Age-dependent oral manifestations of neurofibromatosis type 1: a case–control study

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Abstract

Introduction: Most craniofacial manifestations of neurofibromatosis type 1 (NF1) are considered as a result of tumor compression. We sought to determine salivary changes, caries, and periodontal complications in NF1 patients without tumors in the oral cavity.

Objective and methods: Eleven NF1 patients without tumors in the oral cavity and 29 matched controls without NF1 were enrolled in this case–control study. Demographic information, medical history, and data of intraoral examinations, including the Decayed, Missing, and Filled Teeth (DMFT) scores and Russell’s periodontal index (PI), were recorded. The functional salivary analysis was performed for sialometry, salivary pH values, and amylase activity. Ingenuity Systems Pathway Analysis (IPA) was conducted to identify mutually activated pathways for NF1-associated oral complications.

Results: NF1 patients were associated with periodontitis (OR = 1.40, 95% CI = 1.06–1.73, \( P = 0.04 \)), gingivitis (OR = 1.55, 95% CI = 1.09–2.01, \( P = 0.0002 \)), and decreased salivary flow rates (OR = 1.40, 95% CI = 1.05–1.76, \( P = 0.005 \)). Periodontal destruction, salivary changes, and dental caries in NF1 patients were age-dependent. Subgroup analyses based on age stratification suggested that salivary flow rates and salivary amylase activities were significantly low in NF1 patients aged over 20 years and that salivary pH values, PI and DMFT scores were significantly high among NF1 controls aged over 20. All oral complications were not significantly presented in NF1 patients aged below 20 years. IPA analyses suggested that cellular mechanisms underlying NF1-associated oral complications involved chronic inflammatory pathways and fibrosis signaling pathway.

Conclusion: NF1 patients without tumors in the oral cavity presented a comparatively high prevalence of age-dependent oral complications, including periodontal destruction and salivary gland dysfunction, which were associated with chronic inflammatory pathogenesis.

Keywords: Neurofibromatosis type 1, Oral complications, Periodontitis, Salivary gland dysfunction, Dental caries
which is underlain by germline mutations in the NF1 gene (locus 17q11.2) [1]. NF1 is inherited in an auto-
somal dominant pattern, with a penetrance of 100% by
age of 20 years [1]. In 50% of cases, the mutated gene
is inherited from the parents, and in the other 50% of
cases, mutations develop spontaneously [1]. The
mutated genes expressing rapidly in females [1].

The NF1 gene is ubiquitously expressed in all cells of
the body, but the phenotypic expression differs from
cell to cell and within a cell through different develop-
mental stages of life [1, 2]. Hence, apart from tumours
involving the nervous system, NF1 is associated with
cutaneous angiomas, subcuta neous leiomyomas, car-
cinoid tumours, and pheochromocytoma, as well as
auditory, visionary, cognitive, sleep [3], musculoskel-
etal, endocrinical and cardiovascular complications [4].
Endocrinological, neurological, and ophthalmological
complications of NF1 are age-dependent, as driven by
hormone imbalance at puberty or during pregnancy,
and usually accompanied with the progression of
neurofibromas in terms of their numbers and sizes [5].
Severe complications of NF1 also include the risk of
malignant peripheral nerve sheath tumours (MPNSTs)
[1].

Due to its rarity, there has been a lack of studies
reporting the oral complications of NF1, with findings
of these works including an asymmetrical enlarged
face with multiple cutaneous/dissemi nated and plexi-
iform neurofibromas [1, 2, 8–10, 13, 14]. In addition to
sphenoid and orbital bone dysplasia, mandibular bony
changes such as deformities of the mandibular ramus
and glenoid fossa (56%), condylar head (50%), increased
dimensions of the coronoid notch, decreased jaw angle,
and enlarged mandibular canals (25%) have been noted
[1, 2, 8–10, 13, 14]. These changes are accompanied by
facial plexiform neurofibromas along the trigeminal
nerve that can cause cephalometric alterations. Other
common intraoral findings include pigmentation of
oral mucosa [6, 7, 15] and tongue, enlarged tongue and
fungiform papillae, malposed teeth with an increased
incidence of dental caries, periapical cemental dyspla-
sia, perineural fibrous thickening of pulpal tissue and
class III malocclusion [8–10]. Given that periodontal
disease may take place following reported craniofacial
complications of NF1 such as malocclusion and hypo-
asalivation [11], and that functional salivary changes were
yet to be determined in previous studies [12, 13], we
conducted this study to identify the above-mentioned
orofacial complications in NF1 patients, and evaluated
whether these symptoms were, similar to that observed
within endocrinological complications of NF1 [5], age-
dependent [1].

