UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE MEDICINA

BIOMEDICINA

“ANÁLISIS DEL MICROBIOMA ORAL Y SU CORRELACIÓN CON EL ESTADO ODONTOLÓGICO EN PACIENTES CON SÍNDROME DE MARFÁN”.

TESIS

(POR ARTÍCULO CIENTÍFICO)

“ALTERATIONS IN THE ORAL MICROBIOTA OF MEXICAN INDIVIDUALS WITH MARFAN SYNDROME”

QUE PARA OPTAR POR EL GRADO DE:

MAESTRO EN CIENCIAS BIOLÓGICAS

PRESENTA:

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CIUDAD UNIVERSITARIA, CD. MX. MAYO, 2023

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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS

ENTIDAD (FACULTAD DE MEDICINA)

OFICIO CPCB/254/2023

ASUNTO: Oficio de Jurado

M. en C Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **7 de noviembre de 2022** se aprobó el siguiente jurado para el examen de grado de **MAESTRO EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **Biomedicina** del alumno **ORDAZ ROBLES ERICK RICARDO** con número de cuenta **307109495** por la modalidad de graduación de **tesis por artículo científico** titulado: **"ALTERATIONS IN THE ORAL MICROBIOTA OF MEXICAN INDIVIDUALS WITH MARFAN SYNDROME",** que es producto del proyecto realizado en la maestría que lleva por título: **"ANÁLISIS DEL MICROBIOMA ORAL Y SU CORRELACIÓN CON EL ESTADO ODONTOLÓGICO EN PACIENTES CON SÍNDROME DE MARFÁN,** ambos realizados bajo la dirección de la **DRA. MARÍA MAGDALENA AGUIRRE GARCÍA,** quedando integrado de la siguiente manera:

Presidente:	**DRA. MARÍA DEL CARMEN MALDONADO BERNAL**
Vocal:	**DR. RICARDO JASSO CHÁVEZ**
Vocal:	**DRA. NORMA DEL CARMEN GALINDO SEVILLA**
Vocal:	**DR. EMILIANO TESORO CRUZ**
Secretario:	**DRA. GLADIS DEL CARMEN FRAGOSO GONZÁLEZ**

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 14 de marzo de 2023

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

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Resumen

Antecedentes: El síndrome de Marfan (MFS, por sus siglas en inglés) es uno de los trastornos genéticos del tejido conectivo que afecta a múltiples órganos y sistemas, además, presenta alteraciones importantes en la cavidad oral, lo cual afecta su salud bucal y otros sistemas. El objetivo de este estudio es caracterizar la microbiota oral en individuos mexicanos con MFS, para conocer la diversidad bacteriana presente en estos individuos, ya que es uno de los nichos menos explorados en esta enfermedad.

Métodos: Se diseñó un estudio observacional descriptivo, para colectar las muestras de placa se le dio a cada uno de los individuos la instrucción de no realizar un cepillado dental, ni el uso de productos de higiene oral como enjuagues, chicles o el uso de hilo dental en un periodo mínimo de 36 horas, para garantizar la formación de una PDB madura. Las muestras se tomaron con instrumental estéril, colocadas en tubos eppendorf con etanol al 70% y almacenadas inmediatamente en refrigeración a -20 °C hasta su posterior procesamiento. Obteniendo un total de 42 muestras de personas con MFS y personas sin MFS, 36 y 6 muestras respectivamente. La microbiota oral se evaluó mediante secuenciación de la región V3-V4 del gen 16S rRNA, las secuencias se analizaron con QIIME2 (quantitative insights into microbial ecology), los datos estadísticos se trabajaron con los programas SPSS y RStudio.

Resultados: La evaluación odontológica mostró que los individuos con MFS tienen un alto riesgo de padecer caries dental. La asignación taxonómica del DNA extraído de la placa dentobacteria mostró a *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, *Actinobacteria* como las principales phyla identificadas. *Prevotella* y *Streptococcus* fueron los géneros más abundantes en ambos grupos. *Veillonella* se destacó como género discriminatorio en ambos grupos.

Conclusión: Este es el primer estudio que caracteriza a la microbiota oral en individuos mexicanos con síndrome de Marfan. Los resultados obtenidos de esta investigación abren nuevas perspectivas para un estudio a nivel traslacional para la búsqueda de nuevas condiciones y tratamientos de salud oral que puedan evaluarse a través de ensayos clínicos en estos individuos con alto riesgo de padecer caries dental y disbiosis oral.

Abstract

Background: Marfan syndrome (MFS) is one of the connective tissue disorders that affect multiple organs and systems. The oral cavity presents important alterations in these individuals which affects their oral health and other systems. The objective of this study is to characterize the oral microbiota in Mexican individuals with MFS, since it is one of the least explored niches in this disease.

Methods: Methods: A descriptive observational study was designed, in order to collect plaque samples, each individual was given instructions not to brush their teeth, nor to use oral hygiene products such as rinses, chewing gum or the use of thread tooth in a minimum period of 36 hours, to guarantee the formation of a mature PDB. The samples were taken with sterile instruments, placed in eppendorf tubes with 70% ethanol and immediately stored refrigerated at -20 °C until further processing. Obtaining a total of 42 samples from individuals with MFS and without MFS, 36 and 6 samples respectively. The oral microbiota was evaluated by sequencing the V3-V4 region of the 16S rRNA gene, the sequences were analyzed with QIIME2 (quantitative insights into microbial ecology), the statistical data was processed with the SPSS and RStudio programs.

Results: Results: The dental evaluation showed that individuals with MFS have a high risk of dental caries. The taxomic assignment of the DNA extracted from the dentobacterial plaque showed *Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria* as the main phyla identified. *Prevotella and Streptococcus* were the most abundant genera in both groups. *Veillonella* stood out as a discriminatory gender in both groups.

Conclusion: This is the first characterization of the oral microbiota in Mexican individuals with Marfan syndrome. This first avenue of microbiota in MFS individuals research opens up perspectives for study at the translational level where oral health conditions and treatments can be evaluated through clinical trials in these individuals at high risk of suffering from dental caries and oral dysbiosis.

Introducción

El síndrome de Marfan (MFS) es una enfermedad genética autosómica dominante con una incidencia estimada de 1 de cada 5,000 personas sin preferencia por etnia o ubicación geográfica [1,2]. Actualmente, la prevalencia de esta enfermedad en México no está definida. El MFS es causada por una mutación del gen FBN1 que codifica para la proteína microfibrilar fibrilina-1, glicoproteína principal de la matriz extracelular, cuya pérdida de función o ausencia conduce al deterioro del tejido conectivo [3]. La extensión del daño o condición es variable incluso dentro de la misma familia y afecta múltiples sistemas, incluidos los sistemas musculoesquelético, pulmonar, ocular, dental, nervioso central y cardiovascular [4].

Las alteraciones en el sistema cardiovascular asociadas con MFS conducen a una variedad de condiciones patológicas como arritmias cardíacas, enfermedad arterial coronaria, hipertrofia ventricular izquierda, insuficiencia cardíaca congestiva, dilatación aórtica y disección aórtica se identifica como las principales causas de mortalidad en estos individuos. [5].

Los criterios utilizados para el diagnóstico de MFS han evolucionado durante un cuarto de siglo y actualmente fueron objeto de tres conferencias internacionales, que concluyeron con la última revisión de Bruselas de la Nosología de Genth en el 2010 [6]. Los criterios para diagnosticar MFS incluyen características clínicas importantes, como la mutación del gen FBN1, la ectopia del cristalino y la enfermedad de la raíz aórtica. Sin embargo, los criterios de diagnóstico cambian con la edad, por lo que el diagnóstico de MFS en un niño puede ser más difícil que en un adolescente o adulto. La presencia de una variante patogénica en el gen FBN1, o el antecedente de un familiar diagnosticado de MFS, facilita la detección de este síndrome independientemente de la edad del individuo [7].

