

Infant Gut Microbiome Association with Emotional Behavior

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Abstract

Previous Studies demonstrate associations between the gut microbiota and cognition in human infants, which microorganisms inhabiting the gut influence neurodevelopment [1]. In current study, we tested whether microbial composition at neonate and year 1 of age are associated with emotional behavior tests. There are 18 neonates and 14 typically developing 1-year-olds infants have completed both microbial and behavioral tests. Different models (simple linear regression, linear mixed effect regression, multiple linear regression, COX regression, COX mixed regression, PCA) were adopted to identify association of microbiome diversity and emotional behavior. The results consistently demonstrated the shann. metrics of alpha-diversity in neonate group have significantly association with behavior results. And Wu.P1 metrics of beta -diversity in year 1 group has significant association. The observations give us a clue to understand the dynamic relationship of gut colonization and the neuro-system development.

1. Introduction

The first years of human life are a dynamic time in gut colonization and brain development, but little is known about the relationship between these two processes. The gut microbiome is a complex microbial ecosystem that varies between individuals and may be a key modulator of neurodevelopment through the microbiome-gut-brain axis. The first year of life is the foundational period for microbial colonization of the gut. In current study we test if the microbial diversity has association with the emotional behavior with linear regression.

1.1 Explanatory Variables: Microbial diversity

The diversity of microbiome results are summarized into alpha and beta categories. The alpha diversity is used to measure the diversity within a sample. It is calculated as a value for each sample. Different metrics are developed to calculate diversity in different ways. There are four used diversity metrics [2].

Beta diversity is a term for the comparison of samples to each other. It provides a measure of the distance or dissimilarity between each sample pair. (Table 1.)

Diversity	Metrics Name	Meaning
Alpha	Obs_sp	Measure richness only
	Chao1	Measures richness and evenness
	Shan.	Measures richness and evenness
	PD.wh	Measures richness and evenness
Beta	Wu.P1	Weighted UniFrac first principle component of PCA
	Wu.P2	Weighted UniFrac second principle component of PCA

TABLE 1. Different microbial diversity, their meaning and abbreviations used in the study.

1.2 Response Variables: Emotional behaviors Testing: Mask Task

Previous research has indicated that the incongruity inherent in viewing a mask elicits fear in some children. Here multiple mask tests were performed to test the children emotional control and response. This episode provides such an opportunity for the expression of fear in a non-social context with relatively mild, non-intrusive stimulation. 4 masks presented to subject and name of subject said 3 times. Approximately 7 seconds in every mask, the outcome of emotional behavior was coding by review the video and base on below criterial:

Characteristics	Range	Description
Latency to Fear Response	1-7 " and 12	1 sec is an immediate or ongoing response, other time marks start of first fear response that is a 2 or above in Facial, Vocal, or Bodily fear. "12" means no latency to fear response
Intensity of Facial Fear	0-3	0 – no response 1 – only one facial region shows codable movement, expression is different from baseline but ambiguous 2 – 2 facial regions show codable movement, or expression in one region is very clear – not an extreme fear reaction 3 – facial fear reaction with all 3 facial regions (brows, eyes, mouth) showing strong facial fear
Intensity of Vocal Distress	0-3	0 – no response 1 – Mild vocalization that may be difficult to identify, whimpering, limited short duration 2 – Longer low intensity cry to non-muted crying 3 – Full intensity cry/scream – subject is losing it
Intensity of Bodily Fear	0-3	0 – No response 1 – Decreased activity: sudden decrease from baseline of previous episode, will be coded often – probably not meaningful for measuring fear/anxiety 2 – Tensing: muscles tense, along with decreased movement 3 – Freezing/trembling: more pronounced than 2, often involves entire body, trembling from extreme tension
Intensity of Escape Behavior	0-3	0 – no behavior 1 – Mild: turning away or sinking in chair (in combo with distress, not turning around smiling at mom) 2 – Moderate escape: full body movements, arching back 3 – Vigorous escape: linked, continuous full body movements, arching back, attempts to escape (not common)

TABLE 2. The Coding of Behavioral Testing: Mask Task, and the description of the variables.

2. Method

2.1 Study Population

The neonates were enrolled a prospective longitudinal study of early brain development at the University of North Carolina at Chapel Hill. Exclusion criteria for the parent studies included fetal ultrasound abnormalities and major medical illness of the mother. Informed written consent was obtained from the parent/legal guardian of each subject. Microbiome analysis was performed on neonates. One year later, the infants were followed up

and microbiome analysis was performed again and emotional behaviors were tested. Finally, we have 18 neonates and 14 1-year-olds children who have both microbial and behavior test results.

2.2 Microbiome Analysis

Parents collected approximately 200 mg of fecal material from a single diaper, placed it in a tube filled with Allprotect reagent (Valencia, CA). 16S ribosomal RNA amplicon sequencing of the V1-V2 gene region was performed on the MiSeq platform (Illumina, San Diego, CA) for identification and relative quantification of bacterial taxa. Bioinformatics was performed with Quantitative Insights into Microbial Ecology software. Taxonomic assignment and alpha and beta diversity analysis were performed using Quantitative Insights into Microbial Ecology software.

2.3 Data Manipulate

Due to every subject has four episodes' mask task, and every subject has four behavioral test results. Therefore, the behavioral data was manipulated to meet the request of different analysis models.

- 1). Aggregate the four behavioral data with max, average and sum within subject.
- 2). Append the four test results in time sequence within same subject.

2.4 Linear Regression Model

Simple linear regression analysis (Univariate) is performed to determine how changes in the microbial diversity (predictor variables) are associated with changes in the aggregated behavioral results (dependent variable) respectively. Results are shown in section 3.2.

2.5 Linear Mixed Effects Model (LMM)

Linear mixed models are an extension of simple linear models to allow both fixed and random effects. LMM is particularly used when there is dependence in the data, such as arises from a hierarchical structure. Due to every subject having four mask task tests, the individual subject test results are multiple levels. The variability in the outcome can be thought of as being either within subjects or between subjects. Subject level observations are not independent, as within a subject mask task tests are more similar. The core of mixed models is that they incorporate fixed and random effects. In this study, the fix effect will be the subject level association between microbiome and behaviors. And within subject are set to random effects. With these assumptions, LMMs allow us to explore and understand the difference between effects within and between subjects.

2.6 Cox and Mixed effects Cox regression model

Maximum of latency fear response is 7 second, and time last longer than 7 second will non information. Therefore, the variable data is censoring. Cox regression is method of survival analysis for investigating the

effect of variables upon the time a specified event takes to happen, which is utilized to analyze the association of microbiome diversity and latency in the aggregated data.

Mixed effects cox regression models combine the idea of Cox and LMM. It is used to model survival data when individuals are nested within some other hierarchy, or some other reason to have both fixed and random effects. Mixed Cox Model is utilized to explore the correlation of microbiome and fear response latency at subject level. Results are shown in section 3.4.

2.7 Multiple Linear Regression

Multiple linear regressions were fit to investigate the association of combination of alpha and beta diversities with the subject emotional behavior. Because we find the highly correlation between the alpha diversity so every time only one alpha diversity was involved in the model. Beta diversity variable is the result of PCA, so the independent each other and alpha diversity variables. All two beta variables were involved in the model. Summarily, in the model the predict variable is one alpha plus two beta diversity variables, and response variable is behavioral test result.