Materials and methods
Study design
In this case–control study, 11 NF1 patients of NF1+ status
that met the diagnostic criteria for NF1 by the National
Institutes of Health [1, 12, 13], and 29 matched controls
without NF1 (NF1-), were recruited at Panineeya institute of
dental sciences and research centre, India. The diagnosis of
NF1 required at least two of the following criteria: (1) Six or
more café-au-lait spots or hyperpigmented macules greater
than 5 mm in diameter in prepubertal children and greater
than 15 mm post-pubertal, (2) axillary or inguinal freck-
les, (3) at least two typical neurofibromas or one plexiform
neurofibroma, (4) optic nerve glioma, (5) at least two iris
hamartomas or Lisch nodules in slit-lamp examinations, (6)
sphenoid dysplasia or typical long-bone abnormalities. The
29 NF1- controls were matched on their age, gender, resid-
ing region, socioeconomic status, personal and deleterious
habits, and history of medications [16–18] which can influ-
ence our primary outcome, the salivary flow rate. To ensure
the observed oral complications did not result from direct
tumour compression, it was required in this study that all
enrolled NF1 patients did not have tumors in the maxilla
or mandible. None of the NF1 patients had a diagnosis of
Legius syndrome. This study follows the Strengthening
the Reporting of Observational Studies in Epidemiology
(STROBE) Statement observational studies, the Declaration
of Helsinki, and the National Statements of Ethical Conduct
Elements. Informed consent was acquired from all partici-
pants and was approved by the ethical committee.

Data and intraoral findings
Demographic information and medical history were col-
lected. After conglomeration of data, clinical examination
was performed using a mouth mirror and William's peri-
odontal probe. For the intraoral findings, the Decayed,
Missing, and Filled Teeth (DMFT) index and Russell's
periodontal index (PI) values were recorded. Accordingly,
the diagnoses of gingivitis and periodontitis [19–23] were
made based on Russell's periodontal index score. RPI
scores of 0–0.2, 0.3–0.9, 1–1.9, 2–4.9, 5–8 were classified
as clinically normal supportive tissues, simple gingivitis,
beginning of the destructive periodontal disease, estab-
lished destructive periodontal disease, terminal stage of
periodontal disease, respectively [24].

Collection of saliva for salivary analysis
To evaluate their salivary flow rates, sialometry using
whole unstimulated saliva was performed in the morn-
ing between 10 to 11 AM. Patients were instructed not to
brush or use alcohol/ non-alcohol-based mouthwashes,
consume food or liquids 3 hours before the examination.
The individuals were asked to rinse their mouths with
water and after waiting 10 minutes, the saliva was collected in a sterile test tube for one minute [13, 25]. Accordingly, the salivary flow rate was defined as normal when the whole unstimulated salivary flow rate was greater than 0.3 milliliter per minute (ml/min), decreased salivary rate if the values were in between 0.3 and 0.1 ml/min, and hyposalivation if the values were less than 0.1 ml/min [13].

To identify the salivary pH values and salivary amylase activity (1/min), 1 ml of whole unstimulated saliva was collected in a sterile test tube and the pH value of saliva was measured using a digital pH meter (Electronics India, Model 101). The entire solution was made to 50 ml by adding sterile water. To this solution, 5 drops of 1% starch solution (QUALI-TECH CHEM Starch Indicator 1% Solution 99% PURE) and 2 drops of iodine reagent (Bio balance Lugol’s 2%) were added along the walls of the test tube. After mixing thoroughly, the colour change of the solution into the blue was set as the index time-point, and from that point, the time taken for the solution to become transparent was recorded [26].