La complejidad sindrómica de esta condición involucra diversos cambios patológicos en la cavidad bucal que han sido moderadamente abordados. La literatura odontológica actual sobre MFS se ha enfocado en determinar la presencia de una bóveda palatina alta como manifestación clínica menor de MFS; sin embargo, debemos recalcar que existen más manifestaciones clínicas a nivel oral, como apiñamiento dental, dientes largos, maloclusiones, prognatismo mandibular, trastornos de la articulación temporo-mandibular y cambios estructurales de la úvula [8]. Estas manifestaciones orales asociadas a MFS aumentan el

riesgo de padecer enfermedades orales como caries y periodontitis y complicaciones cardiovasculares como endocarditis entre otras [9].

Según la base de datos de la microbiota oral humana, a partir de abril de 2022, hay 774 especies de bacterias orales divididas en diferentes filos, como *Firmicutes, Actinobacteria, Proteobacteria y Bacteroidetes* [10,11]. Dos géneros pertenecientes a las phyla de *Firmicutes: Streptococcus y Veillonella* son colonizadores iniciales y están asociados con la formación inicial de placa bacteriana dental (DBP). Los cultivos mixtos de *Streptococcus mutans y Veillonella alcalescens* producen niveles de ácido láctico, más altos que los cultivos que contienen solo una de estas especies, lo que nos muestra una relación sinérgica entre las especies para aumentar el riesgo de caries dental [12].

Las caries afectan las superficies duras de los dientes y como tal, no conducen a una exposición a nivel sistémico de estas bacterias. Sin embargo, si no se trata, la caries dental progresa a la pulpa dental (nervios y vasos sanguíneos del diente), lo que lleva a una infección del conducto radicular que se propaga a las estructuras de soporte, incluido el hueso, y aumenta significativamente el nivel de exposición sistémica [13].

En relación con el desarrollo de caries, se han identificado géneros bacterianos como *Streptococcus, Actinomyces, Bifidobacteria, Neisseria y Veillonella* [14]. Varios taxones bacterianos que se encuentran comúnmente en la cavidad oral se detectaron en tejidos vasculares enfermos, incluidas las especies *Streptococcus, Prevotella, Capnocytophaga, Veillonella y Porphyromonas*, lo que sugiere que la cavidad oral puede ser una fuente de diseminación bacteriana al tejido vascular [15].

Los procesos de adhesión y formación de biopelículas se rigen por interacciones complejas entre diferentes bacterias, incluido el intercambio de señales y metabolitos y la producción de compuestos que inhiben o estimulan el crecimiento [16]; cuando se altera este proceso, se promueve la formación de lesiones cariosas en los órganos dentarios. *Veillonella* además de ser un comensal de la cavidad oral, también se ha identificado en el tracto digestivo y urogenital, y también se describe comúnmente como microorganismos con patogenicidad limitada y generalmente se asocian con infecciones polimicrobianas [17, 18]. Asimismo, el género *Veillonella* juega un papel importante en la formación de placa dental, la cual es un

proceso complejo y dinámico que involucra la colonización secuencial y ordenada de colonizadores primarios y secundarios por procesos de adherencia selectiva.

Dadas las anomalías orofaciales del MFS y el hecho de que el MFS se caracterice por varios trastornos, como dilatación aórtica, endocarditis y una mayor prevalencia de caries y periodontitis, es relevante comprender si la microbiota oral está alterada en el MFS, ya que podría proporcionar información relevante para cuidado bucal de estos individuos [19]. Nuestro principal objetivo fue caracterizar la microbiota oral, así como reportar las características clínicas y condiciones dentales en individuos mexicanos con MFS.

Alterations in the Oral Microbiota of Mexican Individuals with Marfan Syndrome

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Abstract

Background: Marfan syndrome (MFS) is one of the genetic connective tissue disorders that affects multiple organs and systems. The oral cavity presents important alterations in these individuals which affects their oral health and other systems. The objective of this study is to characterize the oral microbiota in Mexican individuals with MFS, since it is one of the least explored niches in this disease.

Methods: Descriptive observational design, dental plaque samples were collected from 36 individuals with MFS and individuals without MFS. The oral microbiota was evaluated by region sequencing V3-V4 of the gene 16S rRNA, the sequences were analyzed with QIIME2, SPSS y RStudio.

Results: Caries risk was higher in individuals with MFS. It was observed at *Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria* as the main phylum identified and *Prevotella* and *Streptococcus* were the most abundant in both groups. It was found to *Veillonella* as a discriminatory genus in both groups.

Conclusion: This is the first characterization of the oral microbiota in Mexican individuals with Marfan syndrome. This first avenue of microbiota in MFS individuals research opens up perspectives for study at the translational level where oral health conditions and treatments can be evaluated through clinical trials in these individuals at high risk of suffering from dental caries and oral dysbiosis.

1 Introduction

Marfan syndrome (MFS) is an autosomal dominant genetic disease with an estimated incidence of 1 in 5,000 individuals with no preference for ethnicity or geographic location. [1,2]. Currently, the prevalence of this disease in Mexico is not defined. It is caused by a mutation of the *FBN1* gene that codes for the microfibrillar protein fibrillin-1, a main glycoprotein of the extracellular matrix, whose loss of function or absence leads to the deterioration of the connective tissue [3]. The extent of the damage or condition is variable even within the same family and affects multiple systems including musculoskeletal, pulmonary, ocular, dental, central nervous and cardiovascular systems. [4].

Alterations in the cardiovascular system associated with MFS lead to a variety of pathological conditions such as cardiac arrhythmias, coronary artery disease, left ventricular hypertrophy, congestive heart failure, aortic dilatation and aortic dissection, which are the main cause of mortality in these individuals. [5].

Criteria used for the diagnosis of MFS have evolved over a quarter of a century and were currently the subject of three international conferences, which concluded with the latest Brussels revision of the Ghent Nosology [6]. Criteria to diagnose MFS include important clinical features such as gene mutation *FBN1*, ectopia lentis and aortic root disease. However, diagnostic criteria change with age, so diagnosis of MFS in a child may be more difficult than in an adolescent or adult. The presence of a pathogenic variant in the *FBN1* gene, or the history of a relative diagnosed with MFS, facilitates the detection of this syndrome regardless of the individual's age [7].

The syndromic complexity of this condition involves diverse pathological changes to the oral cavity which have been moderately addressed. The current dental literature on MFS has focused on determining the presence of a high palatal vault as a minor clinical manifestation of MFS, however, we must emphasize that there are more clinical manifestations at the oral level, such as dental crowding, long and narrow teeth, malocclusions, mandibular prognathism, disorders of the temporomandibular joint and structural changes of the uvula [8]. These MFS associated oral manifestations increase the risk of suffering from oral diseases such as caries and periodontitis and cardiovascular complications such as endocarditis among others [9].

According to the human oral microbiota database, as of April 2022, there are 774 oral bacterial species divided into different phyla such as *Firmicutes, Actinobacteria, Proteobacteria* and *Bacteroidetes* [10,11]. Two genera belonging to the *Firmicutes* phylum: *Streptococcus* and *Veillonella* are initial colonizers and are associated with initial formation of dental bacterial plaque (DBP). Mixed cultures of *Streptococcus mutans* and *Veillonella alcalescens* produce higher lactic, acid levels than cultures

containing only one of these species, showing us a synergistic relationship between the species to increase the risk of dental caries [12].