2.8 Principle Component Analysis (PCA)

PCA is a statistical procedure that uses an orthogonal transformation to convert a set of observations of variables into a set of values called principal components. In current study, there are six behavior variable. When regression is performed, only one variables can be worked as response variable. With PCA, we use will first three components to do the regression. Results are shown in section 3.6.

2.9 Multiple Comparisons¹

When we do multiple statistical tests, some may have p value less than 0.05 purely due to chance and lead to false positive results. To address this issue, we use Bonferroni adjustment and also adjusted false discovery rate to take this into account.

Bonferroni adjustment is a common way to control the familywise error rate. We find the critical value (alpha) for an individual test by dividing the familywise error rate (usually 0.05) by the number of tests. An alternative approach is to control the false discovery rate (FDR), Benjamini-Hochberg², the proportion of “discoveries” (significant results) that are actually false positives. Bonferroni adjustment is used here.

2.9 Statistical Software

R version 3.4.0 is used for data analysis. Packages used include: “lme4”, “Coxme”, “Coxph”, “gplot”.

3. Results

¹ <http://www.biostathandbook.com/multiplecomparisons.html>

² <http://www.statisticshowto.com/benjamini-hochberg-procedure/>

3.1 Data overview

- Microbial Diversity Variables correlation

Figure 1 shows the person's correlation between different groups' microbial diversity. The subjects are divided into two groups. Neonate group is labeled within red box. Year 1 group is within green box. Every group subjects have six microbial diversity variables. First four are alpha diversity (Black box). Last two are beta diversity (Yellow box). The correlation of diversity was analyzed in pairwise way. As we expected, within group, the four alpha diversity are correlated, and beta diversity are not show strong correlation. Between groups there are no significant correlation. In figure 1, the larger font of the number represents stronger correlation.

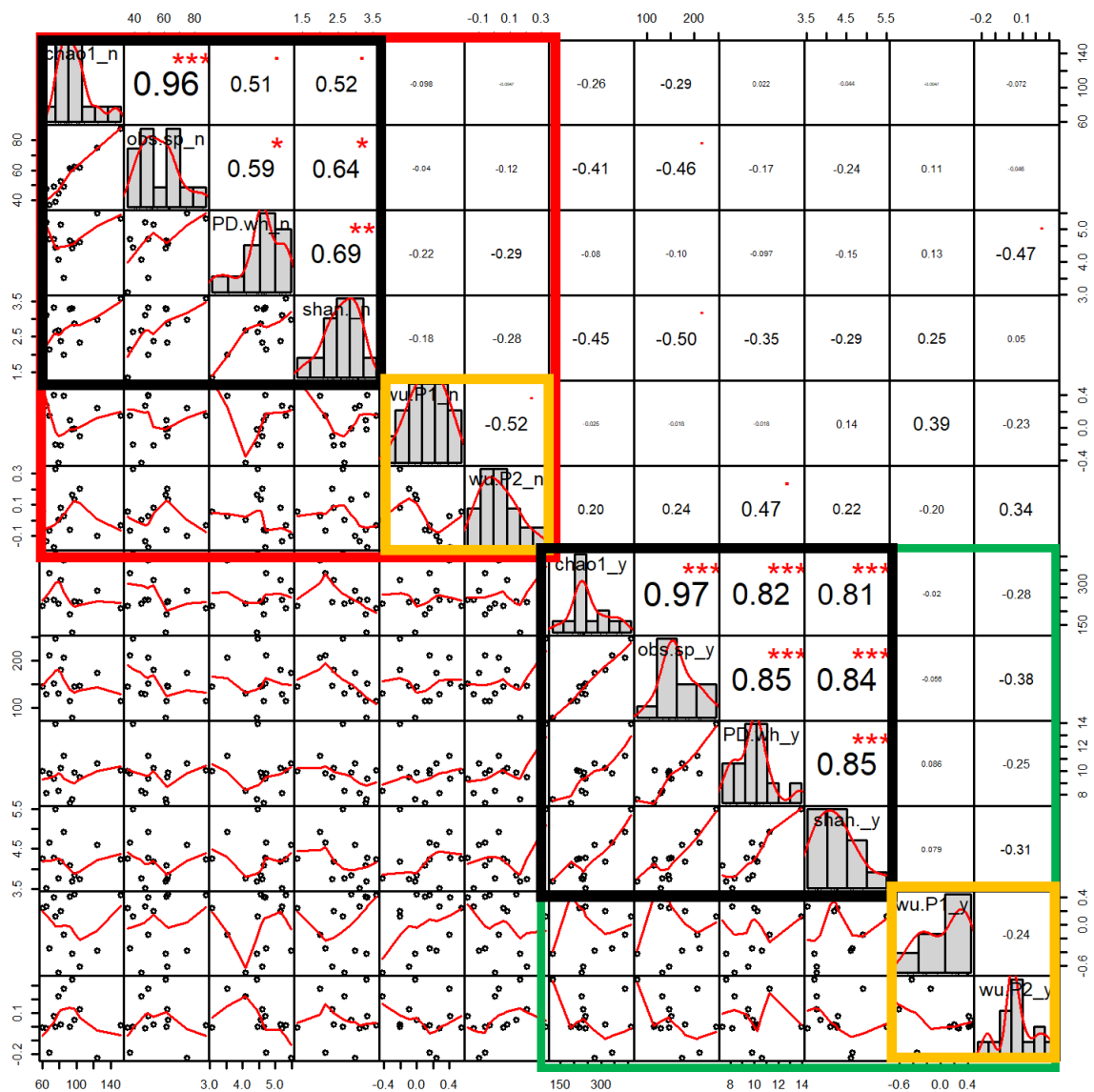


Figure 1. The correlations of microbial diversity variables. The four alpha diversities are correlated with each other. And alpha and beta diversity variables do not show obvious correlation. Significant

code: '***':0.001; '**':0.01; '*':0.05; '.':0.1.

- Behavioral Variables boxplot and correlation

There are five behavioral variables presents the subject emotion behavioral activity in mask task test. The dataset was summarized in 4 mask test episodes (Figure 2) of box plot. The blue point represents subjects. The black bar within box is the media of every group. We can find the median of latency variable tend to get small with the mask test. And ‘Facial’ and ‘vocal’ variables tend to get larger. However, “Body” and “Escape” variables do not show obvious tendency.

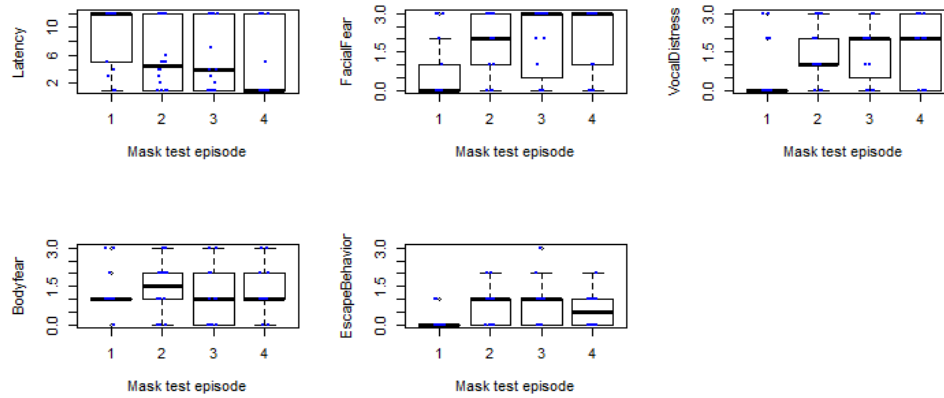


Figure 2. The behavioral test results were show in the level of four mask test episodes. The y-axis is the behavioral test and x-axis is the four mask test episodes’ group.

