

1                   **Generative Adversarial Network augmented the gut**  
2                   **microbiome-based health index by profoundly improved**  
3                   **discrimination power**

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13                   **Abstract**

14                   **Summary:** Gut microbiome-based health index (GMHI) has been applied with success, while the  
15                   discrimination powers of GMHI varied for different diseases, limiting its utility on a broad  
16                   spectrum of diseases. In this work, a Generative Adversarial Network (GAN) model is proposed to  
17                   improve the discrimination power of GMHI. Built based on the batch corrected data through GAN  
18                   (<https://github.com/HUST-NingKang-Lab/GAN-GMHI>), GAN-GMHI has largely reduced the  
19                   batch effects, and profoundly improved the performance for distinguishing healthy individuals and  
20                   different diseases. GAN-GMHI has provided results to support the strong association of gut  
21                   microbiome and diseases, and indicated a more accurate venue towards microbiome-based disease  
22                   monitoring.

23                   **Availability and implementation:** GAN-GMHI is publicly available on GitHub:  
24                   <https://github.com/HUST-NingKang-Lab/GAN-GMHI>.

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26                   **Supplementary information:** Supplementary data are available at Bioinformatics online.

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28                   **Introduction**

29                   There are important links between many complex chronic diseases and the human gut microbiome  
30                   (Gupta, et al., 2020). Specific sets of gut microbes could directly or indirectly influence the  
31                   complex chronic diseases, such as the microbiome dysbiosis in the development of rheumatoid  
32                   arthritis (Bergot, et al., 2019), thus it is nature that gut microbiome could be utilized for disease

33 prediction (Gupta, et al., 2020; Shreiner, et al., 2015). However, a general microbiome-based  
34 index for prediction of a broad spectrum of diseases is lacking.

35

36 A previous work has reported the Gut Microbiome Health Index (GMHI) (Gupta, et al., 2020), a  
37 robust index for assessing health status, based on the species-level taxonomic profile of stool  
38 metagenomic sequencing samples. GMHI values can be used to classify samples as healthy  
39 ( $GMHI > 0$ ), non-healthy ( $GMHI < 0$ ), or neither ( $GMHI = 0$ ), and its results have shown strong  
40 reproducibility on the validation datasets. However, GMHI has limited power to distinguish  
41 samples from different diseases, largely due to the existence of batch effects: as the stool  
42 metagenomes in that study were collected from over 40 published studies, it is nearly impossible  
43 to exclude experimental and technical inter-study batch effects (Gupta, et al., 2020). Thus, the  
44 overall prediction accuracy of GMHI is still far from perfect: 70.72% for distinguishing healthy  
45 individuals and non-healthy individuals.

46

47 Therefore, we introduced GAN-GMHI, based on the Generative Adversarial Network (GAN), for  
48 improved discrimination power of GMHI. GAN was applied to reduce the batch effects on a large  
49 collection of gut microbiome samples from multiple cohorts containing both health and disease  
50 individuals. Then GMHI could be applied on the batch corrected data for prediction. Compared  
51 with original GMHI, GAN-GMHI makes the distribution of GMHI values within the group more  
52 concentrated and the distinction between healthy and non-healthy samples more clearly. The  
53 effectiveness of GAN for cross-cohort batch correction has been demonstrated: the prediction  
54 accuracy of GAN-GMHI has been improved to 88.70% for distinguishing healthy individuals and  
55 non-healthy individuals, compared to the accuracy of 70.95% achieved by GMHI. In summary,  
56 batch effect does exist in data sets from different sources, and GMHI can better predict the status  
57 of health based on GAN corrected data sets.