Caries affect the hard surfaces of teeth and as such do not lead to systemic exposure to bacteria. However, if left untreated, tooth decay progresses to the dental pulp (nerves and blood vessels of the tooth), leading to root canal infection that spreads to supporting structures, including bone, and significantly increases the level of systemic exposure [13].

In relation to the development of caries, bacterial genera have been identified as *Streptococcus, Actinomyces, Bifidobacteria, Neisseria* and *Veillonella* [14]. Several bacterial taxa commonly found in the oral cavity were detected in diseased vascular tissues, including species *Streptococcus, Prevotella, Capnocytophaga*, *Veillonella* and *Porphyromonas,* suggesting that the oral cavity may be a source for bacterial dissemination to vascular tissue [15].

The processes of adhesion and biofilm formation are governed by complex interactions between different bacteria, including the exchange of signals and metabolites and the production of growth-inhibiting or growth-stimulating compounds [16]; when this process is altered, the formation of carious lesions in the dental organs is promoted. *Veillonella* in addition to being an oral cavity commensal, has also been identified in the digestive and urogenital tracts, and is also commonly described as microorganisms with limited pathogenicity and are generally associated with polymicrobial infections [17, 18]. Also, *Veillonella* genus plays an important role in dental plaque formation, which is a complex and dynamic process that involves the sequential and orderly colonization of primary and secondary colonizers by selective adherence processes.

Given the orofacial anomalies of MFS, the fact that MFS is characterized by several disorders, like aortic dilatation, endocarditis and a higher prevalence of caries and periodontitis, understanding if the oral microbiota is altered in MFS is relevant, as it might provide relevant information for oral care [19]. Our main objective was to characterize the oral microbiota, as well as to report the clinical characteristics and dental conditions in Mexican individuals with MFS.

2 Article types

Original research article.

3 Materials and Methods

A comparative descriptive observational study of cases and controls was designed, which included individuals of Mexican nationality who attended the "Ignacio Chávez" National Institute of Cardiology, male and female, between 5 and 55 years of age, previously diagnosed with Marfan syndrome by a rheumatologist from of the department of immunology of the “Ignacio Chávez” National Institute of Cardiology with experience in connective tissue diseases (MES).

3.1 Study population

The MFS group is made up of individual with MFS met more than two Ghent criteria plus genetic confirmation of mutation in the FBN1 gene. The individuals belonging to the non-MFS group was made up of 6 unrelated healthy subjects in whom connective tissue disease was ruled out clinically and were negative for FBN1, who work in the UNAM-INC Research Unit, in addition to two family children of the workers of this unit.

All individuals with characteristics such as missing teeth, chronic alcohol or drug intake, individuals who had undergone antibiotic treatment in the last month, as well as pregnant or lactating individuals were excluded.

This study was approved by the ethics and scientific committees of the National Institute of Cardiology with protocol number PT-18-079.

Informed consent was obtained for all participants, and a directed clinical history, a dental evaluation, and DBP samples were taken.

3.2 Dental bacterial plaque (DBP) sampling

Samples of supragingival dental bacterial plaque were collected from the four quadrants of the oral cavity using a disposable sterile spoon. Individuals were instructed to avoid tooth brushing, flossing,

and mouth washing for 48 to 72 hours prior to sampling. Samples were placed in Eppendorf tubes with 70% ethanol (Sigma) and frozen at -20°C until processing.

3.3 DNA isolation

DBP DNA isolation was performed following the manufacturer's protocol using the commercial kit EZ-10 SPIN COLUMN GENOMIC DNA MINIPREPS KIT (Bio Basic Inc, Markham, Ontario, Canada). DNA concentration and purity was determined using ultraviolet–visible spectrophotometry using the NanoDrop 2000c (ThermoFisher Scientific Waltham, MA, US). The absorbance ratio as an indicator of protein contamination (A260/280) was determined for each sample, only samples with a A260/280 between 1.8-2.0 were considered. DNA integrity was determined by electrophoresis on a 1% agarose gel stained with ethidium bromide (Sigma) and visualized on a ChemiDoc MP™ UV transilluminator (Bio-Rad Hercules, CA, US).

3.4 16S rRNA sequencing

DNA was sent to NOVOGENE Co. (Beijing, China) for library preparation and sequencing. Primers targeting the V3-V4 region were used: 341F: CCT AYG GGR BGC ASC AG; 806R GGA CTA CNN GGG TAT CTA AT.Paired-end sequencing was performed using the Platform Novaseq 6000 and 500 cycles (Illumina, San Diego, CA, USA).

3.5 16S rRNA data analysis

Demultiplexed raw FastQ files (R1 and R2) were processed using “Quantitative Insights Into Microbial Ecology 2” (QIIME2) v.2020.11 (Quantitative Insights into Microbial Ecology). Dada2 plugin was used to merge paired-endfastq files, denoising by removing chimeras and construct a table of amplicon sequence variants (ASV). Taxonomy was assigned using the Human Oral Microbiome (eHOMD) v.15.2 at 99% identity pre-trained for the V3-V4 region. Rarefaction curves were performed at a sampling depth of 108,869 sequences. Alpha-diversity was estimated using 3 metrics: Observed features, Shannon and Chao1. Beta-diversity was performed using the Bray Curtis dissimilarity index and visualized using principal coordinate analysis (PCoA).

3.6 Statistical analysis

Data was analyzed using R v.4.1.2, for the taxonomic profiles between the two study groups and for the alpha diversity data, the test of U Mann-Whitney; Linear Effect Size Discrimination Analysis (LEfSe) algorithm was also applied with a value LDA de 3.5 and $p<0.05$, to identify taxa that may be differentially present between groups.

Beta diversity analysis was determined using the Bray Curtis discrimination index; in the PCoA, the distribution of study subjects grouped by gender and health status is defined; it was determined that the dispersion of the populations is homogeneous (BETADISPER), p value was obtained by the Permutation Multivariate Analysis of Variance (PERMANOVA).

4 Results

4.1 Population

Forty-two male and female subjects, aged between 5 and 55 years, were included: 36 were diagnosed with MFS making up the MFS group (85.7%) and 6 subjects were included in the non-MFS group (14.3%) with an average age of 18.93 years in both groups, the distribution by gender was represented by 26 men (61.9%) and 16 women (38.1%) of the total population (**Table 1**). Oral health status was determined by dentists and assessed using standardized indices as recommended by the World Health Organization to express caries experience, likewise, the permanent teeth/deciduous teeth (DMF) index of Klein and Palmer was determined to objectively indicate the rate of dental caries. [20]. In this evaluation, 8 caries-free individuals (19.05%) were found and 34 of them presented a minimum of one caries (80.95%). The individuals of the MFS group showed a high number of caries, being the subjects >25 years old with the highest risk of caries, while the non-MFS group had a lower risk of caries. (**Table 2**).

4.2 Composition of oral microbiota

In order to verify the subsampling performed, a rarefaction curve was performed based on the observed species, which was almost asymptotic with increasing sequencing, indicating that the sequencing data was sufficient to cover practically all species in all samples and determine the taxonomic identification at a sequence depth of 108,869 (**Figure supplementary 1**).

Phylum

We found that the main phyla in both groups are represented by *Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria,* which correspond to an abundance of 98.16% of all the samples.

For the non-MFS group, the frequency of the main phyla corresponds to *Bacteroidetes* with 28.74%, *Firmicutes* (28%), *Proteobacteria* (16.76%) and *Fusobacteria* (15.74%).

The MFS group presented an abundance of *Firmicutes* (27.62%) *Bacteroidetes* (23.45%), *Proteobacteria* (19.67%) and *Fusobacteria* (16.62%) (**Figure 1A**).

a) Genus

A total of 132 genera were found, with 87.93% of the relative abundance represented by 15 genera including *Prevotella* (13.04%), *Streptococcus* (11.27%), *Veillonella* (9.77%), *Leptotrichia* (8.5%) *Fusobacterium* (8%) *Haemophilus* (7.02%), *Neisseria* (6.38%), *Corynebacterium* (5.65%), *Porphyromonas* (4.5%) and *Capnocytophaga* (3.13%), the remaining represented by the 22.74%.

