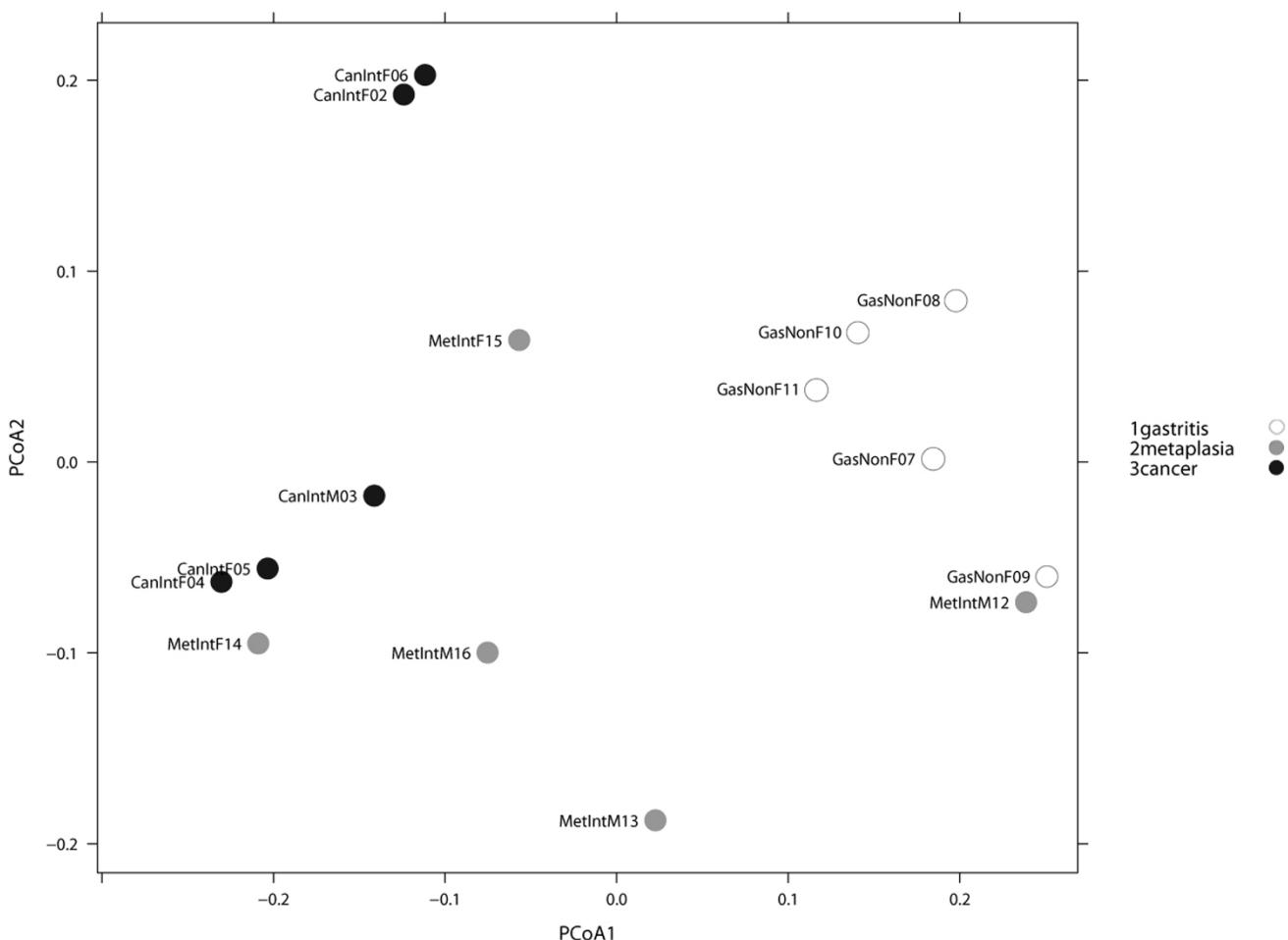
## Discussion

In this study we tested the hypothesis that the microbiota of the gastric mucosa changes from patients with NAG to patients with IM and to patients with GC. To test this we used the generation G3 PhyloChip™, with an increased sensitivity for low abundance bacteria<sup>10</sup>. This is particularly relevant in the study of stomach samples where the microenvironment is restrictive for bacterial colonization. Indeed, we found low microbiota diversity, with a total of 12 phyla and 283 OTUs detected, whereas genus diversity varied from 8 up to 57 in all patients. Our results are not comparable with previous studies because of differences in techniques and populations; still, agreement exist in the low number of phyla detected and in the predominance of Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria in stomach microbiota<sup>7,11,12</sup>. However, with the use of generation 3 PhyloChip we were able to detect more OTUs and higher diversity at the genus level. Thus, it has been reported that in the gastric mucosa the most abundant families are Streptococcaceae and Prevotellaceae<sup>8,11</sup>, which contrast with our results where Lachnospiraceae was the most abundant, representing almost 20%

of the microbiota in all patients. The Lachnospiraceae family is formed by strict anaerobes, of which a new genera has been recently described in the mouth<sup>13</sup>. In the intestine, members of this family have been reported to exert a protective role against *C. difficile* colonization after microbiota has been modified with antibiotics<sup>14</sup>. In addition, it has been suggested that members of Lachnospiraceae family may be associated with a reduced intestinal carcinogenesis<sup>15</sup>.