58

## 59 **Methods**

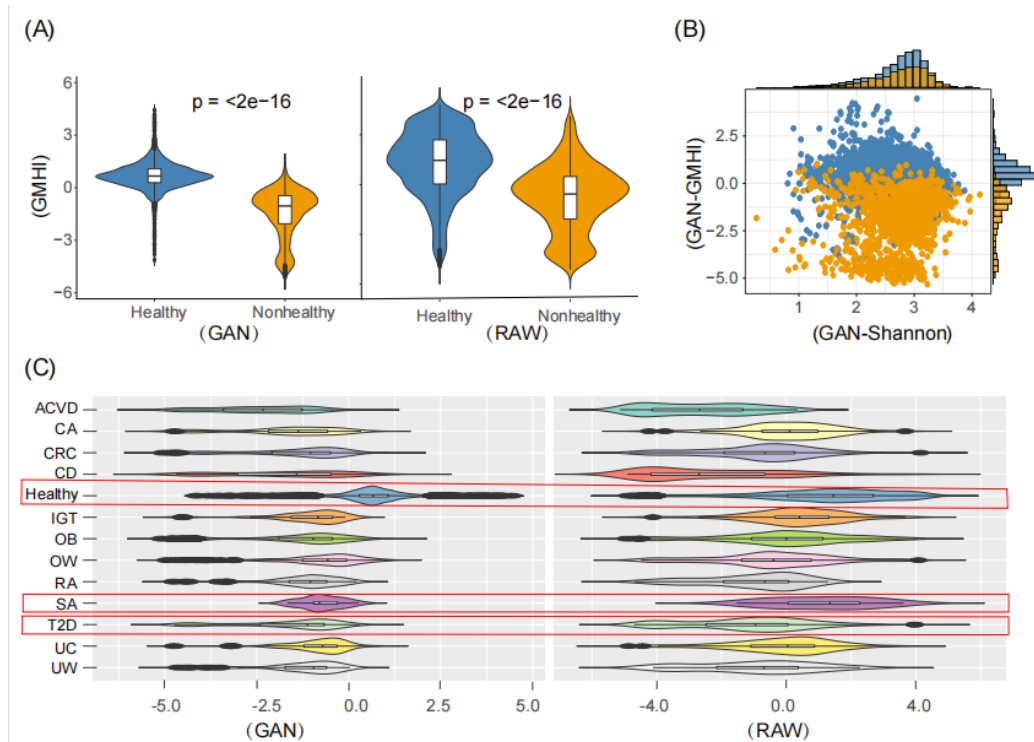
60 Our GAN-GMHI framework consists of three stages, constructing a dataset containing phenotype  
61 and batch information for all samples, and then GAN guiding the batch effect correction of raw  
62 data, the corrected datasets are output as the training data set for GMHI prediction  
63 (**Supplementary Figure 1**). The batch effect removal method of iMAP (Wang, et al., 2021), a  
64 GAN method previously applied on single-cell RNA-Seq data, was adapted for batch effect  
65 removal in this study. It is worth noting that the datasets to be batch-corrected by GAN must be  
66 classified based on the phenotype first, and the sub-data sets of each phenotype are regrouped  
67 according to the batch. To ensure that the unwanted technical variations among different datasets  
68 are eliminated, but the biological differences between different phenotypes are not diminished.

69

## 70 **Results**

71 We have performed a comprehensive analysis on the integrated dataset of 2,636 healthy and 1,711  
72 diseased (including 12 disease phenotypes) individuals' stool metagenomes from 34 published  
73 studies (Gupta, et al., 2020). All of these samples are used as analytical datasets. Additionally, we

