# Comparison of Oral Microbiome In Stable vs Declining Kidney Function In Renal Transplant Recipients

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## Introduction

- Transplantation causes dysbiosis of the oral microbiome and has been correlated with worse allograft outcomes[1].
- Hypothesis: There are differences in dental subgingival plaque microbes between renal transplant recipients with stable versus declining allograft function

**Primary Objective:**

- To compare the subgingival dental plaque taxa of renal transplant recipients with stable vs declining allograft function

**Secondary Objective:**

- To compare transplant subjects' plaque and other oral microbiomes to those of healthy subjects

## Methods

- Case-control study evaluated subginvival plaque samples from 20 adult patients from two existing prospective study cohorts (DeKAF Genomics) at the University of Minnesota in Minneapolis, MN.
- *Stable kidney function (SKF)*: creatinine (SCr) stable from 3 month post-tx baseline
- *Declining kidney function (DKF)*: SCr ≥ 25% above 3 month post-tx baseline
- DNA extracted from plaque and other oral sites (nasal, buccal swab, hard palate, tongue), amplified, and compared to 16S V4 bacterial rRNA libraries
- Healthy subject data taken from the Human Microbiome Project (HMP) 16S V3V5 rRNA
- Samples were grouped into operational taxonomic units (OTUs) and compared to known reference libraries of microorganisms.

## Statistical Analysis

- For each individual OTU, we applied the Wilcox rank-sum test to compare differences between SKF and DKF groups
- We also looked at the aggregated OTU distribution difference at the genus level between the two groups using the kernel RV test[2].

## Results

Selected participant data is listed below.

**Table 1: Selected demographics of study participants**

| Characteristic | SKF (n=9) | DKF (n=11) |
|---|---|---|
| Race: | | |
| White | 8 (88.9%) | 11 (100%) |
| Multiracial | 1 (11.1%) | 0 (0%) |
| Male | 0 (0%) | 5 (45.5%) |
| Donor age, mean (std) | 41.3 (12.8) | 44.6 (11.9) |
| Serum creatinine (mg/dL) (std) | 1.01 (0.23) | 1.76 (0.49) |

### Subgingival Plaque in SKF vs DKF

- In 11 cases of DKF, genus *Tanerella* was more prevalent in subgingival plaque samples compared to those with SKF (p=0.046, KRV – not significant if FDR 0.05)
- By oligotyping, *Tanerella forsythia*

Figure 1: Significant OTUs determined by LEfSe analysis[3] of subgingival plaque in SKF vs DKF renal transplant recipients. Linear discriminant analysis (LDA) threshold > 2.

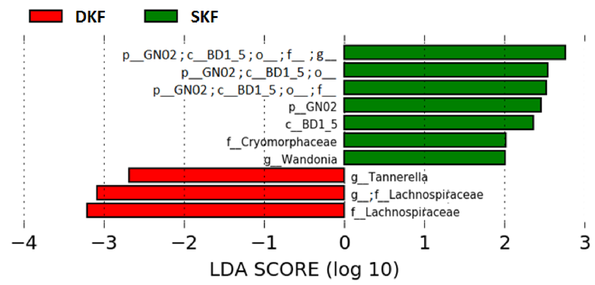
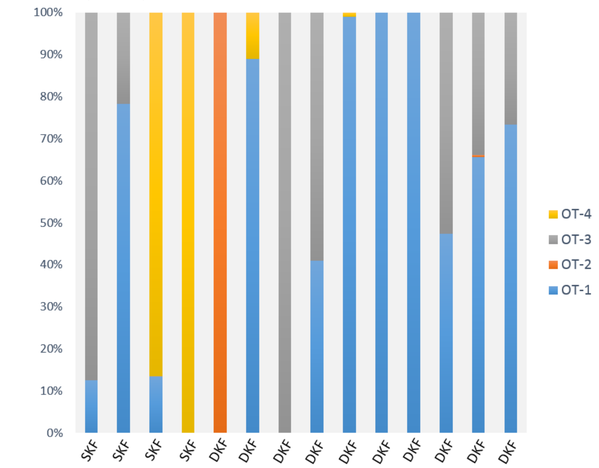