The person’s correlation analysis was performed on the five behavior variable (Figure 3). Correlation between the behavioral variables are observed. And all correlations are significant.

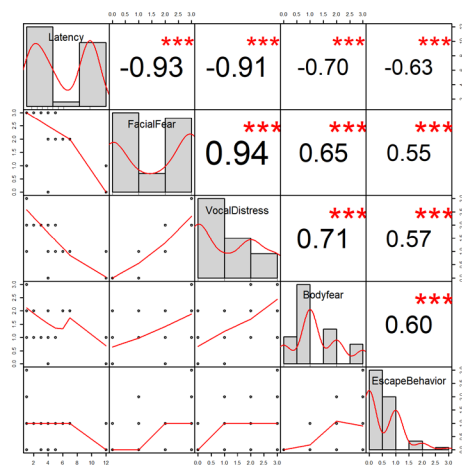


Figure 3. The correlations of behavioral variables. The behavioral variables are highly correlated.

- Missing value

The Microbial diversity data do not have missing value. For behavior data, 4 of 19 subjects have missing value. And 3 subjects (ID:1,24,38) miss 3th and 4th episodes' mask test data, due to the test stop at 1 and 2. 1 subject (ID: 16) miss 4th episode's mask test data. And the missing part value will be removed when perform the analysis.

3.2 Simple linear regression model of aggregated mask task data

Simple linear regressions were performed based on the aggregated behavioral data and microbial diversity. The coefficients estimation of each regression and raw p values for association were shown as heat-maps. The p-value for each term tests the null hypothesis that the coefficient is equal to zero (no association). A low p-value (< 0.05) indicates that you can reject the null hypothesis. In other words, changes in the predictor's value are related to changes in the response variable.

Comparing the raw p value heat-maps of the two groups, there are dramatically difference. In the neonate group, the Shann. of alpha diversity shows a relative smaller p value in all the behavioral test results. However, in the year 1 group, the Wu.P1 of beta diversity demonstrates a smallest p value with the behavioral test data. Specifically, from the heat map, the row of alpha diversity Shan. is the only one the p value is smaller than 0.2. And variable Max_Body has smallest value (0.01) (Figure 4B). On the contrary, in the year 1 group, the row of beta diversity Wu.P1 is the only one significantly associated to most behavioral variables (most columns), which p value smaller than 0.05. And variable Ave_Escape has smallest p value < 0.005 (Figure 6B). Moreover, shan. of alpha diversity have negative coefficients in all regression against behavioral variables in neonate group. The negative coefficient indicates that as the value of the predictor variable (microbial diversity) increases, the mean of the dependent variable (behavior) tends to decrease. Similarly, the beta diversity Wu.P1 is also negative coefficients in regression model. From biological interpretation, the higher microbial diversity associated with more stable emotional behavior.

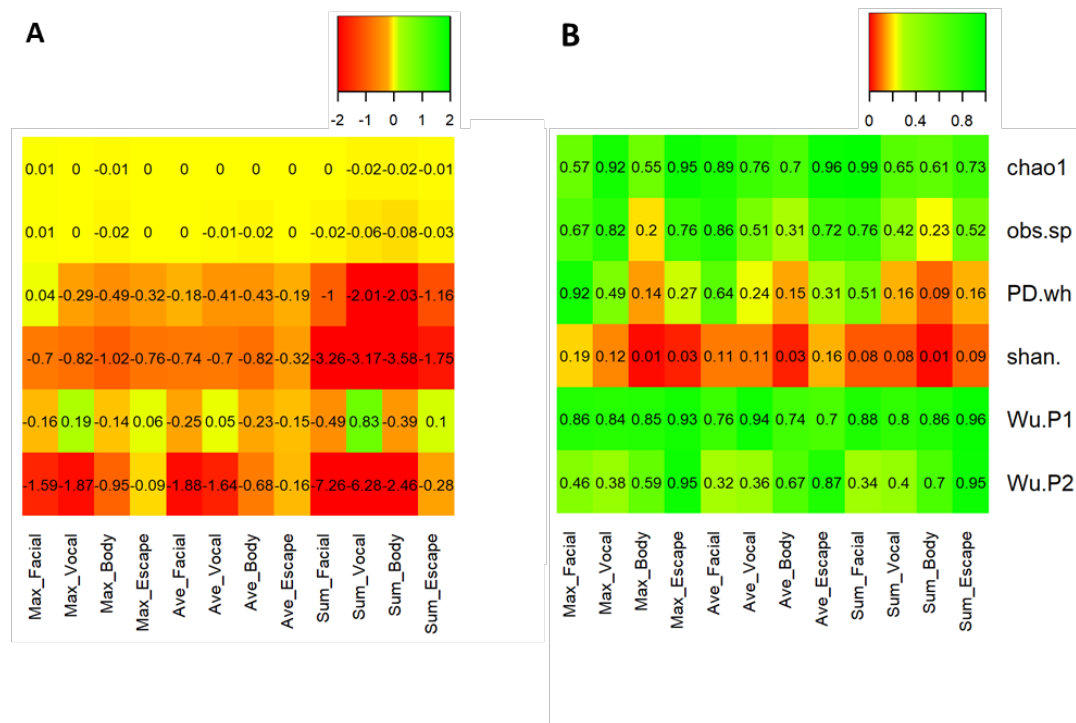


FIGURE 4. A) Coefficients estimation of simple linear regression model of microbiome diversity (row) and behavior variable (column) in Neonates group. B) The raw p value of the estimation.

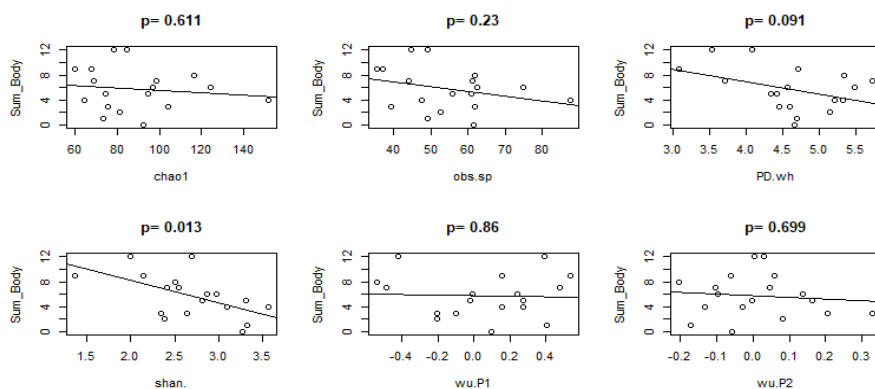


FIGURE 5. Scatter plot of linear regression with sum_Body variable against all six microbial diversity variables in neonate group.