In the non-MFS group, *Streptococcus* (16.04%), *Prevotella* (15.76%) and *Fusobacterium* (8.23%) have the highest abundance, while in the MFS group the most abundant genera correspond to *Prevotella* (12.59%), *Veillonella* (10.68%), *Streptococcus* (10.47%); It is worth noting that *Veillonella* (4.3%) is less abundant in the non-MFS group (**Figure 1B**).

4.3 Diversity and richness of the oral microbiota

Richness determined by Observed features and Chao1, was lower in the MFS group with a median of 571.5 and 586.8 species, respectively. Similarly, the abundance of each group was defined by means

of the Shannon index, where a lower abundance was found in the Marfan group, showing a metric of 4,739 units. On the contrary, the non-MFS individuals presented a median of 590 and 614.6, in relation to the Observed features and Chao1 index; while the median of the Shannon index was 4,904 units. The comparison of alpha diversity between both groups did not show statistically significant differences (**Figure 2A**).

The analysis of variance between the MFS and non-MFS groups, observed in PCoA defined by the distance matrix by Bray Curtis, shows that 3.7% of the variance is influenced by the state of health among the subjects of the non-MFS group and MFS (PERMANOVA, R2= 0.037, p=0.026) (**Figure 2B**)

4.4 Characterization of oral microbiota in non-MFS and MFS individuals

In the frequency analysis of the main taxa, we found *Prevotella*, *Streptococcus* and *Veillonella* as the most abundant genus in both groups, the first two being more abundant in the non-MFS group, however, *Veillonella* is more abundant in the MFS group (**Figure 3A**).

To compare the composition of the oral microbiota of the non-MFS group and the MFS group, linear discrimination analysis with effect size was used (LEfSe) [21]. This analysis revealed *Veillonella* as a discriminatory genus for MFS both groups both groups (**Figure 3B).**

5 Discussion

The relationship between the oral microbiota and its host is known to be dynamic. The microbiota in subjects without alterations or diseases is usually very stable; however, alterations in the oral ecosystem such as anatomical malformations, poor hygiene, physiological and genetic alterations, lifestyles can cause dysbiosis and loss of balance or diversity of microorganisms present in dental plaque and increase the associated risk of disease [22, 23, 24]. Recent data from the scientific literature suggest that there is a link between the development and/or aggravation of certain systemic pathologies and the appearance of an imbalance in the oral microbiota [16, 25, 26].

In our study we were able to characterize for the first time the taxonomic profile of the oral microbiota in individuals with MFS, which can serve as a basis for monitoring the oral microbiota in relation to various systemic alterations.

Our results show that *Firmicutes* was the more abundant phylum in our cohort without significant differences between MFS and non-MFS groups; this suggests that the heterogeneity of the data could be due to the size of the sample. The relative abundance of phyla *Firmicutes* and *Bacteroidetes* is highly variable between subjects of the same population, and is related to factors associated with lifestyle, diet, hygiene habits, among others. This could explain the results observed in both groups, making it difficult to associate the ratio *Firmicutes/Bacteroidetes* with a certain state of health [17].

In this population, it was observed that genera such as *Prevotella* and *Streptococcus* were more abundant in both groups; this finding may be related to the participation of the *Streptococcus* in the formation of an acid medium, which is conducive to acid-tolerant genders such as *Prevotella* [27].

In relation to the taxa identified at the genus level, a significant difference was found in *Veillonella* between the non-MFS and MFS groups by analyzing LEfSe, being discriminant of the MFS group; at this point, significant differences have been described in the comparison of children with and without caries, in the genus *Veillonella, Lactobacillus* and *Lachnoanaerobaculum*. This coincides with our population, where *Veillonella* is significantly more frequent in MFS individuals, who presented a higher caries index [28]. On the other hand, genera associated with an oral health condition have been reported, such as *Corynebacterium*, which was identified more frequently in non-MFS individuals of this population [29].

The analysis of alpha diversity in individuals with and without caries, did not determine a statistical difference using the Shannon and Chao1 index, which coincides with the analysis of the present population [28]. On the other hand, the beta diversity analysis determined that the dispersion of the populations is homogeneous, this suggests that the heterogeneity of the data could be due to the size of the sample groups.

Species of *Veillonella* genus have also been associated with cardiovascular diseases, such as in individuals with prosthetic valve endocarditis or prosthetic atrial infection [30, 31]. Similarly, multiple cases of endocarditis caused by *Veillonella* species have been reported [32]. It should be noted that the authors mention that, on rare occasions, *Veillonella ssp* have been the only etiological agents in serious cardiovascular infections. The possible sources of infection with this microorganism are still unknown, although *Veillonella* is often involved in periodontal disease and dental plaque formation. This gives us a possible starting point by giving you the importance of characterizing the oral microbiota of individuals with MFS and its multiple systemic repercussions, without forgetting cardiac conditions as the main cause of mortality. Species from the oral cavity, such as *Streptococcus periodonticum, S. mutans, Streptococcus sinensis, Streptoccoccusinfantis, Streptococcus parasanguinis, Fusobacterium nucleatum, Fusobacterium periodonticum, Porphyromonas pasteri,* and *Aggregatibacter segnis*, have been isolated from subjects undergoing aortic replacement; These species also correspond to the most frequent genus in our population, so it is suggested that they may have the same participation in individuals with MFS and some cardiovascular alteration. [33].

6 Conclusion

This is the first characterization of the oral microbiota in people with FMS in Mexico. Showing that caries risk in this population (MFS) is high. The main taxa at the phyla and genus level were identified, and in MFS a relatively higher abundance of the genus *Veillonella* was found, which corresponds to a bacterium related to heart disease. This first avenue of investigation of the microbiota opens perspectives for future studies at the translational level where oral and systemic health conditions and treatments can be evaluated through clinical trials, as well as studies directed at the mechanisms of immune response that participate at the oral level. oral.

However, there were some limitations in this study. First, the healthy subject samples were only 6, which is too small to minimize experimental bias and make a group comparison. Second, to determine

the association of *Veillonella* with MFS, it is necessary to analyze the clinical, biological, and social variables associated with cardiovascular disease.

Given the limitations, studies with more individuals are needed to confirm the results of this study.

Future implications in this type of studies will allow new hypotheses based on the association of oral damage with cardiovascular alterations.