**Statistical analysis**
The comparison of the NF1 patients and matched controls (NF1-) for each age subgroup was done by using t tests. P-values less than 0.05 were considered to be significant. Age distribution of sicca syndrome and periodontitis among NF1 patients and NF1- controls were demonstrated, and the chi-square test was used to test the significance. The odds ratios (ORs) were derived to estimate the risk of sicca syndrome and periodontitis between the NF1 patients and NF1- controls [20, 27–29]. The statistical software used was GraphPad Prism, version 5 (GraphPad Software, Inc).

**Ingenuity pathway analysis**
Canonical Pathway Analysis was conducted by comparing differentially expressed (DE) ribonucleic acid (RNA)-sequencing (RNA-seq) data registered in Gene Expression Omnibus of the National Centre for Biotechnology Information. We compared RNA-sequencing data from whole-transcriptome expression profiling using z-score and P-value visualization to identify underlying mechanisms among peripheral blood from patients with NF1, periodontium tissue isolated from patients with periodontitis, and parotid gland tissue from patients with sicca syndrome, compared with healthy controls. The DE RNA-seq was derived by normalizing the RNA expression level of patient samples with that of healthy controls. RNA-seq data with values of $-\log_{10}(P)$ larger than 1.3 were considered significant, and positive z-scores indicated up-regulation while negative scores indicated down-regulation. The canonical pathway analysis was conducted with Ingenuity Systems Pathway Analysis (IPA) software (QIAGEN, Hilden, Germany). Additionally, the network between NF1 and periodontitis or sicca syndrome/Sjögren’s syndrome was constructed through Path Explorer and Molecular Activation Prediction (MAP) within IPA, based on reported gene–gene interactions in Gene Expression Omnibus.

**Results**

**Demographics of NF1 patients and NF1- controls**
Out of the 11 NF1 patients, 5 were females and 6 were males. In these 11 individuals, 4 aged below 21 years, 4 aged between 21 and 40, and 3 aged over 40 years. Controls were matched for age (+/− 3 years), gender, residing region, socio-economic status, personal and deleterious habits, and medications, including calcium channel blockers and caffeine. The case-to-control ratio was 1-to-2.6 (Appendix Table 2). The mean ages of NF1 patients and NF1- controls were 35.18 and 38.96, respectively. After adjusting for the above-mentioned matching criteria, there was no significant difference between NF1 patients and NF1- controls (Appendix Table 2).

**Periodontal findings and dental caries in NF1 patients and NF1- controls**
The prevalence of gingivitis (OR = 1.56, 95%CI = 1.10–2.02, $P = 0.0002$) (Fig. 1A) and periodontitis (OR = 1.40, 95%CI = 1.06–1.73, $P = 0.04$) (Fig. 1B) were significantly higher in NF1 patients compared to that of NF1- controls. Subgroup analyses based on age stratification suggested that the PIs and DMFT scores were age-dependent, which were both significantly higher among NF1 patients aged over 20 (Fig. 1C for PI scores and Fig. 1D for DMFT).

Specifically, the PIs were 3.7 for NF1 patients versus 1.45 for NF1- controls aged between 20–40 (mean difference $= 2.24$, 95%CI $= 0.70–3.80$, $P = 0.009$), and 6.53 for NF1 patients versus 4.8 for controls aged over 41 (mean difference $= 1.73$, 95% CI $= 0.16–3.30$, $P = 0.03$). In contrast, the PIs were not significantly different among NF1 patients below 20 years old (0.65 versus 0.49, mean difference $= 0.16$, 95%CI $= −0.01–0.34$, $P = 0.069$) (Table 1). Likewise, the DMFT scores were 9.5 for NF1 patients versus 4.63 for NF1- controls aged between 20 and 40 (mean difference $= 4.87$, 95% CI $= 3.33–9.41$, $P = 0.03$), and 22.67 for NF1 patients versus 7.71 for NF1- patients aged over 41 (mean difference $= 14.95$, 95%CI $= 12.44–17.47$, $P < 0.0001$). In contrast, the DMFT scores were not significantly different among NF1 patients and NF1- participants below 20 years old (1.25 versus 1.85, mean difference $= −0.61$, 95% CI $= −2.80–1.59$, $P = 0.55$) (Table 1).