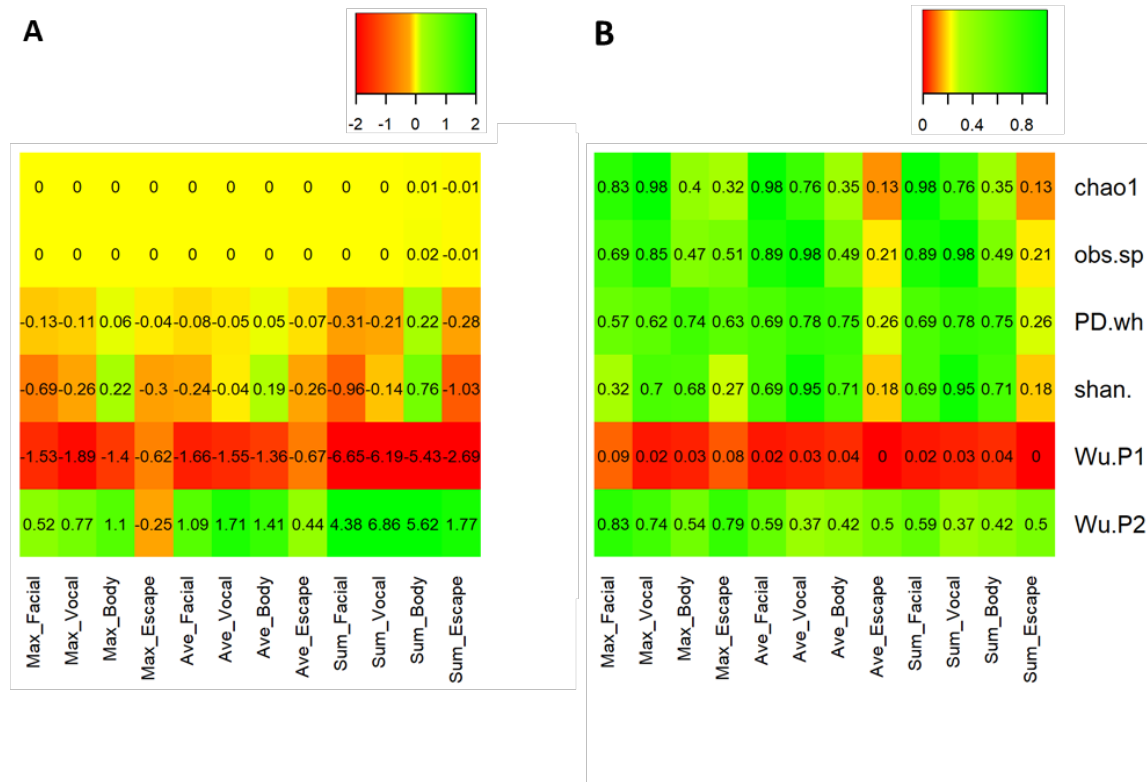


FIGURE 6. A) Coefficients estimation of simple linear regression model of microbiome diversity (row) and behavior variable (column) in year 1 group. B) The raw p value of the estimation.

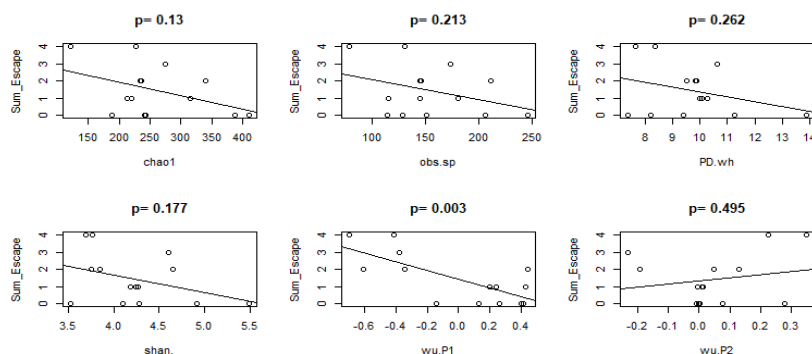


FIGURE 7. Scatter plot of linear regression with sum_Escape variable against all six microbial diversity variables in year 1 group.

Residual plots can be used to assess the quality of a regression. You can examine the underlying statistical assumptions about residuals such as constant variance, independence of variables and normality of the distribution. For these assumptions to hold true for a particular regression model, the residuals would have to be randomly distributed around zero.

Different types of residual plots can be used to check the validity of these assumptions and provide information on how to improve the model. For example, the scatter plot of the residuals will be disordered if the regression is good. The residuals should not show any trend. A trend would indicate that the residuals were

not independent. On the other hand, QQ-normal plot lies on a straight diagonal line, indicating that the normality assumption is likely to be true. We show above linear regressions' residual plots, most of them holds the assumptions (appendix Figure 11).

3.3 Linear Mixed Effects Model

LMM were performed to fit the separate of behavioral test results within subject with microbial diversity. The coefficients of fixed part of each regression and raw p values with LMM were shown in same way of simple linear regression. As expect, the results are strongly consistent with simple linear regression. Furthermore, stronger significant association was observed in both neonate and year 1 group.

In neonate group: The Shan. of alpha diversity shows a relative high significant relationship with all the behavioral test data. Moreover, the much smaller p value is observed compare to the corresponding simple linear regression (Figure 8B).

In year 1 group: Similar to simple linear regression, the Wu.P1 of beta diversity demonstrates a highest association the behavioral test data. And the p value generally smaller than simple linear regression, which is same to neonate. Two significant variables even after Bonferroni correction are: Facial ($p = 0.001$) and Escape ($p = 0.0001$). The coefficients of LMM model have similar results of simple linear regression (Figure 9B).

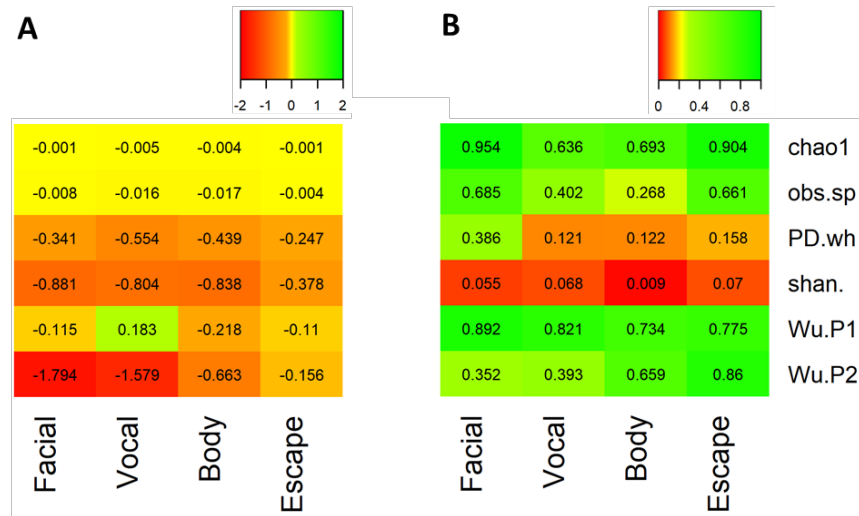


FIGURE 8. A) Coefficients estimation of LMM model of microbial diversity (row) and behavioral variable (column) in neonate group. B) The raw p value of the estimation.