Data availability statement

The datasets presented in this study can be found in online repositories. Repository: SRA accession number: PRJNA869709 The link to the data can be found below: http://www.ncbi.nlm.nih.gov/bioproject/869709.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Ordaz-Robles Erick R. performed the design, experiments, data analysis, and wrote the manuscript; Soto-Lopez María E. contributed to the recruitment and diagnosis of the participants, translation of the manuscript, Hernández-Ruiz Paulina designed the figures and data analysis, Pinto-Cardoso Sandra contributed to the genomic analysis and the results, Wong-Chew Rosa M. analyzed the results and Aguirre-García María M.contributed to the supervision of the project, funding, conception, and design of the study.All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Table 1. Demographics characteristics of the individuals

	non-MFS	MFS	*p-value*
Sample size	6 (14.3)	36 (85.7)	
Gender			✦0.18
Male	2 (4.8)	24 (57.1)	
Female	4 (9.5)	12 (28.6)	
Age,years			▷0.409
0 to 10	2 (4.8)	9 (21.6)	
11 to 20	0 (0)	16 (38.4)	
21 to 30	2 (4.8)	6 (14.4)	
31 to 40	2 (4.8)	3 (7.2)	
41 to 50	0 (0)	1 (2.4)	
51 to 60	0 (0)	1 (2.4)	

Note. Data is presented as n (%)

✦Fisher exact test

▷U- Mann Whitney

Table 2. Caries experience in group with non-MFS versus MFS group

Age groups	Health status	n	Median	Range	DMF index
All individuals	non-MFS	6	0.5	0-3	1.3
	MFS	36	6.19	0-18	8.8
0-17 y§	non-MFS	2	1.5	0-3	3
	MFS	23	5.83	0-18	7.34
18-25 y§	non-MFS	1	0	0	0
	MFS	5	8.2	0-13	9.8
>25 y§	non-MFS	3	0	0	0.66
	MFS	8	6	0-14	12.75

Note. DMF index: decay-missing-filled index (Very low 0-1.1, Low 1.2-2.6, Moderate 2.7-4.4, High 4.5-6.5, Very high >6.6)
§ Leveled according to epidemologic significance of index. [20, 34]

Figure legends

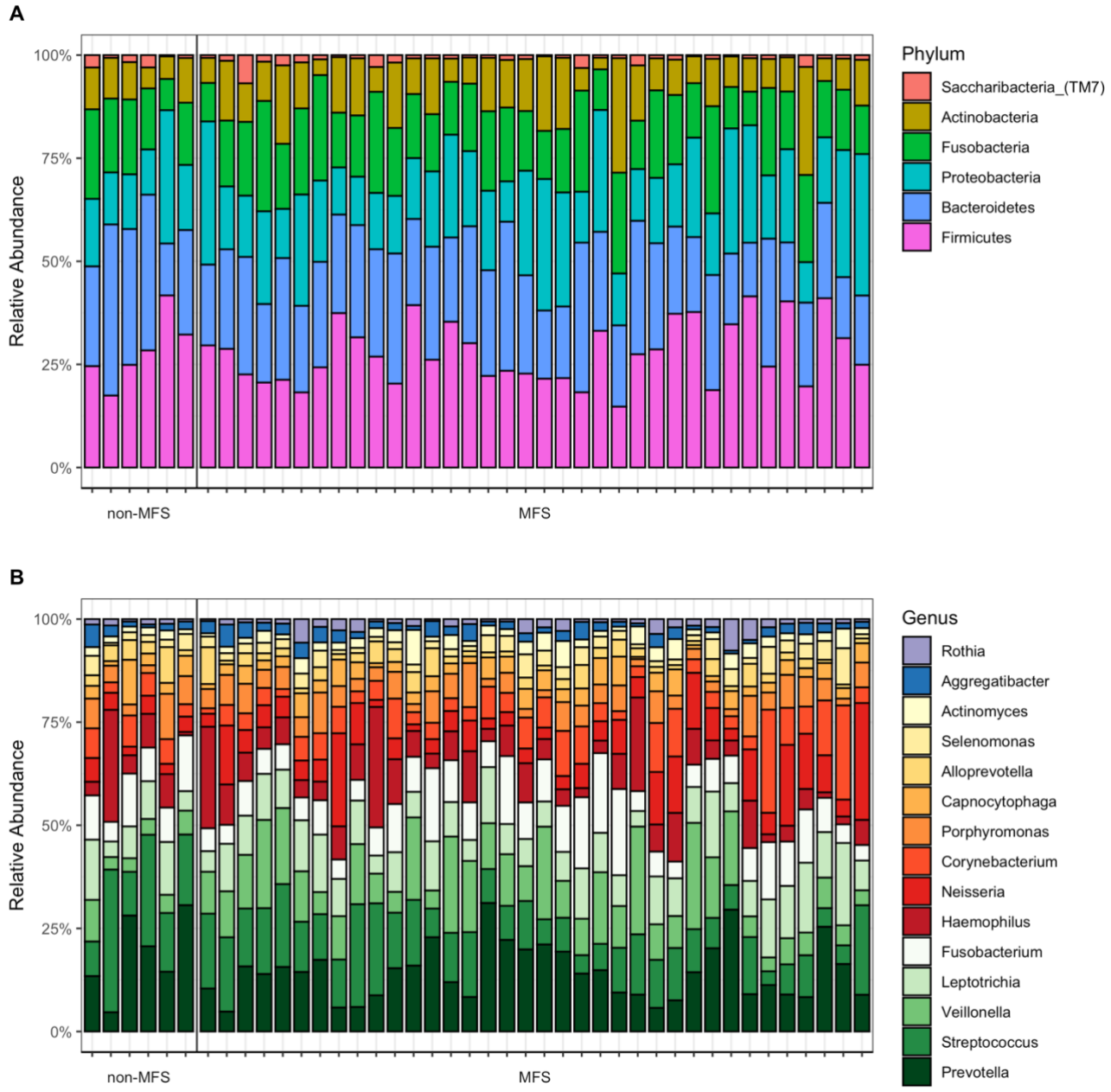


Figure 1. Relative abundance of oral microbiota in non-MFS and MFS groups. A) Phylum level and B) Genus level.