**Salivary changes in NF1 patients and NF1- controls**
Significantly decreased salivary flow rates in NF1 patients (OR $= 1.40$, 95%CI $= 1.05–1.76$, $P = 0.005$) (Fig. 2A)
Table 1  Presentation of oral complications among NF1 patients and NF1- controls

| Index                  | Age subgroups | Mean of NF1- controls | Mean of NF1+ cases | Mean difference (95% CI) values of NF1+—that of NF1- | P value |
|------------------------|---------------|-----------------------|--------------------|------------------------------------------------------|---------|
| Salivary flow rate     | < 20          | 0.340                 | 0.335              | −0.005 (−0.019–0.0092)                                | 0.45    |
| Salivary flow rate     | 21–40         | 0.321                 | 0.293              | −0.029 (−0.047–−0.011)                               | 0.005   |
| Salivary flow rate     | > 40          | 0.292                 | 0.173              | −0.12 (−0.15–0.09)                                   | <0.0001 |
| Amylase activity       | < 20          | 0.387                 | 0.374              | −0.013 (−0.03–0.0083)                                | 0.15    |
| Amylase activity       | 21–40         | 0.377                 | 0.323              | −0.053 (−0.075–−0.033)                               | 0.0008  |
| Amylase activity       | > 40          | 0.360                 | 0.292              | −0.068 (−0.089–−0.047)                               | <0.0001 |
| Salivary pH value      | < 20          | 7.04                  | 7.08               | 0.032 (−0.068–0.13)                                  | 0.49    |
| Salivary pH value      | 21–40         | 7.10                  | 7.80               | 0.70 (0.46–0.94)                                     | <0.0001 |
| Salivary pH value      | > 40          | 7.17                  | 8.27               | 1.10 (0.911.27)                                      | <0.0001 |
| PI                     | < 20          | 0.49                  | 0.66               | 0.16 (−0.016–0.35)                                   | 0.07    |
| PI                     | 21–40         | 1.46                  | 3.70               | 2.25 (0.69–3.80)                                     | 0.009   |
| PI                     | > 40          | 4.80                  | 6.53               | 1.73 (0.16–3.31)                                     | 0.03    |
| DMFT score             | < 20          | 1.86                  | 1.25               | −0.61 (−2.80–1.59)                                   | 0.55    |
| DMFT score             | 21–40         | 4.62                  | 9.5                | 4.88 (0.33–9.91)                                     | 0.04    |
| DMFT score             | > 40          | 7.71                  | 22.67              | 14.95 (12.44–17.47)                                  | <0.0001 |

Fig. 1  Periodontal diseases and DMFT among NF1 patients and NF1- controls. A Prevalence of gingivitis diagnosed clinically among NF1 patients and NF1- controls (OR = 1.56, 95% CI = 1.10–2.02, P = 0.0002). B Prevalence of periodontitis among NF1 patients and NF1- controls (OR = 1.40, 95% CI = 1.06–1.74, P = 0.04). C Periodontal indices among NF1 patients and NF1- controls. Y axis represents PIs. X axis represents age of participants. D DMFT scores among NF1 patients and NF1- controls. Y axis represented DMFT scores. X axis represented age of participants.