We also found in several of the patients the species *Haemophilus parainfluenzae* and *Veillonella ratti*, which have been reported previously only in the urogenital tract<sup>16,17</sup>, and members of the families Desulfobacteraceae and Acidobacteriaceae (data not shown), which have been described as free living bacteria<sup>18,19</sup>. The presence of these low abundance bacteria in several cases suggests they are not a contaminant, and their identification might be due to the improved sensitivity of G3 PhyloChip<sup>10</sup>. Results from this and other studies suggest that the gastric mucosa may be colonized with bacteria previously undescribed in the stomach or even in humans<sup>7</sup>.

The low diversity observed in this study contrasts with a recent report using the G2 PhyloChip that detected substantially higher diversity in adults with gastritis<sup>20</sup>, as many as 44 phyla, many of them in very low frequencies, although there was agreement in the dominant phyla. Differences with our study might be explained in part because of differences in population, since they sampled Amerindians from the Venezuelan Amazon with a particular ancestry and poor socioeconomic and hygiene conditions.



**Figure 4 | PCoA analyses Weighted Unifrac.** PCoA analyses of the gastric microbiota in non-atrophic gastritis vs intestinal metaplasia vs gastric cancer, based on Weighted Unifrac distance between samples given abundance of 27 taxa with significant abundance difference across at least one of the categories. Axis 1, explained 55% of variation and axis 2, 22%. An entire separation of the microbiota of non-atrophic gastritis and gastric cancer groups along the PCoA1 axis was observed.