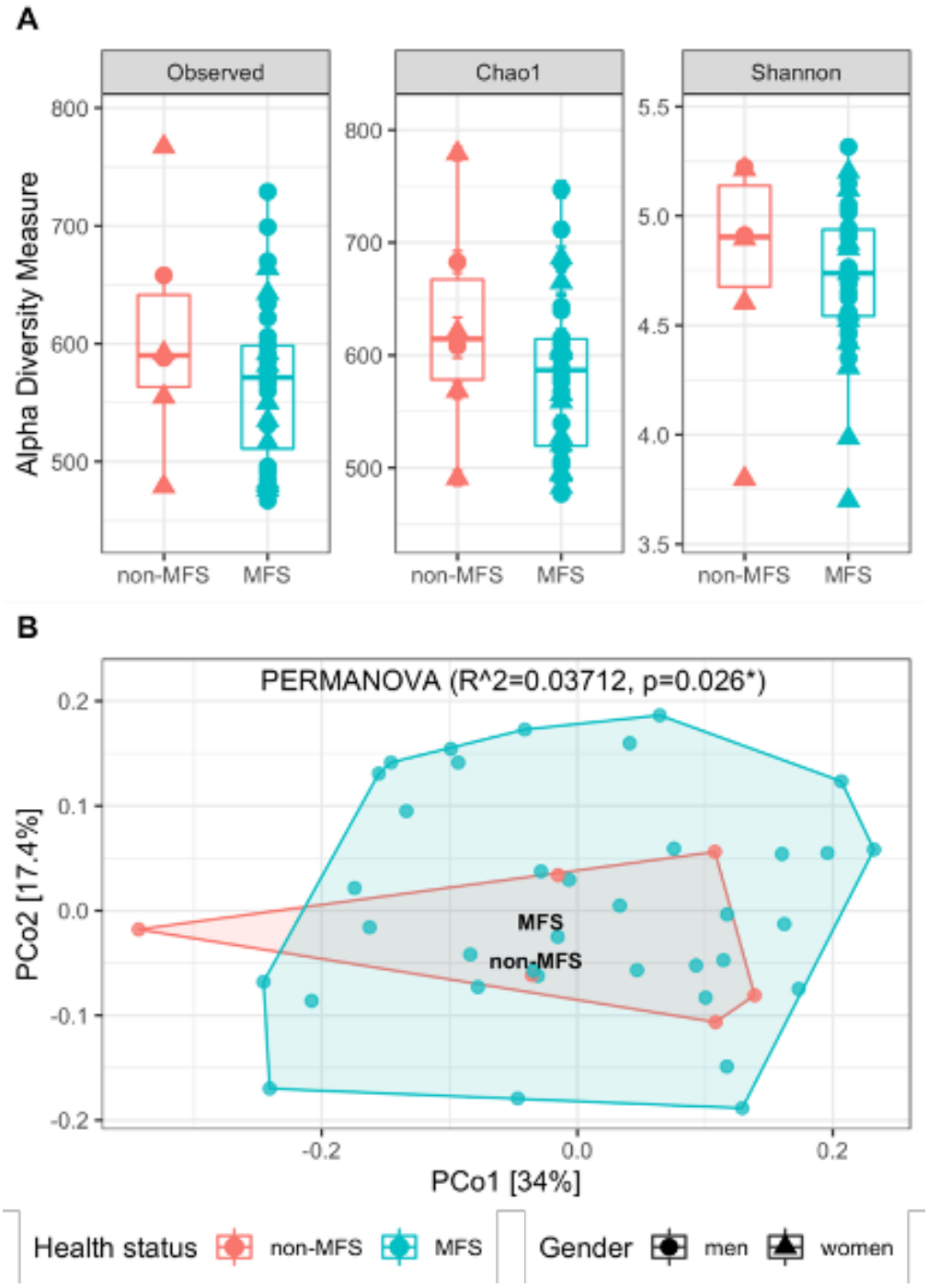


Figure 2. Oral microbiota diversity analysis in non-MFS and MFS subjects. A) Alpha diversity based on Observed features, Chao1 and Shannon index. B) Principal coordinate analysis (PCoA) based on Bray Curtis dissimilarity index distributed by health status. PERMANOVA (R2 = 0.037, p=0.026).

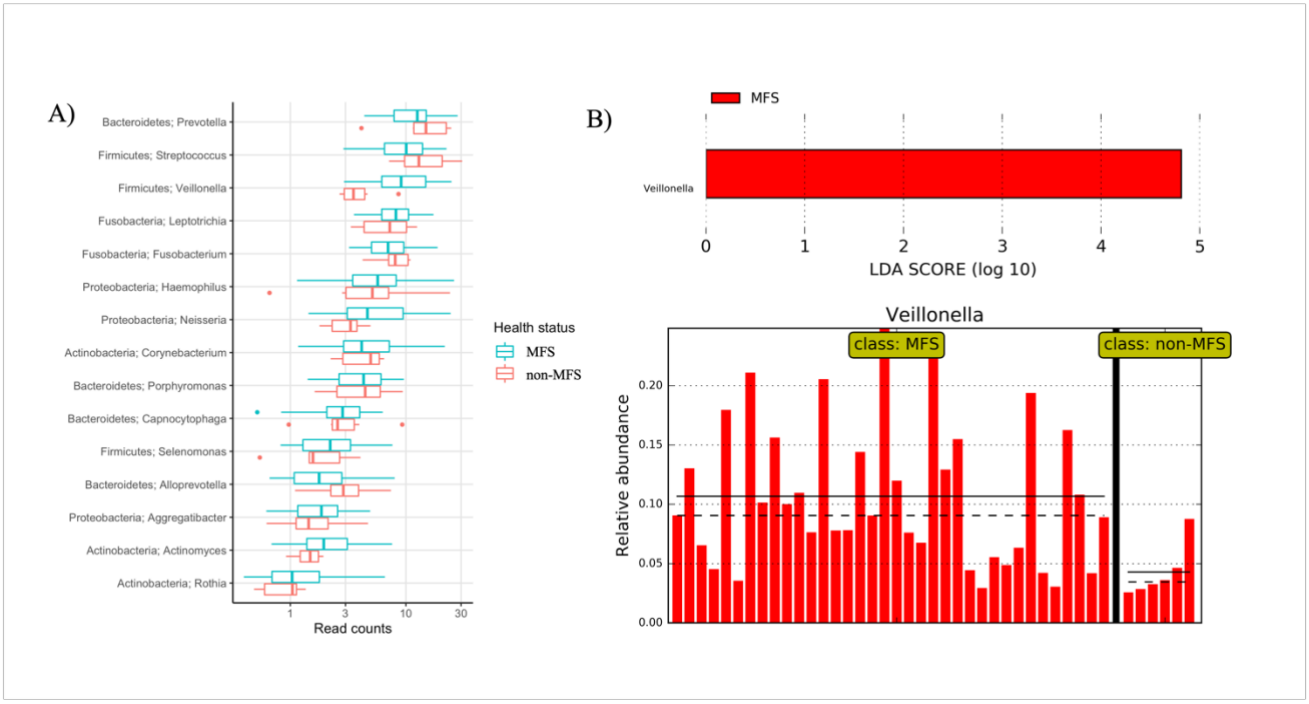
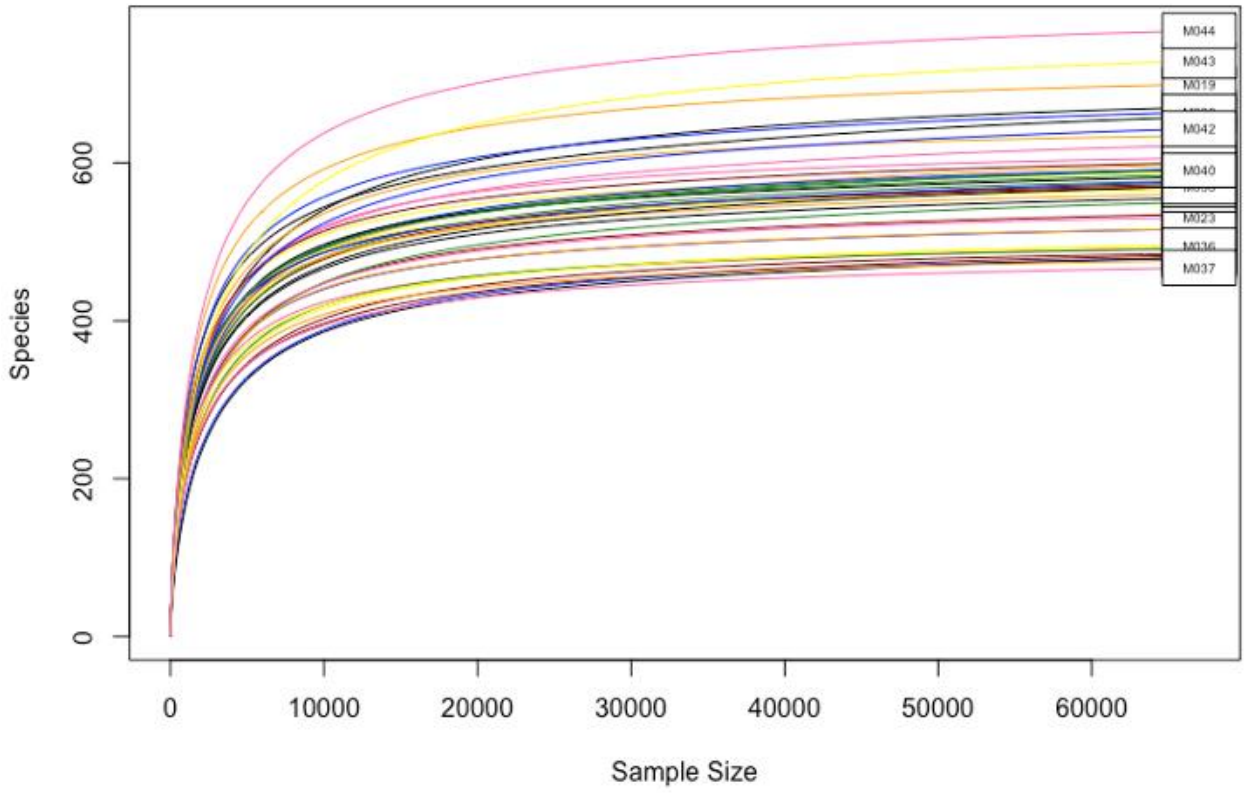


Figura 3. Analysis of the principal genus in non-MFS and MFS subjects. A) Frequency of more abundant genus by health status. B) LEfSe analysis referring the genus associated to non-MFS and MFS subjects; the upper graph refers the genus shown as biomarkers by health status; the lower graph refers the relative abundance of the genus *Veillonella* in MFS subjects (LDA score 3.5, $p<0.05$, black line refers median, dot line refers median).