74 have used 679 samples (118 healthy and 561 diseased) as validation datasets (Gupta, et al., 2020).  
75 The analytical and validation datasets configuration is the same as in (Gupta, et al., 2020).  
76  
77 We have first assessed and compared prediction results based on the discovery cohort (training  
78 data). By comparison of the species-level GMHI before (RAW) and after (GAN) batch correction  
79 (**Figure 1 (A)**) for distinguishing samples from healthy and non-healthy individuals, we observed  
80 that the accuracy for prediction of the healthy and diseased groups after correction is 87.03% and  
81 91.29% (with overall accuracy of 88.70%), respectively, compared with 75.61% and 63.76%  
82 before correction (overall accuracy of 70.95%), which has proven the advantage of GAN-GMHI  
83 over GMHI (**Supplementary Table 1**). Additionally, we compared the abilities of GAN-GMHI  
84 and Shannon diversity indicators to differentiate the gut microbiome of healthy and non-healthy  
85 individuals. The results demonstrated that GAN-GMHI could yield clearer separation compared  
86 with Shannon's diversity in differentiating healthy and non-healthy individuals (**Figure 1 (B)**).  
87 Furthermore, results on comparison among the healthy group and the 12 non-healthy phenotypes  
88 have showed that: when GMHI was applied, the GMHI values were dispersed over a wide range,  
89 and GMHI values for healthy samples were slightly higher than those for non-healthy samples  
90 except for SA. On the other hand, when GAN-GMHI was applied, the GMHI values were  
91 concentrated for each group, and the healthy group was significantly higher than the 12 disease  
92 phenotypes ( $p$ -value  $< 0.05$  for all disease groups), and the third quartile of GMHI was lower than  
93 0 for all disease phenotypes (**Figure 1 (C)**). Moreover, GMHI's results are easier for clinical  
94 interpretation. For example, on Type 2 diabetes (T2D), GAN-GMHI has captured *Lactobacillus* as  
95 biomarkers, which are well founded by published works (Wang, et al.).



96

97 **Figure 1. Comparison of GAN-GMHI with other methods under different settings.** (A) Violin  
98 plots of GMHI for the healthy and non-healthy groups before (left) and after (right) batch

99 correction by GAN. (B) the distribution of corrected GMHI and Shannon diversity. (C) Violin  
100 plots of GMHI index for the healthy and 12 disease phenotypes before (right) and after (left) batch  
101 correction by GAN. ACVD: Arteriosclerosis Cardiovascular Disease, CA: colorectal adenoma, CC:  
102 colorectal cancer, CD: Crohn's disease, IGT: Impaired glucose tolerance, OB: obesity, OW:  
103 overweight, RA: rheumatoid arthritis, SA: Symptomatic arteriosclerosis, T2D: Type 2 Diabetes,  
104 UC: ulcerative colitis, UW: underweight.

105

106 Additionally, we have compared GAN-GMHI and GMHI on the validation datasets. Cross-cohort  
107 batch correction by GAN profoundly improved the performance for distinguishing healthy  
108 individuals and different diseases. The prediction accuracy of GAN-GMHI has been significantly  
109 improved to 88.70% for distinguishing healthy individuals and non-healthy individuals, compared  
110 to the accuracy of 70.95% achieved by GMHI, and GAN-GMHI still outperforms GMHI on the  
111 independent validation cohort (**Supplementary Table 1**).

112

113 Moreover, GAN is not only applicable for GMHI disease prediction model, but could also be  
114 easily adapted to other models, such as Random Forest (RF). It has already been observed that  
115 GMHI and RF have similar performances on the validation datasets, while GMHI's results are  
116 easier for clinical interpretation (**Supplementary Table 1**). We emphasize that although the results  
117 of GAN-GMHI and GAN-RF also have similar accuracies on the validation datasets, GAN-GMHI  
118 has inherited the interpretability of the GMHI method, and thus is more suitable for clinical  
119 interpretation. For example, on Type 2 diabetes (T2D), GAN-GMHI has captured *Lactobacillus* as  
120 biomarkers, which are well founded by published works (Wang, et al.).

121

## 122 **Conclusion**

123 The association of gut microbiome and diseases has been proven for many diseases, while  
124 transformation of such association to a robust and universal disease prediction model has  
125 remained illusive, largely due to the batch effects presents in multiple microbiome cohorts.  
126 GAN-GMHI is a novel method built based on the batch corrected data through GAN, as well as  
127 GMHI for prediction of a broad spectrum of diseases. Results have shown that it has largely  
128 reduced the batch effects, and profoundly improved the performance for distinguishing disease  
129 and healthy individuals. In summary, Generative Adversarial Network augmented the gut  
130 microbiome-based health index, and GAN-GMHI has indicated a more accurate venue towards  
131 microbiome-based disease monitoring.