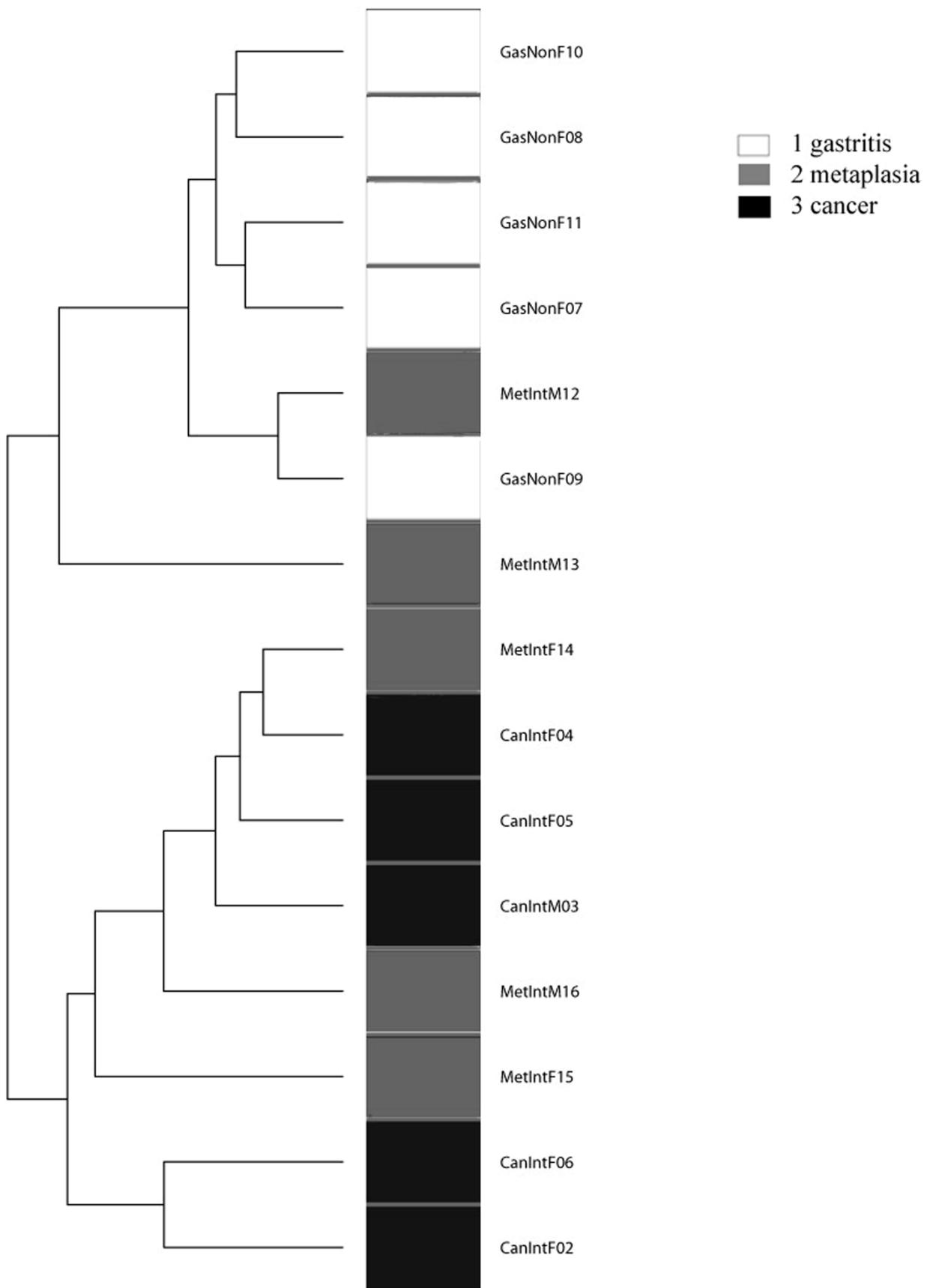
Concerning changes in the microbiota, we found evidence of a gradual change in patients from NAG to IM to GC, bacterial diversity showed a trend to diminish from NAG to IM to GC, and was significantly higher in NAG than in GC patients. These results would suggest that changes in the gastric mucosa as patients move to pre-neoplasia and to cancer render the environment more restrictive to bacterial growth, contrary to what others and we have hypothesized<sup>21</sup>. Mucins are produced by normal epithelial cells along the gastrointestinal tract and serve as a protective barrier and as specialized niche for colonization of microbiota<sup>22</sup>. A healthy gastric mucosa produces MUC5AC and MUC6 mucins<sup>23</sup>, which the gastric microbiota colonizes; but when normal mucosa is replaced by atrophy or pre-neoplastic lesions the mucins change to metaplastic expression of intestinal MUC2<sup>24</sup>, and we suggest this makes the mucosa less suitable for colonization by the normal microbiota.

When whole microbiota profile was studied, ordination analyses based on both, presence/absence or abundance, revealed a significant separation of GC from NAG, whereas IM did not separate from either NAG or GC. A cluster analyses also showed two separate groups for NAG and GC with the IM cases intermingled with GC and NAG groups. These findings would suggest a change from NAG to GC, with IM as an intermediate group. This observation was possible because our study included IM patients, in contrast to a previous report where GC was compared only with NAG patients<sup>8</sup>. Recent studies in a transgenic hypergastrinemic mouse model found

that infection with *H. pylori* in the presence of intestinal flora caused the development of gastrointestinal intraepithelial neoplasia<sup>25</sup>; even colonization with a very restricted intestinal flora was found sufficient to induce neoplasia<sup>26</sup>. These observations suggest that presence of other bacteria in addition to *H. pylori* are needed for the development of gastric cancer, although they do not really help elucidate whether an increased or a decreased bacterial diversity is responsible for this adjuvant effect.