were noted. Overall, the salivary changes in NF1 patients were age-dependent. Subgroup analyses based on age stratification suggested that the whole unstimulated salivary flow rates (ml/min) and the salivary amylase activity (1/min) were age-dependent, which were both significantly low among NF1+ patients, aged over 20
Moreover, the salivary pH values were significantly high among NF1 patients aged over 20 (Fig. 2D). For instance, the flow rates were 0.29 for NF1 patients versus 0.32 for controls aged between 20 and 40 (mean difference $= -0.029$, $95\%$ CI $= -0.047$–$-0.011$, $P = 0.005$), and 0.17 for NF1 patients versus 0.29 for controls aged over 41 (mean difference $= -0.12$, $95\%$ CI $= -0.14$–$-0.088$, $P < 0.0001$). On the contrary, the flow rates were not significantly different among NF1 patients below 20 years old (0.34 versus 0.34, mean difference $= -0.005$, $95\%$ CI $= 0.019$–$0.0091$, $P = 0.44$) (Table 1).

Likewise, amylase activity, which was defined as the reciprocal number of the time for amylase to function, was 0.32 (min$^{-1}$) for NF1 patients versus 0.38 (min$^{-1}$) for NF1- controls aged between 20 and 40 (mean difference $= -0.054$, $95\%$ CI $= -0.075$ to 0.033, $P = 0.0008$), and 0.29 (min$^{-1}$) for NF1 patients versus 0.36 (min$^{-1}$) for NF1- controls aged over 41 (mean difference $= -0.068$, $95\%$ CI $= -0.089$ to $-0.046$, $P < 0.0001$). But the times were not significantly different among NF1 patients below 20 years old (0.37 versus 0.39, mean difference $= -0.013$, $95\%$ CI $= -0.034$–$-0.0082$, $P = 0.15$) (Table 1).

Finally, the pH values were 7.8 for NF1 patients versus 7.1 for NF1- controls aged between 20 and 40 (mean difference $= 0.7$, $95\%$ CI $= 0.46$–$0.94$, $P < 0.0001$), and 8.27 for NF1 patients versus 7.17 for controls aged over 41 (mean difference $= 1.10$, $95\%$ CI $= 0.91$–$1.27$, $P < 0.0001$). In contrast, the pH values were not significantly different among NF1 patients below 20 years old (7.07 versus 7.04, mean difference $= 0.032$, $95\%$ CI $= -0.068$–$0.13$, $P = 0.48$) (Table 1 & Fig. 2).

**Genetic and cellular mechanisms underlying NF1, salivary gland dysfunction, and periodontal destruction**

IPA analyses indicated that the possible mechanisms underlying the correlation between NF1 with cutaneous neurofibromas and oral complications including (1) periodontal destruction, and (2) salivary gland dysfunction, involved multiple chronic inflammatory pathways (Fig. 3A). As for the top 20 canonical pathways mutually expressed in the pathogenesis of NF1, periodontitis, and sicca syndrome, which were ordered based on the sum of z scores, fibrosis signaling pathway was highly
expressed in the three disease groups when compared to healthy donors, with Z scores being 3.70 for NF1, 4.54 for periodontitis, and 3.5 for sicca syndrome, and significant $-\log_{10}(P)$ values being 11.28 for NF1, 10.66 for periodontitis, and 3.30 for sicca syndrome, respectively.

Path Explorer performed using IPA suggested that the downregulation of NF1 gene in NF1 patients may induce periodontitis via upregulation of TNF Superfamily Member 13b (TNFSF13B) while linking to sicca syndrome or Sjögren's syndrome with upregulated TNFSF13B, promyelocytic leukaemia (PML) and signal transducer and activator of transcription 1 (STAT1) (Fig. 3B). Moreover, MAP within IPA demonstrated that the lack of NF1 expression resulted in the upregulation of fibrosis pathway through TNFSF13B-, PML-, or STAT1-involved pathways (Fig. 4). These results demonstrated the upregulated pathways and genes underlying the association between NF1 and the observed oral complications in this study, including periodontal destruction and sicca-like symptoms.