Supplementary Material

Supplementary Figures



Supplementary Figure 1. Supplementary figure 1. Rarefaction curves of oral microbiota in non-MFS and MFS subjects.

Discusión

Se sabe que la relación entre la microbiota oral y su huésped es dinámica, en sujetos sin alteraciones ni enfermedades suele ser muy estable; sin embargo, alteraciones en el ecosistema oral como malformaciones anatómicas, mala higiene, alteraciones fisiológicas y genéticas, estilos de vida pueden causar disbiosis y pérdida del equilibrio o diversidad de microorganismos presentes en la placa dental y aumentar el riesgo de enfermedad asociadas a la placa. [22, 23, 24]. Datos recientes de la literatura científica sugieren que existe un vínculo entre el desarrollo y/o agravamiento de determinadas patologías sistémicas y la aparición de un desequilibrio en la microbiota bucal [16, 25, 26].

En nuestro estudio pudimos caracterizar por primera vez el perfil taxonómico de la microbiota oral en individuos con MFS, lo que puede servir como base para monitorear la microbiota oral en relación con diversas alteraciones sistémicas. Nuestros resultados muestran que *Firmicutes* fue el phylum más abundante en nuestra cohorte sin diferencias significativas entre los grupos MFS y no MFS; esto sugiere que la heterogeneidad de los datos podría deberse al tamaño de la muestra. La abundancia relativa de las phyla *Firmicutes y Bacteroidetes*es muy variable entre sujetos de una misma población, y está relacionada con factores asociados al estilo de vida, dieta, hábitos de higiene, entre otros. Esto podría explicar los resultados observados en ambos grupos, dificultando la asociación de la relación *Firmicutes/Bacteroidetes* con un determinado estado de salud [17].

En esta población se observó que géneros como *Prevotella y Streptococcus* fueron más abundantes en ambos grupos; este hallazgo puede estar relacionado con la participación de *Streptococcus* en la formación de un medio ácido, lo que favorece géneros tolerantes al ácido como *Prevotella* [27].

En relación con los taxones identificados a nivel de género, el análisis LEf Se mostró diferencia significativa en *Veillonella* entre los grupos no MFS y MFS, siendo más frecuente en el grupo MFS; en este punto se han descrito en otros estudios diferencias significativas en la comparación de niños con y sin caries, en los géneros *Veillonella y Lactobacillus.* Esto coincide con nuestra población, donde *Veillonella*

es significativamente más frecuente en individuos con MFS, quienes presentaron un mayor índice de caries [28]. Por otro lado, se han reportado géneros asociados a una condición de salud oral, como *Corynebacterium*, que se identificó con mayor frecuencia en individuos sin MFS de esta población [29].

El análisis de la diversidad alfa en individuos con y sin caries, no determinó diferencia estadística mediante el índice de Shannon y Chao1, lo cual coincide con el análisis de la población actual [28]. Por otro lado, el análisis de diversidad beta determinó que la dispersión de las poblaciones es homogénea, esto sugiere que la heterogeneidad de los datos podría deberse al tamaño de los grupos muestrales.

Las especies del género *Veillonella* también se han asociado con enfermedades cardiovasculares, como en individuos con endocarditis de válvula protésica o infección auricular protésica [30, 31]. De manera similar, se han informado múltiples casos de endocarditis causada por especies de *Veillonella* [32]. Cabe señalar que los autores mencionan que, en raras ocasiones, *Veillonella ssp* han sido los únicos agentes etiológicos en infecciones cardiovasculares graves. Las posibles fuentes de infección por este microorganismo aún se desconocen, aunque *Veillonella* suele estar implicada en la enfermedad periodontal y la formación de placa dental. Esto nos brinda un posible punto de partida al brindarles la importancia de caracterizar la microbiota bucal de los individuos con MFS y sus múltiples repercusiones sistémicas, sin olvidar las afecciones cardíacas como principal causa de mortalidad.

Se han aislado especies de la cavidad oral, como *Streptococcus periodonticum, S. mutans, Streptococcus sinensis, Streptoccoccus infantis, Streptococcus parasanguinis, Fusobacterium nucleatum, Fusobacterium periodonticum, Porphyromonas pasteri y Aggregatibacter segnis*, de sujetos sometidos a reemplazo aórtico; Estas especies también corresponden al género más frecuente en nuestra población, por lo que se sugiere que puedan tener la misma participación en individuos con MFS y alguna alteración cardiovascular. [33].

Conclusión

Este es el primer estudio que caracteriza a la microbiota oral en individuos mexicanos con síndrome de Marfan, el cual demuestra que el riesgo de caries en MFS es alto. Se identificaron los principales taxones a nivel de phyla y género, y en MFS se encontró una abundancia relativamente mayor del género *Veillonella,* que corresponde a una bacteria relacionada con enfermedades cardiovasculares. Esta primera vía de investigación de la microbiota abre perspectivas para futuros estudios a nivel traslacional donde se puedan evaluar condiciones y tratamientos de salud bucal y sistémica a través de ensayos clínicos, así como estudios dirigidos a los mecanismos de respuesta inmune que intervienen a nivel oral.

Sin embargo, hubo algunas limitaciones en este estudio. Primero, las muestras de sujetos sanos solo fueron 6, lo cual es demasiado pequeño para minimizar el sesgo experimental y hacer una comparación de grupos. En segundo lugar, para determinar la asociación de *Veillonella* con MFS, es necesario analizar las variables clínicas, biológicas y sociales asociadas a la enfermedad cardiovascular.

Dadas las limitaciones, se necesitan estudios que incluyan un número mayor de pacientes MFS que permita conocer más sobre la diversidad de la microbiota oral y pueda ser asociada con las variables clínicas de este síndrome, lo que permitirá la correlación del daño oral con las alteraciones cardiovasculares.

Referencias bibliográficas

[1] Zeigler, S. M., Sloan, B., & Jones, J. A. (2021). Pathophysiology and Pathogenesis of Marfan Syndrome. Advances in experimental medicine and biology, 1348, 185–206. https://doi.org/10.1007/978-3-030-80614-9_8

[2] Coelho, S. G., & Almeida, A. G. (2020). Marfan syndrome revisited: From genetics to the clinic. Síndrome de Marfan revisitada – da genética à clínica. Revista portuguesa de cardiologia, 39(4), 215–226. https://doi.org/10.1016/j.repc.2019.09.008

[3] Soto, M. E., Ochoa-Hein, E., Anaya-Ayala, J. E., Ayala-Picazo, M., & Koretzky, S. G. (2021). Systematic review and meta-analysis of aortic valve-sparing surgery versus replacement surgery in ascending aortic aneurysms and dissection in patients with Marfan syndrome and other genetic connective tissue disorders. Journal of thoracic disease, 13(8), 4830–4844. https://doi.org/10.21037/jtd-21-789

[4] Pyeritz R. E. (2016). Recent progress in understanding the natural and clinical histories of the Marfan syndrome. Trends in cardiovascular medicine, 26(5), 423–428. https://doi.org/10.1016/j.tcm.2015.12.003

[5] Soto, M. E., Iturriaga Hernández, A. V., Guarner Lans, V., Zuñiga-Muñoz, A., Aranda Fraustro, A., Velázquez Espejel, R., & Pérez-Torres, I. (2016). Participation of oleic acid in the formation of the aortic aneurysm in Marfan syndrome patients. Prostaglandins & other lipid mediators, 123, 46–55. https://doi.org/10.1016/j.prostaglandins.2016.05.001

[6] Loeys, B. L., Dietz, H. C., Braverman, A. C., Callewaert, B. L., De Backer, J., Devereux, R. B., Hilhorst-Hofstee, Y., Jondeau, G., Faivre, L., Milewicz, D.