Of particular interest is to highlight that one of the taxa that was significantly more abundant in GC than in NAG was the genus *Pseudomonas* (data not shown), which is relevant in light of a recent report in patients with stomach adenocarcinoma that found specific integration of *Pseudomonas*-like DNA in somatic cells, in the 5'-UTR and 3'-UTR of four proto-oncogenes that are up-regulated in GC<sup>27</sup>. In fact, this is the first study to document bacterial-human somatic cell lateral gene transfer in cancer patients, and our results on predominance of *Pseudomonas* in GC would be in agreement with this important report.

A further selection of the OTUs with the lowest p-values showed 6 taxa with a clear decreasing trend from NAG to IM to GC (2 *TM7*, 2 *Porphyromonas* sp, *Neisseria* sp and *Streptococcus sinensis*), and 2 with an increasing trend (*Lactobacillus coleohominis* and *Lachnospiraceae*). *TM7* is a recently described phylum present in the mouth and intestine of humans; and a small fraction of its genes have sequence similarity to genes found in members of the classes



**Figure 5 | Hierarchical Clustering of the gastric microbiota.** Analysis based on Weighted Unifrac distance between samples given abundance of 27 taxa with significant abundance differences across at least one of the categories. A distinct clustering was observed between non-atrophic gastritis and gastric cancer, whereas intestinal metaplasia overlapped with these two groups.



**Table 1 | Description of the top 12 OTUs with the lowest p-value (parametric Welch test) that were significantly increased or decreased across at least one disease group; comparison of gastric cancer with intestinal metaplasia and with non-atrophic gastritis**

Taxa ID	Phylum	Class	Order	Family	Genus	Species	Trend
56	TM7	TM7-3	CW040	F16	unclassified	unclassified	Decrease
31	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	unclassified	Decrease
116	TM7	TM7-3	EW055	unclassified	unclassified	unclassified	Decrease
63	Proteobacteria	Betaproteo-bacteria	Neisseriales	Neisseriaceae	Neisseria	unclassified	Decrease
283	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	unclassified	Decrease
282	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	pallens	Decrease
265	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	coleohominis	Increase
277	Proteobacteria	Gammaproteo-bacteria	Pasteurellales	Pasteurellaceae	Haemophilus	unclassified	Decrease
180	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	Increase
57	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	sinensis	Decrease
279	Proteobacteria	Betaproteo-bacteria	Neisseriales	Neisseriaceae	Bergerilla	denitrificans	Decrease
159	Tenericutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Bulleidia	unclassified	Decrease

*Bacilli*, *Clostridia* and *Fusobacteria*<sup>28</sup>. TM7 encodes genes for Type IV pili that is recognized as a virulence factor in some pathogenic bacteria, and may also have a role in inflammatory diseases such as Crohn's disease<sup>29</sup> and colitis<sup>30</sup>. *Streptococcus sinensis* has been reported in the oral cavity<sup>31</sup> and associated with infective endocarditis<sup>32</sup>. On the other hand, *L. coleohominis* is a relatively novel species recovered from the vagina<sup>33</sup> and from urine of healthy woman<sup>34</sup>, but no reports exist on any disease association. Lachnospiracea family has been reported to significantly diminish in patients with inflammatory disease<sup>35</sup>. Thus, many of the bacterial groups we found altered in the gastric mucosa of patients with GC have been reported associated to inflammatory processes, and may conceivably play a role in the regulation of the inflammatory response observed in the gastric wall associated with the development of GC.

A previous study reported no difference in stomach microbiota between GC and dyspeptic patients, although in some cancer patients species of *Streptococcus*, *Lactobacillus* and *Veillonella* were more prevalent<sup>8</sup>. This study was done based on cloning, sequencing and restriction fragment length polymorphisms of 16S rRNA, which has important limitations in sensitivity and coverage compared with more recently developed techniques, including the G3 PhyloChip. Still, in agreement with our results, they also reported dominance of *Lactobacillus* in GC. To our knowledge, there are no additional reports describing microbiota in patients with GC, and studies are necessary to learn more on the role of stomach microbiota in health and disease. Studies of microbiota in other human tissues revealed important diversity between individuals and significant differences among different human populations, even in studies using the same sample handling and technological platform<sup>36,37</sup>. We need to address the study of microbiota in the stomach using a similar approach, with studies involving larger groups and different human populations. We acknowledge that an important draw back of our study is the reduced number of patients, which allowed us finding differences between disease groups, but fall short to study the effect of other variables within groups; e.g., we had a small number of males in the NAG and GC groups. These issues should be addressed by studying a larger number of cases.