**Discussion**

In this case–control study, NF1 patients were associated with periodontal disease, decreased salivary flow rates, compromised amylase activities, and high prevalence of caries, all in an age-dependent manner. Significantly high PI scores and DMFT scores, low unstimulated salivary flow rates and amylase activities, and high salivary pH values, were observed among NF1 patients aged over 20 years old. For those aged below 20 years, the above-mentioned measurables were not significantly different between NF1 patients and NF1- controls. Moreover, the difference in the means was the highest for those aged over 40, followed by those aged between 20 and 40, finally those aged below 20. With RNA-seq data from peripheral blood in NF1 patients, gingival tissues from patients in periodontitis, and parotid glands in patients with sicca syndrome, IPA analysis predicted that several chronic inflammatory pathways were involved for the association. These pathways included the fibrosis pathway, which may initiate periodontitis and sicca-like symptoms through the upregulation of TNFSF13B, PML and STAT1.

Although a case of gingival neurofibroma in the attached gingiva has been reported [30, 31], so far there is a lack of evidence regarding whether NF1 patients may present periodontal disease without tumors directly involving the oral cavity. In our study, as all NF1 patients did not have neurofibromas in the maxilla or mandible, our findings for the first time demonstrated that NF1 patients were at a high risk of periodontitis. As periodontitis is an inflammatory disease triggered by dysbiosis in the oral cavity [32, 33], our findings on periodontitis in patients with NF1 were in accordance with previous studies pinpointing that NF1 patients presented with

![Fig. 3 NF1-associated pathways and genes in periodontitis or sicca syndrome identified by IPA.](image-url)
low-grade chronic inflammation [34]. On the other hand, our findings on salivary gland dysfunction and dental caries were in phase with a previous case–control study in which the prevalence of hyposalivation in NF1 patients was four-fold higher than their respective NF1- controls [13].

Apart from the above-mentioned theory that low-grade chronic inflammation in NF1 patients may initiate or exacerbate periodontal disease [13], a previous cross-sectional study conducted on 150 individuals demonstrated that the saliva was more alkaline among individuals with periodontitis, which was similar to our study [25] in that high salivary pH values were noted in the enrolled NF1 patients. Importantly, alkaline changes to pH values of the saliva can lead to a decrease in the activity of various salivary anti-microbial proteins and enzymes, as evidenced by salivary amylase activities in starch-iodine-saliva experiments. The basic environment of saliva can also increase the proteolytic activity of the organism, which promotes the deposition of calcium phosphate, thereby placing NF1 patients at great risk of dental caries and periodontal destruction as a feedback loop [25].

Other factors contributing to periodontitis and caries in NF1 patients include fused tooth, misregulation of amelogenesis leading to enamel hypoplasia, microdontia [14], low levels of serum 25-hydroxy vitamin D3 and increased osteoclastic activity [35]. Specifically, low levels of serum 25-hydroxy vitamin D3 among NF1 patients may result from increased pigmentation,
cutaneous neurofibromas-associated impaired synthesis or accelerated catabolism, with its severity increasing with age, making NF1 patients susceptible to periodontitis in an age-dependent pattern [36]. Moreover, histological evidence revealed that the infiltration of mast cells through stem cell factor (SCF) can drive STAT1 activation, creating an inflammatory microenvironment in neurofibroma that is considered a clinicopathological hallmark [37, 38]; on the other hand, the increase of mast cells, also known as mastocytosis, is as well critically involved in the pathogenesis of periodontitis through secreting inflammatory cytokines and participating in periodontal tissue destruction [39]. As such, mast cells may play an essential pathological role in connecting neurofibromatosis and periodontitis.