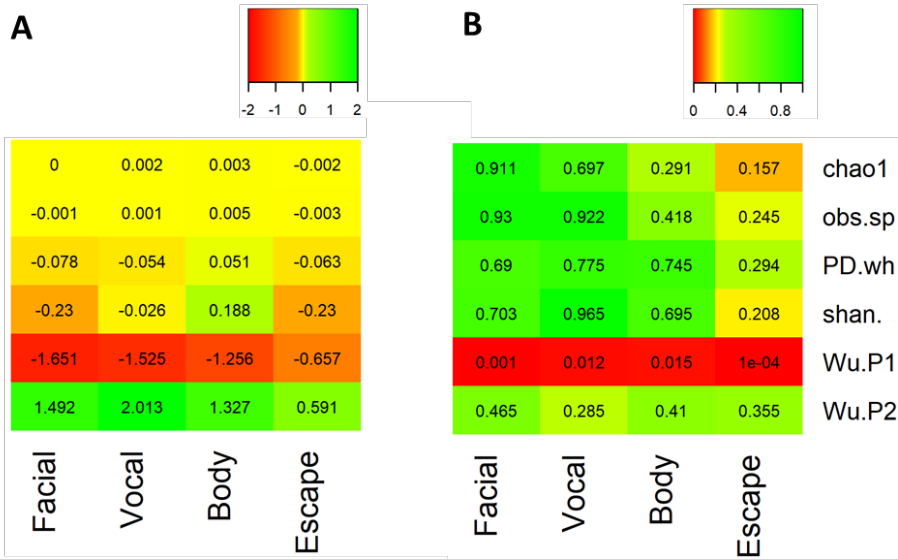


FIGURE 9. A) Coefficients estimation of LMM model of microbial diversity (row) and behavioral variable (column) in neonate group. B) The raw p value of the estimation.

3.4 Cox and Mixed effects Cox regression model

The ‘Latency’ variable was censored. There are 3 subjects (ID: 7,8,30) have reached maximum time (7’’) and no more information after that. The Cox regression was conducted. The results are consistent with the results of other linear regressions. The Shan. diversity association to segregate latency in the neonate group have smallest p value, although it is not significant (Table 3). The Wu.P1 row has smaller p value in year 1 group. The Cox mixed-effects model has similar results of Cox model. As expected, Cox mixed-effects model generates more significant results than Cox model, especially in year 1 group; the p value is 0.0037 (Table 4) vs 0.026 in Cox model.

As expected, shan. of alpha diversity have negative coefficients in Cox regression in neonate group. It can be interpreted that exponent of coefficient small than 1, which means that an increase in one-unit microbial diversity will decrease the probability of subject ending experiment throughout the observation period. That means the increase microbial diversity associated with decrease of infants’ fear behavior.

		Max		Average		Sum	
		coefficients	p value	coefficients	p value	coefficients	p value
Neonate (Latency)	Chao1	-0.002	0.834	0	0.77	0	0.89
	Obs.sp	-0.004	0.846	0	0.8	0	0.92
	PD.wh	-0.138	0.774	-0.2	0.67	-0.46	0.38
	Shan.	-0.84	0.08	-0.7	0.14	-0.96	0.066
	Wu.P1	0.265	0.797	0.28	0.79	0.5	0.63
	Wu.P2	-1.06	0.547	-1	0.56	-0.85	0.63
Year 1 (Latency)	Chao1	0	0.931	0	0.77	0	0.77
	Obs.sp	-0.002	0.826	0	0.69	0	0.69
	PD.wh	-0.168	0.702	-0.07	0.7	-0.07	0.7
	Shan.	-0.515	0.44	-0.47	0.5	-0.45	0.5
	Wu.P1	-1.42	0.09	-1.97	0.026	-2	0.026
	Wu.P2	0.176	0.951	2.39	0.52	2.35	0.52

TABLE 3. Coefficients estimation and the raw p value of COX regression model of microbial diversity (row) and behavioral variable (column) in neonates and year 1 group.

	Neonate (Latency)		Year 1 (Latency)	
	coefficients	p value	coefficients	p value
Chao1	7.18	0.99	0	0.86
Obs.sp	0.004	0.84	0	0.74
PD.wh	-0.25	0.53	-0.14	0.55
Shan.	-0.76	0.11	-0.35	0.61
Wu.P1	-0.43	0.61	-1.91	0.0037*
Wu.P2	-1.7	0.39	2.27	0.28

TABLE 4. Coefficients estimation and the raw p value of COX mixed-effect regression model of microbial diversity (row) and behavioral variable (column) in neonates and year 1 group.

3.5 Multiple Linear Regression

The results of both neonate and year 1 group multiple linear regressions were show in table of appendix. In neonate group alpha diversity PD.Wh demonstrate highly significant correlation with body variables and with the combination of Wu.P1 and Wu.P2. And Shan. demonstrate highly association with body variables.

In year 1 group beta diversity variable Wu.P1 demonstrate highly significant correlation with Ave_Escapel and Sum_Escape. We can tell from the results of multiple linear regression are highly consistent with simple linear regression (appendix Table 7).

3.6 Principle Component Analysis

From previous analyses, we find that Shan. of neonate group and Wu.P1 of year 1 group universally have a higher significant association with behavioral variables in different models. However, due to multiple comparison adjustment, the results significant power further decrease. Therefore, we performed the PCA in the

five behavioral variables, and only take the first 3 components to do the linear regression with diversity variables respectively. Two advantage of such approach are: 1). PCA components combine all behavioral variable information, and first one has most information. 2). The results do not need the multiple comparison adjustment. From below results, we do observe the first component in different aggregate groups all demonstrate significant results. However, for next two components in every group, none of them is observed significant results. In neonate group, the smallest p value is in the Max group.

		MAX			AVERAGE			SUM		
		PCA1	PCA2	PCA3	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
Neonate (Latency)	Chao1	0.946	0.413	0.341	0.849	0.713	0.291	0.711	0.852	0.253
	Obs.sp	0.712	0.252	0.196	0.518	0.597	0.149	0.408	0.731	0.169
	PD.wh	0.343	0.98	0.138	0.24	0.998	0.103	0.147	0.83	0.112
	Shan.	0.033	0.972	0.211	0.047	0.816	0.808	0.028	0.662	0.993
	Wu.P1	0.919	0.373	0.9	0.809	0.988	0.535	0.991	0.784	0.845
	Wu.P2	0.486	0.408	0.692	0.444	0.812	0.616	0.495	0.73	0.649
Year 1 (Latency)	Chao1	0.887	0.064	0.847	0.907	0.023	0.607	0.907	0.023	0.607
	Obs.sp	0.82	0.096	0.732	0.971	0.076	0.511	0.971	0.076	0.511
	PD.wh	0.71	0.49	0.749	0.725	0.235	0.797	0.725	0.235	0.797
	Shan.	0.532	0.096	0.793	0.764	0.094	0.605	0.764	0.094	0.605
	Wu.P1	0.026	0.89	0.813	0.007	0.153	0.512	0.007	0.153	0.512
	Wu.P2	0.784	0.54	0.71	0.389	0.971	0.317	0.389	0.971	0.317

TABLE 5. First three PCA components of behavioral results is performed simple linear regression with diversity variables. The p value of coefficients estimation was shown by diversity variables (row) and behavior variables PCA components (column).