The current study is the first to show differences in the stomach microbiota of patients with gastric cancer, and suggest a gradual shift in microbiota profile from gastritis to pre-neoplastic lesion to cancer. The study also suggests that a decrease in *Porphyromonas*, *Neisseria*, TM7 group, and *S. sinensis*, as well as an increase in *L. coleohominis* and Lachnospiracea might favor development of gastric cancer. The role of these taxa and its mechanisms of pathogenesis in gastric cancer remain to be studied.

## Methods

**Patients.** We included patients consulting the Oncology and General hospitals in the Medical Center SXXI, Instituto Mexicano del Seguro Social (IMSS), in Mexico City.

Five patients each with NAG, IM, and GC of the intestinal type were selected for the study. For both, the NAG and IM patients we selected tissue samples from the antrum, whereas for the GC group we worked with tissue from the lesion, which in 4 cases extended in both antrum and corpus, and one involved fundus and corpus. The characteristics of these patients are described in Table 3; the groups of patients studied did not differ significantly in body mass index or school years, although there were limited number of males, and patients with NAG were younger than those with IM and GC. We excluded patients with immunodeficiencies, diabetes or other chronic diseases, and patients who received antibiotics or proton pump inhibitors during the last three months, or patients who previously received therapy for *H. pylori* eradication. *H. pylori* diagnosis was done using an enzyme-linked immunosorbent assay previously validated in our population<sup>38</sup>. All patients were Mexican-mestizo with a medium to low socioeconomic level. Patients were informed about the study and those willing to participate signed a consent letter. The IRB committee from IMSS approved the study.

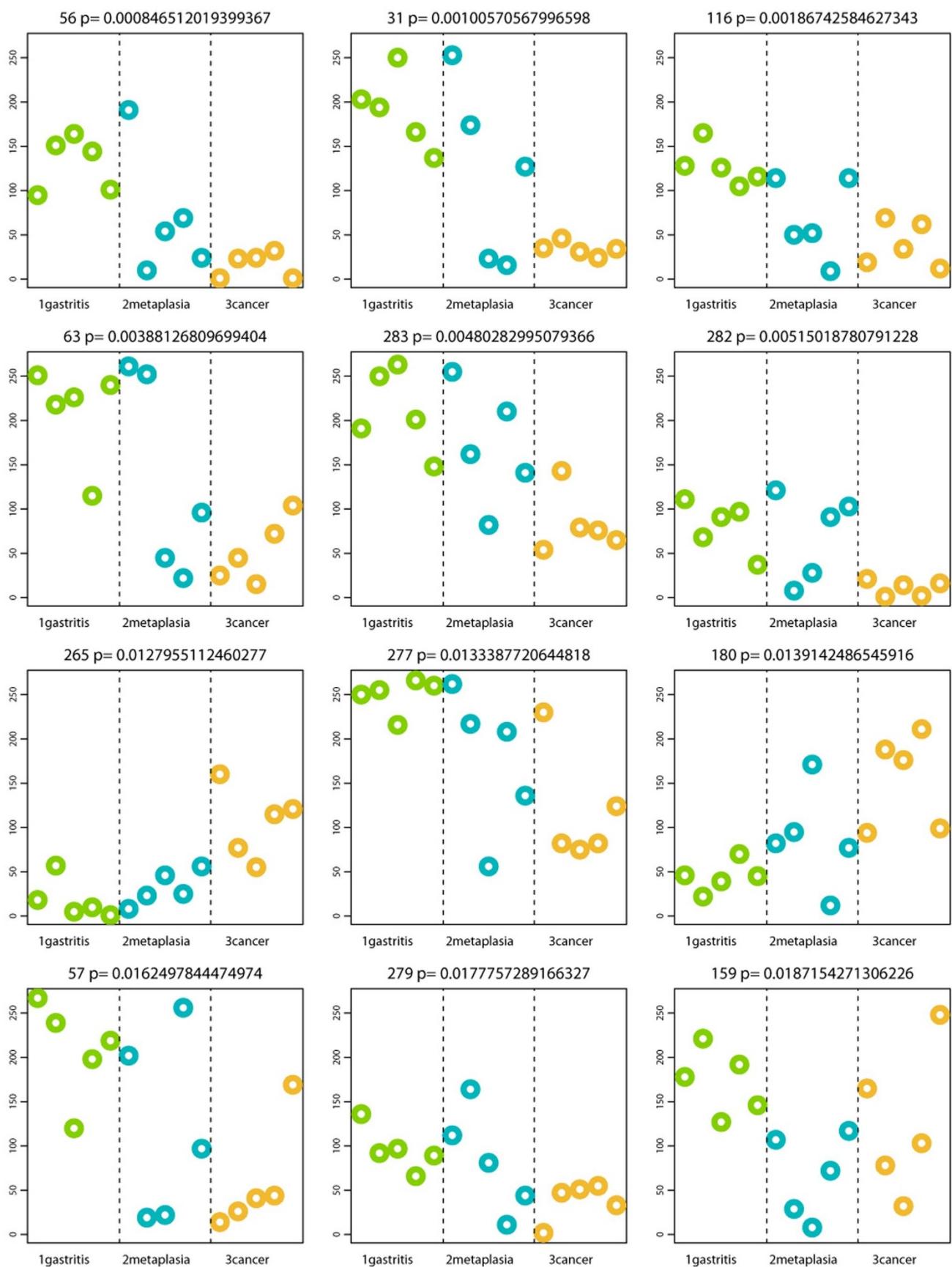
**Gastric samples.** Patients with NAG and with IM were subjected to endoscopy and biopsies from antrum and corpus were taken, whereas patients with GC were subjected to surgery to remove the tumor. Samples of either NAG, IM or GC were processed as follows: one biopsy or a fraction of the tumor lesion was immediately placed in formalin for histological studies, and another tissue fraction placed in liquid nitrogen for transportation to our lab, where both biopsies and tissues were stored at -70°C until tested. Fixed tissue was stained with Hematoxylin and Eosin and modified Giemsa, and with periodic acid-Schiff/Alcan blue staining when metaplasia was suspected. Preparations were examined by a single experienced pathologist and final diagnosis assigned using previously reported criteria<sup>39,40,41</sup>.