132

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135

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141 *Conflict of Interest: none declared.*

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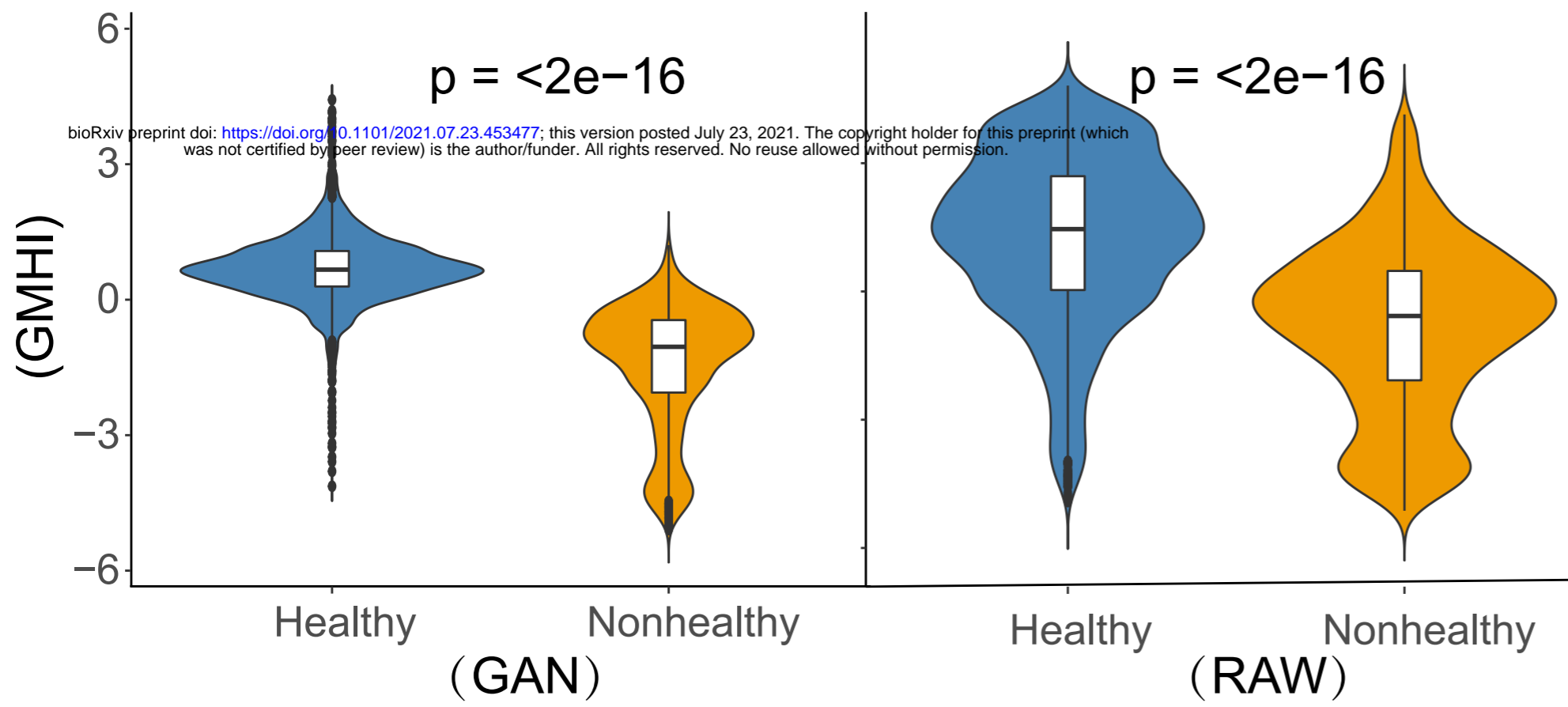
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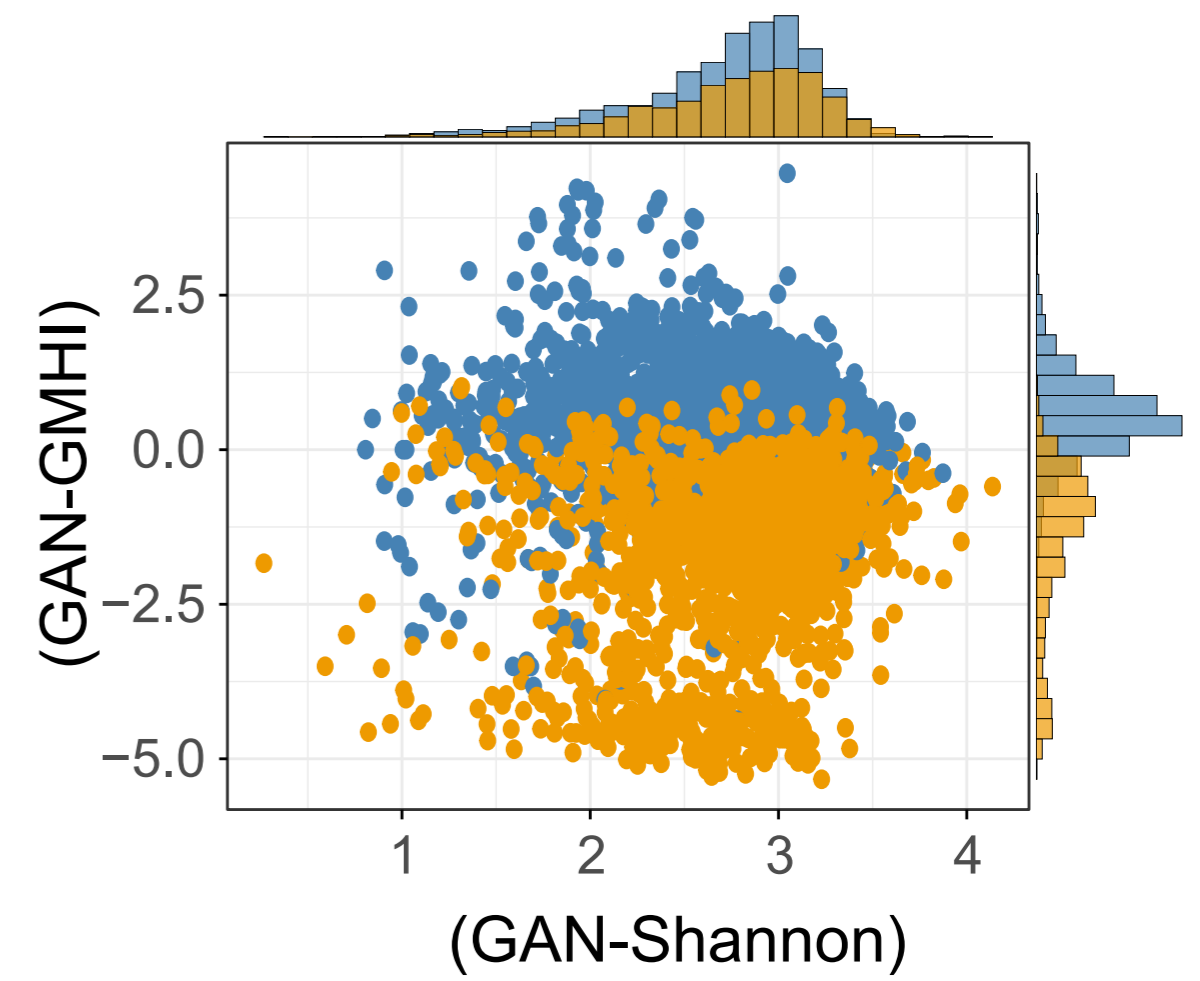
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155

(A)



(B)



(C)