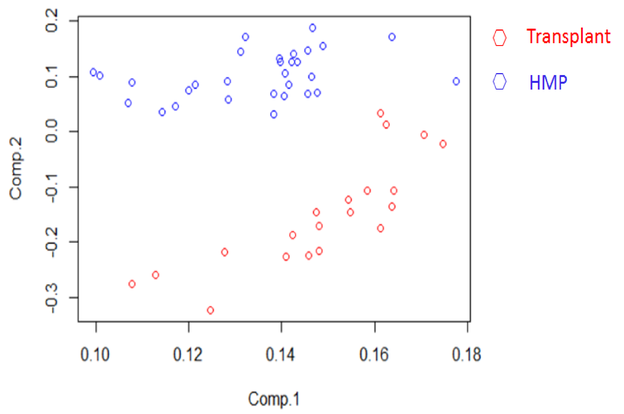

Figure 2: Distribution of *Tannerella forsythia* oligotypes in 4 stable (SKF) and 10 declining kidney function (DKF) subgingival plaque samples. Four distinct oligotypes (OT-1 through OT-4) were identified and relative abundances for each sample are presented.




### Subgingival Plaque in HMP vs Transplant

- 20 transplant (TX) recipient subgingival plaque samples were compared to 30 healthy subjects found in the Human Microbiome Project (HMP)
- The overall subgingival plaque microbiome is significantly different in TX vs HMP hosts (KRV, p-1.28x10$^{-12}$)

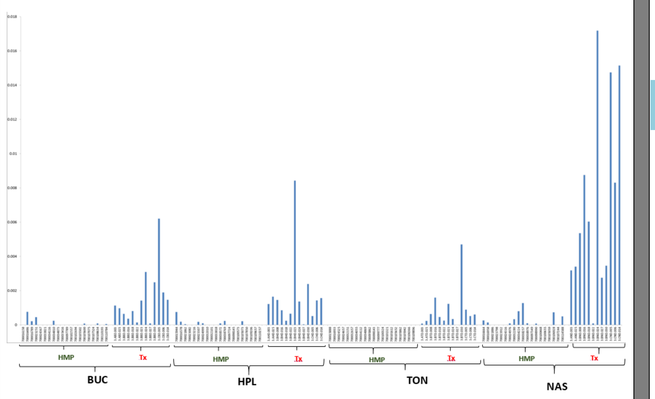
Figure 3: Beta diversity, a measure of difference between differing communities, as measured by principle coordinates of analysis (PCoA) plot with centered log-ratio (CLR) transformation comparing 30 healthy (HMP) to 20 transplant plaque samples.



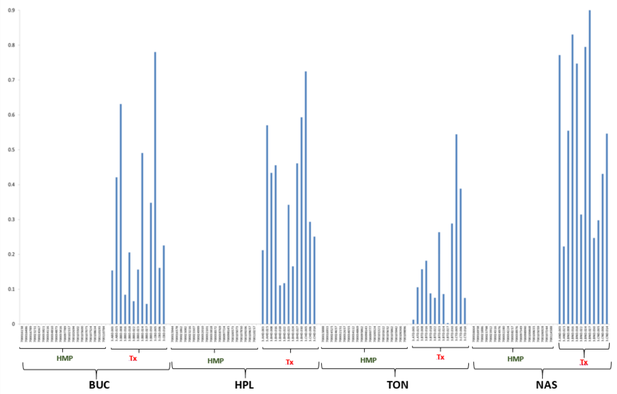

- In non-plaque oral soft tissue (buccal mucosa, tongue, hard palate) and nasal samples, genera *Cellulosimicrobium* and *Pseudonomas* are more abundant in TX subjects when compared to HMP (Wilcox, p<0.0001)

Figure 4: Relative abundance of genera (A) *Pseudomonas* and (B) *Cellulosimicrobium* across all body site samples in renal transplant recipients. (BUC=buccal mucosa, HPL=hard palate, TON=tongue, NAS=nasal).

A) Relative abundance of *Pseudomonas*




B) Relative abundance of *Cellulosimicrobium*




## Conclusions

- There are distinct signatures in the subgingival plaque of renal transplant recipients compared to those of healthy subjects.
- There is a significant prevalence of both *Cellulosimicrobium* and *Pseudomonas* in the non-plaque oral sites of transplant recipients compared to healthy hosts (Wilcox, p<0.0001).
- There is a non-significant greater prevalence of *Tannerella forsythia* in the plaque of DKF vs SKF renal transplant recipients (KRV, p=0.046, not significant if FDR 0.05).

## References

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2. Zhan X, Plantinga A, Zhao N, Wu MC. A fast small-sample kernel independence test for microbiome community-level association analysis. Biometrics. 2017:73(4);1453-1463.

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