**DNA extraction and PCR amplification for 16S rRNA microarray.** The tissue samples from the 15 patients were thawed and DNA extracted using the QIAamp DNA easy mini kit (Qiagen, Hilden, Germany). Isolated lyophilized DNA was then hydrated and quantified using the PicoGreen kit (Life technologies, Carlsbad, CA). Samples were subjected to amplification using degenerate 16S rRNA gene primers 27F1 (5'-AGRGTGTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') for bacteria and 4Fa and 1492R for archaea as described in Hazen et al.<sup>10</sup>. Amplified PCR products were concentrated, purified and quantified, and 500 ng of amplified product were fragmented to a range of 50–200 bp and then biotin labeled (Affymetrix WT Double Stranded DNA Terminal Labeling) for hybridization.

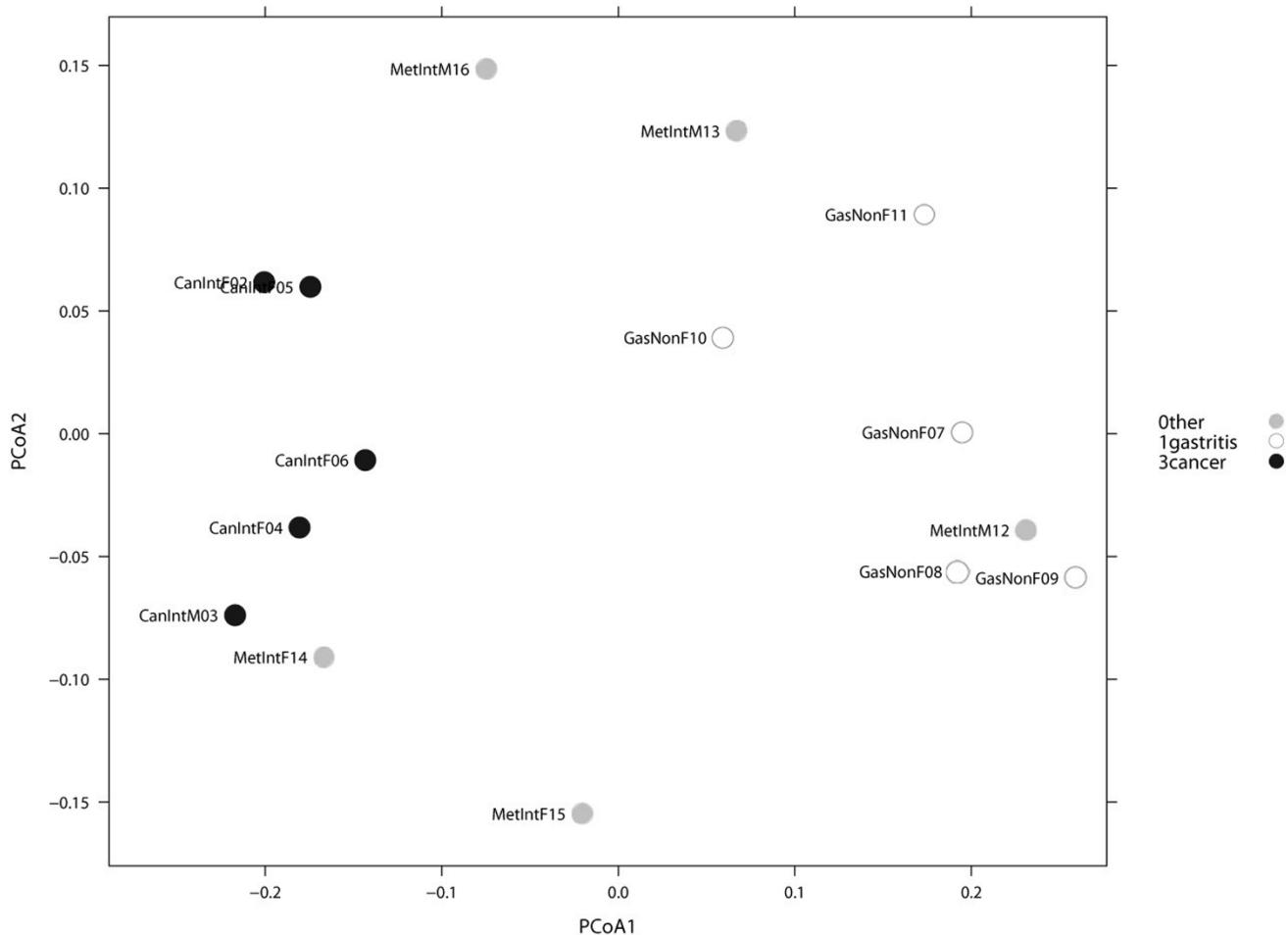
**Microarray hybridization.** The Phylochip Generation 3 (G3) microarray was used for hybridization, and samples were run at Second Genome, INC. (San Francisco, CA). This includes 1.1 million DNA probes able to categorize all known bacteria and archaeal operational taxonomic units (OTUs) into over 50,000 taxa using 59,959 clusters of 17 nucleotides as probes representing 147 phyla, 1123 classes, 1219 orders and 1464 families, for a total of 27938 OTUs. Staining and scanning were performed according to the manufacturer instructions (Affymetrix) and following previously described procedures<sup>10</sup>. PhyloChip has built-in controls