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Altered relationship between MMP-8 and TIMP-2 in gingival crevicular fluid in adolescents with Down's syndrome

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Background and Objective: Periodontitis is more frequently found in subjects with Down's syndrome. The aim was to investigate whether the relationship between MMPs and TIMPs) in the gingival crevicular fluid of subjects with Down's syndrome is altered compared with controls.

Material and Methods: Twenty-one adolescents with Down's syndrome and gingivitis (DS-G), 12 subjects with Down's syndrome and periodontitis (DS-P), 26 controls with gingivitis (HC-G) and eight controls with periodontitis (HC-P) were clinically examined. All patients were between 11 and 20 years of age. Gingival crevicular fluid was collected from each subject and the concentrations of MMPs (2, 3, 8, 9 and 13) and TIMPs (1, 2 and 3) (expressed as pg/μL adjusted for volume* of gingival crevicular fluid) were determined using multianalyte kits from R&D Systems.

Results: The concentrations of MMP-2, MMP-3, MMP-8, MMP-9 and TIMP-2 in gingival crevicular fluid were significantly higher ($p < 0.005$) in the DS-G group compared with the HC-G group. The correlation coefficient between MMP-8 and TIMP-2 differed significantly ($p = 0.006$) between the DS-G group and the HC-G group. On the contrary, the correlation coefficients between MMPs and TIMPs did not differ significantly between the DS-P group and the HC-P group. However, the DS-P group exhibited a significantly lower concentration of TIMP-2 in the gingival crevicular fluid compared with the HC-P group.

Conclusion: Down's syndrome subjects with gingivitis exhibit higher concentrations of MMPs in gingival crevicular fluid with an altered relationship between MMP-8 and TIMP-2, which might impair the periodontal tissue turnover.

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Periodontitis is more frequently found in subjects with Down's syndrome, and it is often diagnosed during adolescence (1). The impaired host response in subjects with Down's syndrome is characterized by reduced chemotaxis, impaired phagocytosis of polymorphonuclear leukocytes and disturbances in T- and B-lymphocyte

[*Correction added after first online publication 26 June 2013: (Concentrations 'expressed as pg/μL of gingival crevicular fluid' have been amended to 'expressed as pg/μL adjusted for volume of gingival crevicular fluid' throughout the article)]

subsets (2–4), altogether contributing to an enhanced risk of periodontitis. Additional factors, such as poor oral hygiene, tongue pressure and the occurrence of periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* have also been suggested as risk factors for periodontal disease in Down's syndrome (5,6).

In periodontal tissue, MMPs are expressed by various inflammatory cells such as monocytes, macrophages, lymphocytes and polymorphonuclear cells, as well as by resident cells such as fibroblasts, epithelial cells and endothelial cells (7). To date, 24 different MMPs have been detected, of which 23 are found in humans (8). MMPs are classified, based on substrate specificity, into several groups, such as collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3 and MMP-10), stromelysin-like MMPs (MMP-7, MMP-11 and MMP-12) and membrane-type MMPs (MMP-14, MMP-15, MMP-16 and MMP-17) (9). MMPs are secreted as latent, inactive pro-enzyme forms, with cytokines such as interleukin-1β and tumor necrosis factor alpha (TNF-α) the probable inducers of MMP expression (10–13). Increasing evidence has implicated MMPs as key mediators in the tissue destruction associated with various forms of periodontal disease, including the progression from gingivitis to periodontitis (14–16). However, *in-vitro* studies have demonstrated anti-inflammatory effects of MMP-8 and MMP-9 (17,18), and MMP-8 has been suggested to play a protective role in *P. gingivalis*-induced alveolar bone loss by processing cytokines and chemokines (19,20).

The activity of MMPs is further modulated by TIMP-1, TIMP-2, TIMP-3 and TIMP-4, which partly control and stabilize MMPs and thereby participate in tissue remodelling during periodontal tissue destruction (8,21,22). In general, most mesenchymal and epidermal cells are capable of producing TIMPs (23), but TIMP-1, TIMP-2 and TIMP-3 are also produced by white blood cells (24,25).

Knowledge regarding MMPs and TIMPs in periodontal tissue in Down's syndrome is limited. However, it has been reported that the levels of MMP-2, MMP-8 and MMP-9 are enhanced in gingival crevicular fluid in Down's syndrome (26,27). In addition, increased immunoreactivity of MMP-8 and MMP-9 in saliva compared with healthy controls has also been demonstrated (28). The aim of this study was therefore to investigate whether the relationship between MMPs and TIMPs in gingival crevicular fluid is altered in subjects with Down's syndrome compared with controls.

Material and methods

Subjects

The study design was cross-sectional and was approved by the local Ethics Committee at Karolinska Institutet, Huddinge University Hospital. The study population comprised subjects with Down's syndrome who had been consecutively referred to the Department of Paediatric Dentistry, Eastmaninstitutet, Stockholm. The control subjects with gingivitis were selected from the Public Dental Health Services, Stockholm. The control subjects with periodontitis were consecutively referred to the Department of Paediatric Dentistry, Eastmaninstitutet, and to the Department of Periodontology, Stockholm. All subjects and/or their parents provided verbal consent before participating in the study.