M., Pyeritz, R. E., Sponseller, P. D., Wordsworth, P., & De Paepe, A. M. (2010). The revised Ghent nosology for the Marfan syndrome. Journal of medical genetics, 47(7), 476–485. https://doi.org/10.1136/jmg.2009.072785

[7] Pyeritz R. E. (2019). Marfan syndrome: improved clinical history results in expanded natural history. Genetics in medicine: official journal of the American College of Medical Genetics, 21(8), 1683–1690. https://doi.org/10.1038/s41436-018-0399-4

[8] Westling, L., Mohlin, B., &Bresin, A. (1998). Craniofacial manifestations in the Marfan syndrome: palatal dimensions and a comparative cephalometric analysis. Journal of craniofacial genetics and developmental biology, 18(4), 211–218.

[9] Herrema, H., Nieuwdorp, M., & Groen, A. K. (2022). Microbiome and Cardiovascular Disease. Handbook of experimental pharmacology, 270, 311–334. https://doi.org/10.1007/164_2020_356

[10] Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W. H., Lakshmanan, A., & Wade, W. G. (2010). The human oral microbiome. Journal of bacteriology, 192(19), 5002–5017. https://doi.org/10.1128/JB.00542-10

[11] Escapa, I. F., Chen, T., Huang, Y., Gajare, P., Dewhirst, F. E., & Lemon, K. P. (2018). New Insights into Human Nostril Microbiome from the Expanded Human Oral Microbiome Database (eHOMD): a Resource for the Microbiome of the Human Aerodigestive Tract. mSystems, 3(6), e00187-18.

[12] Simón-Soro, A., & Mira, A. (2015). Solving the etiology of dental caries. Trends in microbiology, 23(2), 76–82. https://doi.org/10.1016/j.tim.2014.10.010

[13] Debelian, G. J., Olsen, I., &Tronstad, L. (1995). Bacteremia in conjunction with endodontic therapy. Endodontics & dental traumatology, 11(3), 142–149.

[14] F.O. Van Ruyven, P. Lingstr¨om, J. Van Houte, R. Kent, Relationship among mutans streptococci, “low-ph” bacteria, and Iodophilic polysaccharide-producing bacteriain dental plaque and early enamel caries in humans, J. Dent. Res. 79 (2) (2000)778–784).

[15] Kholy, K. E., Genco, R. J., & Van Dyke, T. E. (2015). Oral infections and cardiovascular disease. Trends in endocrinology and metabolism: TEM, 26(6), 315–321.

[16] Gutiérrez de Ferro MI, Ruiz de Valladares RE, Benito de Cardenas IL Recuperación de Veillonella a partir de saliva. Rev. Argent. Microbiol. 2005; 37 :22–25.

[17] Lamont, R.J.; Koo, H.; Hajishengallis, G. The oral microbiota: Dynamic communities and host interactions. Nat. Rev. Genet. 2018, 16, 745–759.

[18] Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., & Balamurugan, R. (2020). The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients?.Nutrients, 12(5), 1474. https://doi.org/10.3390/nu12051474

[19] De Coster, P. J., Martens, L. C., & De Paepe, A. (2002). Oral manifestations of patients with Marfan syndrome: a case-control study. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics, 93(5), 564–572. https://doi.org/10.1067/moe.2002.121430

[20] World Health Organization. Oral Health Surveys, Basic Methods, 2nd ed. Geneva: WHO; 1978.

[21] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):60

[22] Trawicka, A., Lewandowska-Walter, A., Majkowicz, M., Sabiniewicz, R., & Woźniak-Mielczarek, L. (2022). Health-Related Quality of Life of Patients with Marfan Syndrome-Polish Study. International journal of environmental research and public health, 19(11), 6827. https://doi.org/10.3390/ijerph19116827

[23] Wozniak-Mielczarek, L., Sabiniewicz, R., Drezek-Nojowicz, M., Nowak, R., Gilis-Malinowska, N., Mielczarek, M., Łabuc, A., Waldoch, A., &Wierzba, J. (2019). Differences in Cardiovascular Manifestation of Marfan Syndrome Between Children and Adults. Pediatric cardiology, 40(2), 393–403. https://doi.org/10.1007/s00246-018-2025-2

[24] Chimenos-Küstner, E., Giovannoni, M. L., &Schemel-Suárez, M. (2017). Dysbiosis as a determinant factor of systemic and oral pathology: importance of microbiome. Disbiosis como factor determinante de enfermedad oral y sistémica: importancia del microbioma. Medicina clinica, 149(7), 305–309. https://doi.org/10.1016/j.medcli.2017.05.036

[25] Laganà, G., Venza, N., Malara, A., Liguori, C., Cozza, P., & Pisano, C. (2021). Obstructive Sleep Apnea, Palatal Morphology, and Aortic Dilatation in Marfan Syndrome Growing Subjects: A Retrospective Study. International journal of environmental research and public health, 18(6), 3045. https://doi.org/10.3390/ijerph18063045

[26] Cervino, G., Cicciù, M., De Stefano, R., Falcomatà, D., Bianchi, A., Crimi, S., Laino, L., Herford, A. S., Gaeta, M., & Fiorillo, L. (2020). Oral health in patients with Marfan syndrome. Archives of oral biology, 116, 104745. https://doi.org/10.1016/j.archoralbio.2020.104745

[27] Takahashi N. Oral Microbiome Metabolism: From "Who Are They?" to "What Are They Doing?". J Dent Res. 2015 Dec;94(12):1628-37]

[28] Qudeimat MA, Alyahya A, Karched M, Behbehani J, Salako NO. Dental plaque

[29] profiles of children with caries-free and caries-active dentition. J Dent. 2021;104:103539].

[30] Cai Z, Lin S, Hu S and Zhao L (2021) Structure and Function of Oral Microbial Community in Periodontitis Based on Integrated Data. Front. Cell. Infect. Microbiol. 11:663756].

[31] Periasamy, S., &Kolenbrander, P. E. (2010). Central role of the early colonizer Veillonella sp. in establishing multispecies biofilm communities with initial, middle, and late colonizers of enamel. Journal of bacteriology, 192(12), 2965–2972.

[32] Houston, S., Taylor, D., & Rennie, R. (1997). Prosthetic valve endocarditis due to Veillonelladispar: successful medical treatment following penicillin desensitization. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 24(5), 1013–1014. https://doi.org/10.1093/clinids/24.5.1013

[33] Marchandin, H., Jean-Pierre, H., Carrière, C., Canovas, F., Darbas, H., & Jumas-Bilak, E. (2001). Prosthetic joint infection due to Veillonelladispar.

European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology, 20(5), 340–342. https://doi.org/10.1007/pl00011273

[34] Pardo A, Signoriello A, Signoretto C, Messina E, Carelli M, Tessari M, De Manna ND, Rossetti C, Albanese M, Lombardo G, Luciani GB. Detection of Periodontal Pathogens in Oral Samples and Cardiac Specimens in Patients Undergoing Aortic Valve Replacement: A Pilot Study. J Clin Med. 2021;10(17):3874

[35] Altman D. Statistics and ethics in medical research. VI. Presentation of results. BrMed J 1980;281:1542-4.