for all steps of the microarray hybridization process and normalization of the probe intensity values. Fluorescence intensity is coded as integers ranging from 0 to 65,536. Hybridization score (HybScore) values are in the range of 0 to 16000 and changes by 1000 in the OUT's HybScore indicate a two-fold increase in the fluorescence intensity.

**Data reduction and statistical analysis.** Taxa are filtered to those present in at least one of the samples, or to taxa significantly increased in their abundance in one category compared to the alternate categories. For the abundance filter, the parametric Welch test was employed to calculate p-values. Additionally, q-values were calculated using the Benjamini-Hochberg procedure to correct p-values, controlling for false discovery rates<sup>10</sup>.

After the taxa were identified for inclusion in the analysis, the values used for each taxa-sample intersection were defined in two distinct ways. In the first case, the



**Figure 6 | Profiles of the top 12 OTUs generating the lowest p-values distinguishing disease groups.** P-values shown at top of each OTU plot are unadjusted for multiple testing. The y-axis represents the HybScore (integers of fluorescence intensity). Samples are grouped and colored by category along the x-axis in the following order: GasNonF08, GasNonF09, GasNonF10, GasNonF11; MetIntM12, MetIntM13, MetIntM14, MetIntM15, MetIntM16; CanIntF02, CanIntF03, CanIntF04, CanIntF05, CanIntF06.