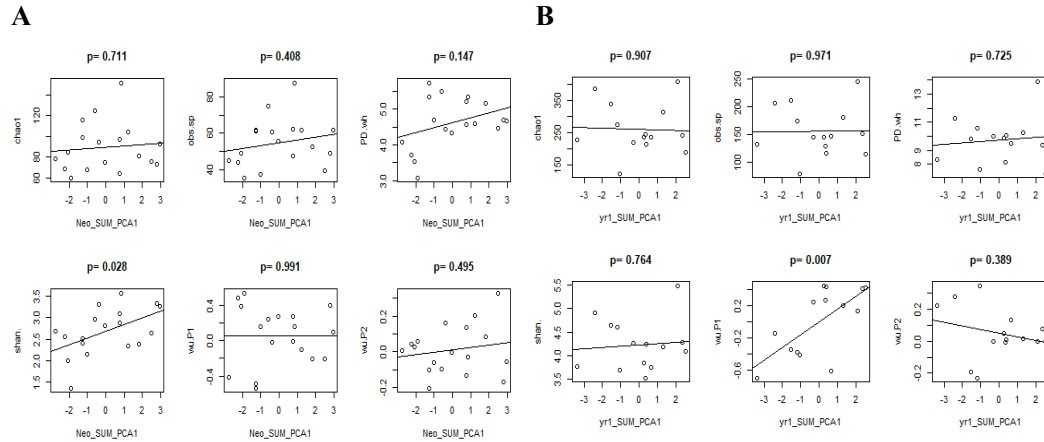


TABLE 10. A. First Sum PCA components of behavioral results in neonate group is performed simple linear regression with diversity variables. B. First Sum PCA components of behavioral results in Year 1 group is performed simple linear regression with diversity variables.

4. Summary

In this report, different models utilized to test the correlation between the microbiome and infant emotional behavior. By compare the respective results of neonate and year 1 group, there are several important observations:

1. Association between microbial diversity and behavior is different in neonates and year 1 group: Shann. and Wu.P1 shows stronger association with behavior in neonate group and year 1 group.
2. Different model gives consistent results.
3. The LMM model got more significant results compare to simple linear regression.

All of the regression information with raw p value smaller than 0.05 are listed below. Bonferroni adjustment is a common way to control the familywise error rate. In our case, we have 5 tests, so critical value for the individual test will be 0.01. Last column showed that significant results after Bonferroni correction in table6:

Model	Group	Diversity	Behavior	p value	Adjusted p value
LM	Neonate	Shan.	Max_Body	0.01	0.05
			Max_Escape	0.032	0.16
			Average_Body	0.026	0.13
			Sum_Body	0.017	0.085
	Year 1	Wu.P1	Max_Vocal	0.021	0.105
			Max_Body	0.03	0.15
			Average_Facial	0.022	0.11
			Average_Vocal	0.027	0.135
			Average_Body	0.037	0.185
			Average_Escape	0.004	0.02
LMM	Neonate	Shan.	Body	0.009	0.045
	Year 1	Wu.P1	Facial	0.001	0.005
			Vocal	0.012	0.06
			Body	0.015	0.075
			Escape	0.0001	0.0005

TABLE 6. Significant correlations identified for each behavior results with diversities and their test p value with Bonferroni adjustment. EscapeBehavior against beta diversity with LMM model in year 1 group and EscapeBehavior against Shannon diversity with LMM model in neonate group have the lowest p values.

5. Reference

- [1] Alexander L. Carlson, Kai Xia ect. Infant Gut Microbiome Associated With Cognitive Development. Biological Psychiatry. January 15, 2018Volume 83, Issue 2, Pages 148–159
- [2] Xochitl C. Morgan, Curtis Huttenhower. Human Microbiome Analysis. PLOS. Published: December 27, 2012. <https://doi.org/10.1371/journal.pcbi.1002808>

6. Appendix

6.1 Microbiome Diversity data set:

Neonate group:

SUBID	chao1	obs.sp	PD.wh	shan.	wu.P1	wu.P2
1	78.52714	44.8	4.078069	2.687361	-0.4191	0.004521
6	94.88952	60.9	4.425893	3.308986	-0.01785	0.163372
7	75.55	39.2	4.45345	2.645096	-0.2028	0.3301
8	92.46845	61.5	4.656041	3.270525	0.096872	-0.05754
12	67.92	37.1	4.704867	2.143581	0.155399	-0.06146
13	98.64543	61.1	5.73443	2.412939	-0.4859	-0.10372
14	124.4354	74.9	5.480818	2.971191	0.24005	-0.09666
16	59.945	35.4	3.07852	1.363877	0.539767	0.058932
23	97.06762	62.4	4.558061	2.874473	-0.00557	0.137654
24	84.64143	49	3.524231	1.989776	0.392024	0.029151
26	81.25472	52.7	5.147565	2.389763	-0.20628	0.082926
29	103.9005	61.9	4.602313	2.337947	-0.0985	0.205139
30	73.32992	49.1	4.693625	3.33095	0.403339	-0.17211
32	64.15937	47.4	5.210736	3.101029	0.276075	-0.13174
36	151.9264	87.8	5.329134	3.571283	0.15418	-0.02989
38	68.88679	44.1	3.71589	2.54755	0.480181	0.044438
39	116.2723	61.8	5.335957	2.511996	-0.53825	-0.20475
45	74.66934	55.8	4.338991	2.810049	0.274248	-0.0017

Year 1 group:

SUBID	chao1	obs.sp	PD.wh	shan.	wu.P1	wu.P2
1	227.6644	131.3	8.36382	3.760285	-0.70107	0.223364
6	121.4911	79.1	7.642605	3.68906	-0.41721	0.349383
7	410.577	245.9	13.90691	5.493653	0.127618	-0.0021
8	188.373	114.4	7.348635	4.093201	0.415342	0.002257
12	339.9649	210.9	9.80586	4.652661	-0.34635	-0.19269
14	274.7382	173.4	10.60556	4.606313	-0.37886	-0.23058
19	219.6534	145.4	10.0016	4.262055	0.24435	-0.00308
23	236.5775	144.8	9.881551	3.847816	0.447123	0.049583
24	386.6384	206.3	11.26585	4.914776	-0.14529	0.278508
26	314.3932	180.3	10.2726	4.182712	0.198	0.014146
29	234.2751	146.4	9.501476	3.751231	-0.61126	0.130728
30	241.218	150.8	9.377224	4.281201	0.404884	0.076402
32	242.8423	128.5	8.183998	3.523464	0.264607	-0.00928
36	212.3631	115.2	10.07344	4.239186	0.433524	0.01099

6.2 Behavioral data set:

Group	SUBID	Mask	Latency	Facial	Vocal	Body	Escape
1 and 2	1	1	1	3	3	3	1
	1	2	1	3	3	3	1
	1	3	NA	NA	NA	NA	NA
	1	4	NA	NA	NA	NA	NA
1 and 2	6	1	4	0	0	2	1
	6	2	5	2	1	1	1
	6	3	3	3	2	1	1
	6	4	1	3	3	1	1
1 and 2	7	1	12	0	0	1	0
	7	2	12	0	0	1	0
	7	3	12	0	0	0	0
	7	4	12	0	0	1	0
1 and 2	8	1	12	0	0	0	0
	8	2	12	0	0	0	0
	8	3	12	0	0	0	0
	8	4	12	0	0	0	0
1 and 2	12	1	12	1	0	1	0
	12	2	4	3	2	2	0
	12	3	1	3	2	3	1
	12	4	1	3	3	3	1
2	13	1	3	3	2	1	0
	13	2	1	3	2	2	1
	13	3	1	3	2	2	1
	13	4	1	3	2	2	1
1 and 2	14	1	12	0	0	1	0
	14	2	3	3	2	1	1
	14	3	1	3	3	2	1
	14	4	1	3	2	2	1
2	16	1	12	0	0	1	0
	16	2	2	3	2	2	1
	16	3	1	3	3	3	3
	16	4	NA	NA	NA	NA	NA
1 and 2	19	1	12	0	0	1	0
	19	2	6	3	2	1	0
	19	3	1	3	2	1	0
	19	4	1	3	2	1	1
1 and 2	23	1	12	0	0	1	0
	23	2	6	2	1	2	1
	23	3	7	2	1	2	1
	23	4	12	1	0	1	0