Several studies proposed mechanisms that can explain salivary gland dysfunction in NF1 patients or models. For instance, animal studies suggested that the NF1 gene played an important role in the organogenesis of salivary glands [12]. Likewise, a study based on salivary gland tissues from NF1 patients pointed out that the neurofibromin gene was expressed in major and minor salivary acinar, ductal epithelium irrespective of age and sex, which indicated the importance of the NF1 gene in the normal function of the salivary gland [40]. Overall, mechanisms that may contribute to hyposalivation in NF1 patients included defective organogenesis of the salivary gland [41, 42], parasympathetic ganglion supplying the gland [42], and neurovascular bundles determining the quality and quantity of saliva that enter the salivary glands [41]. Moreover, mutations of the NF1 gene were reported to bring about complications that resulted from collagen loss or inability to produce collagen fibers, with which the underlying fibrosis pathways may account for pulmonary complications of NF1, such as interstitial lung disease and pre-capillary pulmonary hypertension [43–45], lack of skin elasticity [46], and the susceptibility to osteoporosis and osteomalacia [47]. Thus, similar to how the fibrosis of alveolar epithelium and connective tissue of lungs result in pulmonary complications of NF1 [43–45], the effect of mutations of the NF1 gene on the observed gingival tissue loss/periodontal destruction and sicca-like symptoms can also be underlain by an inadequate collagen amount in the connective tissue of both the periodontium and salivary glands in patients with NF1 [48]. As salivary glands function through squeezing the saliva out of these exocrine glands [49] that are full of type I collagen [50, 51], inadequate collagen production or defective collagen structure [46–48] in the context of NF1 may lead to adverse salivary changes, such as abnormal salivary flow rates and salivary pH values that were noted in the present study.

The major strengths of this study included matching between NF1 patients and NF1- controls, with which the potential effect of confounding factors such as age, gender [52], region, socioeconomic status, medical history, personal and deleterious habits [53] on periodontal health and salivary flow was minimized. However, limitations of this study included the inability to provide robust causal inference in case–control studies of relatively small sample sizes. Moreover, due to the high mutation spectrum of NF1-associated genes, for which genotype–phenotype correlations had been widely investigated for various phenotypic expressions of NF1 [54–58], the lack of genetic sequencing data that can provide information on NF1-mutated subtype limited the ultimate scope to delineate specific mutated regions that were associated with the observed intraoral complications in this study. Future studies incorporating radiographics and genetics are warranted to provide more details about the presenting orofacial complications of NF1, and to provoke patient awareness through communication [59] and education [60].

In conclusion, this study demonstrated age-dependent periodontal destruction and functional salivary changes correlating with carious lesions in NF1 patients, which were evidenced by intraoral examinations and functional salivary tests. These oral complications, instead of resulting from direct compression by neurofibromas, may develop following systemically upregulated chronic inflammatory pathways.

Appendix

See Table 2.

### Table 2 Baseline characteristics for NF1 + and NF1- controls

| Characteristics                      | NF1- controls | NF1 + cases | Case/ control (a) or P-value (b) |
|--------------------------------------|---------------|-------------|---------------------------------|
| Number                               | 29            | 11          | 1.26 (a)                        |
| Age distribution                     |               |             |                                 |
| < 21                                 | 7             | 4           | 1.17 (a)                        |
| 21–40                                | 8             | 4           | 1.2 (a)                          |
| > 40                                 | 14            | 3           | 1.46 (a)                        |
| Mean age (years)                     | 38.96         | 35.18       | –                                |
| Sex (female/male*100%)               | 48.2%         | 45.4%       | –                                |
| Tobacco use                          | 6             | 2           | 0.6 (b)                          |
| Alcohol                              | 5             | 2           | 0.6 (b)                          |
| Medication history of xerostomia-associates drugs | 6 | 2 | 0.6 (b) |
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Authors’ contributions
Conceptualization, E.T., L.T.W. & K.S.M.; Methodology, E.T., J.J.V., B.P.L., J.K., L.T.W. & K.S.M.; Writing—Original Draft, E.T., J.J.V., L.T.W. & K.S.M.; Writing—Review & Editing, Y.W. C., L.T.W. & K.S.M.; Funding Acquisition, Y.W. C., L.T.W. K.S.M. All authors read and approved the final manuscript.

Data sharing
The data used in this study are available upon reasonable requests, and all consent forms will be provided with request.

Declarations
Ethics approval and consent to participate
The Research Ethics Committee approved the current study and informed consents were obtained from the participants.

Competing interests
The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria, educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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