**Figure 7 | Non-atrophic gastritis vs gastric cancer.** PCoA analyses of the gastric microbiota in non-atrophic gastritis vs gastric cancer, based on Weighted Unifrac distance between samples, given abundance of 44 taxa with significant abundance difference across at least one of the categories. Axis 1 explained 62% of variation and axis 2, 14%. A significant separation of microbiota between non-atrophic gastritis and gastric cancer was observed.

abundance metrics were used directly (AT). In the second case, binary metrics were created (absence, 0; presence, 1) (BT). A non-paired, heteroscedastic student's t-test assuming unequal variances was used to test for differences in microbial diversity and hierarchical summarization between two sample categories.

To evaluate sample-to-sample distance all profiles were inter-compared in a pairwise fashion to determine a dissimilarity score and stored it in a distance dissimilarity matrix. The distance functions were chosen to allow similar biological samples to produce only small dissimilarity scores. The Unifrac distance metric utilizes the phylogenetic distance between OTUs to determine the dissimilarity between communities<sup>42</sup>. Unifrac was used for presence/absence, whereas Weighted Unifrac (WUnifrac) was used for abundance data.

Two-dimensional ordinations and hierarchical clustering maps of the samples in the form of dendograms were created to graphically summarize the inter-sample relationships. To create dendograms, the samples from the distance matrix were clustered hierarchically using the average-neighbor (HC-AN) method. Principal Coordinate Analyses (PCoA) is a method of two-dimensional ordination plotting used to visualize complex relationships between samples. PCoA uses the dissimilarity values to position the points relative to each other. The Adonis test was used to evaluate statistically significant differences. In this randomization/Monte Carlo permutation test, the samples are randomly reassigned to the various sample categories, and the between-category differences are compared to the true between-category differences. Adonis utilizes the sample-to-sample distance matrix directly.

**Table 2 | Description of the top 12 OTUs with the lowest p-value (parametric Welch test) that were significantly increased or decreased across at least one disease group; comparison of gastric cancer with non-atrophic gastritis**

Taxa No	Phylum	Class	Order	Family	Genus	Species	Trend
56	TM7	TM7-3	CW040	F16	unclassified	unclassified	Decrease
116	TM7	TM7-3	EW055	unclassified	unclassified	unclassified	Decrease
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282	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	pallens	Decrease
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180	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified	unclassified	Increase
277	Proteobacteria	Gammaproteo-bacteria	Pasteurellales	Pasteurellaceae	Haemophilus	unclassified	Decrease
222	Proteobacteria	Alphaproteo-bacteria	Rhodospirillales	Rhodospirillaceae	unclassified	unclassified	Increase

**Table 3 | General characteristics of the patients included in the study**

Group, ID	Age	Sex	H. pylori, serology	Body mass Index	School years
<i>Non-atrophic gastritis</i>					
F07	76	Female	+	22.8	12
F08	37	Female	+	27.5	8
F09	43	Female	+	21.3	16
F10	32	Female	+	21.6	8
F11	32	Female	-	24.8	3
<i>Intestinal metaplasia</i>					
M12	63	Male	+	23.6	6
M13	71	Male	+	20.7	2
F14	71	Female	+	24.6	12
F15	60	Female	+	27.1	6
M16	70	Male	+	31.9	12
<i>Intestinal Gastric cancer</i>					
F02	79	Female	+	24.25	4
M03	59	Male	-	26.66	8
F04	82	Female	+	26.56	2
F05	52	Female	+	27.39	6
F06	81	Female	+	19.77	6

Data analyses were done using the PhyCA-Stats™ analyses software (Second Genome, San Francisco, CA).

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## Author contributions

J.T. design the study; J.T. and F.A.J. analyzed data and wrote the main manuscript; F.V.J. processed clinical samples for microbiota analyses; R.M.G. was responsible for patient's selection and clinical diagnosis; A.M. was responsible for selection of tissue samples and pathology studies. All authors reviewed the manuscript.

## Additional information

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