1 and 2	24	1	1	3	3	3	0
	24	2	1	3	3	3	0
	24	3	NA	NA	NA	NA	NA
	24	4	NA	NA	NA	NA	NA
1 and 2	26	1	12	0	0	1	0
	26	2	12	0	0	0	0
	26	3	12	0	0	0	0
	26	4	1	3	2	1	1
1 and 2	29	1	12	0	0	1	0
	29	2	5	2	1	1	1
	29	3	4	2	1	0	0
	29	4	12	1	0	1	1
1 and 2	30	1	12	0	0	1	0
	30	2	12	0	0	0	0
	30	3	12	0	0	0	0
	30	4	12	0	0	0	0
1 and 2	32	1	12	0	0	1	0
	32	2	12	1	1	1	0
	32	3	4	3	2	1	0
	32	4	1	3	2	1	0
1 and 2	36	1	12	0	0	1	0
	36	2	5	2	1	1	0
	36	3	12	1	1	1	1
	36	4	5	3	2	1	0
2	38	1	5	2	2	1	1
	38	2	1	3	3	2	2
	38	3	NA	NA	NA	NA	NA
	38	4	NA	NA	NA	NA	NA
2	39	1	12	0	0	1	0
	39	2	4	3	2	2	1
	39	3	1	3	2	2	2
	39	4	1	3	3	3	2
2	45	1	12	0	0	1	0
	45	2	1	1	1	2	2
	45	3	2	3	2	1	1
	45	4	1	3	3	1	0

Note: For column group, number 1 is year 1 group and 2 is neonate group.

6.3 Multiple Linear Regression Results

Behavior	Diversity	Estimate	p value	Diversity	Estimate	p value	Diversity	Estimate	p value	Diversity	Estimate	p value
Max_Facial	chao1	0.005	0.725	obs.sp	0.006	0.789	PD.wh	-0.253	0.665	shan.	-0.789	0.149
	wu.P1	-0.011	0.991	wu.P1	-0.072	0.939	wu.P1	-0.414	0.717	wu.P1	-0.201	0.816
	wu.P2	-1.502	0.505	wu.P2	-1.506	0.508	wu.P2	-2.204	0.396	wu.P2	-2.229	0.295
Max_Vocal	chao1	-0.001	0.956	obs.sp	-0.008	0.736	PD.wh	-0.749	0.189	shan.	-0.912	0.093
	wu.P1	0.24	0.809	wu.P1	0.192	0.84	wu.P1	-0.603	0.58	wu.P1	0.168	0.842
	wu.P2	-1.89	0.408	wu.P2	-2.028	0.377	wu.P2	-3.566	0.158	wu.P2	-2.56	0.223
Max_Body	chao1	-0.009	0.43	obs.sp	-0.027	0.144	PD.wh	-1.112	0.008	shan.	-1.109	0.006
	wu.P1	-0.345	0.66	wu.P1	-0.362	0.613	wu.P1	-1.419	0.065	wu.P1	-0.25	0.665
	wu.P2	-1.208	0.502	wu.P2	-1.509	0.381	wu.P2	-3.493	0.046	wu.P2	-1.815	0.205
Max_Escape	chao1	0	0.964	obs.sp	-0.004	0.812	PD.wh	-0.586	0.165	shan.	-0.763	0.055
	wu.P1	0.166	0.821	wu.P1	0.143	0.84	wu.P1	-0.496	0.537	wu.P1	0.102	0.866
	wu.P2	-0.117	0.944	wu.P2	-0.188	0.911	wu.P2	-1.432	0.43	wu.P2	-0.683	0.643
Ave_Facial	chao1	-0.002	0.899	obs.sp	-0.009	0.644	PD.wh	-0.73	0.131	shan.	-0.872	0.057
	wu.P1	-0.322	0.704	wu.P1	-0.363	0.656	wu.P1	-1.125	0.228	wu.P1	-0.372	0.598
	wu.P2	-1.958	0.319	wu.P2	-2.103	0.286	wu.P2	-3.571	0.097	wu.P2	-2.579	0.144
Ave_Vocal	chao1	-0.005	0.642	obs.sp	-0.017	0.39	PD.wh	-0.993	0.025	shan.	-0.801	0.071
	wu.P1	-0.091	0.91	wu.P1	-0.104	0.892	wu.P1	-1.109	0.175	wu.P1	-0.047	0.946
	wu.P2	-1.785	0.341	wu.P2	-1.972	0.289	wu.P2	-3.892	0.043	wu.P2	-2.251	0.188
Ave_Body	chao1	-0.006	0.572	obs.sp	-0.021	0.225	PD.wh	-0.997	0.01	shan.	-0.901	0.018
	wu.P1	-0.39	0.59	wu.P1	-0.425	0.526	wu.P1	-1.401	0.05	wu.P1	-0.344	0.542
	wu.P2	-0.859	0.603	wu.P2	-1.115	0.486	wu.P2	-2.963	0.063	wu.P2	-1.389	0.316
Ave_Escape	chao1	-0.001	0.811	obs.sp	-0.005	0.646	PD.wh	-0.434	0.076	shan.	-0.344	0.156
	wu.P1	-0.164	0.706	wu.P1	-0.169	0.685	wu.P1	-0.629	0.178	wu.P1	-0.164	0.669
	wu.P2	-0.221	0.823	wu.P2	-0.277	0.78	wu.P2	-1.165	0.262	wu.P2	-0.443	0.634
Sum_Facial	chao1	-0.009	0.849	obs.sp	-0.045	0.588	PD.wh	-3.182	0.107	shan.	-3.755	0.044
	wu.P1	-0.861	0.804	wu.P1	-1.014	0.76	wu.P1	-4.302	0.254	wu.P1	-1.013	0.721
	wu.P2	-7.598	0.345	wu.P2	-8.241	0.306	wu.P2	-14.558	0.095	wu.P2	-10.196	0.152
Sum_Vocal	chao1	-0.025	0.594	obs.sp	-0.076	0.342	PD.wh	-4.297	0.018	shan.	-3.554	0.05
	wu.P1	0.133	0.968	wu.P1	0.098	0.975	wu.P1	-4.221	0.199	wu.P1	0.368	0.894
	wu.P2	-6.862	0.373	wu.P2	-7.689	0.313	wu.P2	-15.925	0.04	wu.P2	-8.887	0.198
Sum_Body	chao1	-0.026	0.529	obs.sp	-0.091	0.194	PD.wh	-4.249	0.006	shan.	-3.87	0.012
	wu.P1	-1.134	0.701	wu.P1	-1.263	0.642	wu.P1	-5.408	0.056	wu.P1	-0.904	0.687
	wu.P2	-3.202	0.635	wu.P2	-4.289	0.509	wu.P2	-12.126	0.057	wu.P2	-5.436	0.325
Sum_Escape	chao1	-0.01	0.71	obs.sp	-0.03	0.528	PD.wh	-2.125	0.056	shan.	-1.808	0.1
	wu.P1	-0.085	0.966	wu.P1	-0.09	0.962	wu.P1	-2.285	0.273	wu.P1	-0.021	0.99
	wu.P2	-0.562	0.901	wu.P2	-0.88	0.846	wu.P2	-5.098	0.276	wu.P2	-1.655	0.691

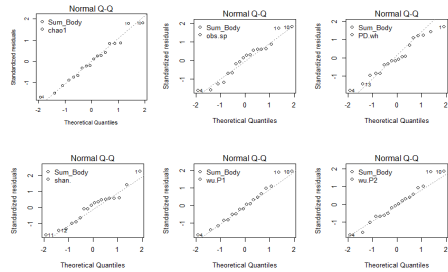
Table 7. Neonate group Multiple linear regression. The predict variable is column of Diversity and response variable is column of behavior. The estimate of coefficients and p values of every model were listed.

Behavior	Diversity	Estimate	p value	Diversity	Estimate	p value	Diversity	Estimate	p value	Diversity	Estimate	p value
Max_Facial	chao1	-0.002	0.675	obs.sp	-0.007	0.444	PD.wh	-0.108	0.639	shan.	-0.716	0.308
	wu.P1	-1.293	0.179	wu.P1	-1.372	0.151	wu.P1	-1.245	0.191	wu.P1	-1.254	0.171
	wu.P2	-0.678	0.785	wu.P2	-1.166	0.648	wu.P2	-0.644	0.792	wu.P2	-1.125	0.638
Max_Vocal	chao1	-0.001	0.875	obs.sp	-0.004	0.62	PD.wh	-0.083	0.671	shan.	-0.213	0.726
	wu.P1	-1.714	0.048	wu.P1	-1.766	0.042	wu.P1	-1.694	0.048	wu.P1	-1.702	0.047
	wu.P2	-0.427	0.84	wu.P2	-0.768	0.725	wu.P2	-0.539	0.794	wu.P2	-0.554	0.793
Max_Body	chao1	0.004	0.227	obs.sp	0.006	0.283	PD.wh	0.094	0.525	shan.	0.363	0.428
	wu.P1	-1.3	0.037	wu.P1	-1.263	0.045	wu.P1	-1.375	0.036	wu.P1	-1.367	0.035
	wu.P2	0.865	0.567	wu.P2	1.019	0.525	wu.P2	0.549	0.725	wu.P2	0.693	0.66
Max_Escape	chao1	-0.003	0.129	obs.sp	-0.005	0.168	PD.wh	-0.047	0.6	shan.	-0.357	0.181
	wu.P1	-0.595	0.084	wu.P1	-0.625	0.079	wu.P1	-0.542	0.146	wu.P1	-0.545	0.116
	wu.P2	-1.044	0.242	wu.P2	-1.172	0.22	wu.P2	-0.753	0.432	wu.P2	-1.012	0.269
Ave_Facial	chao1	0	0.988	obs.sp	-0.002	0.786	PD.wh	-0.045	0.791	shan.	-0.151	0.776
	wu.P1	-1.501	0.048	wu.P1	-1.533	0.045	wu.P1	-1.497	0.047	wu.P1	-1.502	0.046
	wu.P2	0.16	0.931	wu.P2	-0.059	0.976	wu.P2	0.038	0.983	wu.P2	-0.007	0.997
Ave_Vocal	chao1	0.002	0.601	obs.sp	0.001	0.859	PD.wh	-0.004	0.982	shan.	0.14	0.78
	wu.P1	-1.325	0.058	wu.P1	-1.338	0.061	wu.P1	-1.356	0.055	wu.P1	-1.357	0.054
	wu.P2	1.143	0.51	wu.P2	1.007	0.583	wu.P2	0.867	0.615	wu.P2	1.024	0.56
Ave_Body	chao1	0.004	0.19	obs.sp	0.006	0.306	PD.wh	0.1	0.503	shan.	0.362	0.435
	wu.P1	-1.155	0.057	wu.P1	-1.127	0.073	wu.P1	-1.237	0.056	wu.P1	-1.228	0.056
	wu.P2	1.269	0.404	wu.P2	1.35	0.411	wu.P2	0.918	0.562	wu.P2	1.046	0.514
Ave_Escape	chao1	-0.002	0.021	obs.sp	-0.004	0.035	PD.wh	-0.059	0.224	shan.	-0.237	0.11
	wu.P1	-0.667	0.001	wu.P1	-0.692	0.001	wu.P1	-0.622	0.007	wu.P1	-0.627	0.005
	wu.P2	-0.267	0.524	wu.P2	-0.376	0.415	wu.P2	-0.091	0.855	wu.P2	-0.19	0.694
Sum_Facial	chao1	0	0.988	obs.sp	-0.007	0.786	PD.wh	-0.181	0.791	shan.	-0.603	0.776
	wu.P1	-6.005	0.048	wu.P1	-6.131	0.045	wu.P1	-5.989	0.047	wu.P1	-6.006	0.046
	wu.P2	0.642	0.931	wu.P2	-0.235	0.976	wu.P2	0.151	0.983	wu.P2	-0.027	0.997
Sum_Vocal	chao1	0.007	0.601	obs.sp	0.005	0.859	PD.wh	-0.014	0.982	shan.	0.56	0.78
	wu.P1	-5.301	0.058	wu.P1	-5.352	0.061	wu.P1	-5.425	0.055	wu.P1	-5.43	0.054
	wu.P2	4.574	0.51	wu.P2	4.027	0.583	wu.P2	3.469	0.615	wu.P2	4.096	0.56
Sum_Body	chao1	0.016	0.19	obs.sp	0.024	0.306	PD.wh	0.4	0.503	shan.	1.446	0.435
	wu.P1	-4.619	0.057	wu.P1	-4.507	0.073	wu.P1	-4.948	0.056	wu.P1	-4.911	0.056
	wu.P2	5.075	0.404	wu.P2	5.402	0.411	wu.P2	3.674	0.562	wu.P2	4.184	0.514
Sum_Escape	chao1	-0.009	0.021	obs.sp	-0.015	0.035	PD.wh	-0.238	0.224	shan.	-0.947	0.11
	wu.P1	-2.668	0.001	wu.P1	-2.766	0.001	wu.P1	-2.488	0.007	wu.P1	-2.509	0.005
	wu.P2	-1.069	0.524	wu.P2	-1.506	0.415	wu.P2	-0.365	0.855	wu.P2	-0.761	0.694

Table 8. results of year 1 group Multiple linear regression. The predict variable is column of Diversity and response variable is column of behavior. The estimate of coefficients and p value was listed.

6.4 QQ plots for models

A



B

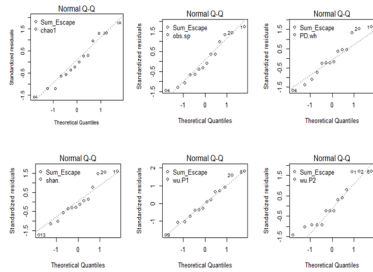


TABLE 11. QQ-plot of residues of linear regression. A:neonate group; B: year 1 group.