The inclusion criteria for all patients were age between 11 and 20 years and, for the controls with gingivitis (HC-G) and subjects with Down's syndrome and gingivitis (DS-G) groups, bleeding on probing (BOP) below 50%. The inclusion criteria for the periodontitis groups [controls with periodontitis (HC-P) and subjects with Down's syndrome and periodontitis (DS-P)] were one or more sites with a periodontal probing depth of > 3 mm and marginal alveolar bone loss on radiographs. Exclusion criteria for all patients were recent use of antibiotics (in the last 3 mo), previous and/or ongoing smoking, as well as ongoing orthodontic treatment. For the controls, the occurrence of a diagnosed medical chronic disorder was also used as an exclusion criterion. A power analysis was performed before the start of the recruitment of subjects. Based on a previous report of MMP-9 in the gingival crevicular fluid of subjects with Down's syndrome (27), a 5% significance level and 80% power required a sample size of 14 subjects/group to detect differences in MMP concentrations (expressed in pg/mL) between subjects with Down's syndrome and controls.

Of the total number of subjects with Down's syndrome ($n = 56$) examined, eight were excluded because of BOP > 50% and 15 were excluded because of a lack of compliance during the clinical examination. The DS-G group comprised 21 subjects with a mean age of 16.1 years and the DS-P group comprised 12 individuals with a mean age of 15.0 years.

Of the total number of control patients ($n = 88$), 12 were excluded because of ongoing orthodontic treatment, 15 were excluded because of a missed appointment, 21 declined to participate in the study, two had a medical disorder, one was a smoker and three patients referred for periodontitis were incorrectly diagnosed. The HC-G group comprised 26 subjects with a mean age of 16.5 years and the HC-P group comprised eight individuals with a mean age of 15.6 years. The characteristics of all study groups are given in Table 1.

Questionnaire

A questionnaire was answered by all the parents/subjects regarding sociodemographic factors, oral hygiene habits and medical conditions (Table 1). In the event of language problems, an interpreter assisted the subjects.

Clinical examination

Gingival inflammation was based on BOP of the gingival sulcus at four sites of all teeth (excluding wisdom teeth). The percentage of surfaces with BOP was calculated for each individ-

Table 1. Characteristics of the subjects with Down's syndrome and the controls

Variables	Down's syndrome with gingivitis (n = 21)	Controls with gingivitis (n = 26)	Down's syndrome with periodontitis (n = 12)	Controls with periodontitis (n = 8)	p-Value
Female/male	7/14	13/13	3/9	6/2	0.374[a]0.065[b]
Age (years)	16.1 (1.9)	16.5 (1.7)	15.0 (2.5)	15.6 (2.5)	0.344[a]
Range	13–20.5	13.5–19	13–19.5	11.5–19	0.440[b]
Age interval (no. of patients)					
11–14 years	5	5	7	1	0.643[a]
15–17 years	13	16	4	5	0.112[b]
18–21 years	3	5	1	2	
Clinical condition					
BOP (%)	32 (9)	29(8)	69(30)	43(31)	0.126[a]
range	15–49	18–46	30–100	10–100	0.032[b]
Probing depth (no. of patients)					
0–3 mm	21	26	0	0	
4 mm	0	0	1	0	
$\geq$ 5 mm	0	0	11	8	
Mean percentage of sites > 3 mm	0	0	7.5 (4.3)	9.3 (5.3)	0.435[b]
ABL ($\geq$ 2 mm)					
1–2 sites	0	0	10	4	
> 2 sites	0	0	2	4	
Mean percentage of sites $\geq$ 2 mm	0	0	4.4 (2.9)	4.4 (2.3)	0.974[b]
Chronic diseases					
Heart disorder	5	0	4	0	
Asthma	3	0	1	0	
Upper airway	2	0	2	0	
Autism	1	0	0	0	
Epilepsy	1	0	2	0	
Thyroid deficiencies	5				
Immigrant background					
Yes	1	0	2	7	0.447[a]
No	20	26	10	1	0.005[b]
Oral hygiene habits					
2/d with assistance	18	0	6	0	
2/d without assistance	3	24	0	6	
1/d with assistance	1	0	5	0	
1/d without assistance	0	2	0	0	
Never	0	0	1	2	

Data are given as mean (standard deviation), unless indicated otherwise.
ABL, alveolar bone loss; BOP, bleeding on probing.
The Mann–Whitney U-test or the chi-square exact test was used as the statistical method.
[a]p-Value for difference between subjects with Down's syndrome and gingivitis and controls with gingivitis.
[b]p-Value for difference between subjects with Down's syndrome and periodontitis and controls with periodontitis.

ual and expressed as BOP%, a reliable indicator of gingival inflammation (29).

Periodontal probing depth was recorded using a graded periodontal probe (Hu-Friedy, Chicago, IL, USA) and measured to the nearest millimetre at four sites of all teeth (excluding wisdom teeth). Pathological probing depth was classified when the subject exhibited one or more sites with a periodontal probing depth of > 3 mm (30).

Radiographic assessment was performed on bitewing or peri-apical radiographs, which were taken by either a digital or a conventional X-ray technique. When subjects in the Down's syndrome group did not cooperate with intra-oral radiographs, panoramic radiographs were taken. The magnification factor of the orthopantomogram used in this study was 1.2, and the panoramic radiographs were adjusted accordingly. Alveolar bone loss (ABL) was classified when the distance from the cemento–enamel junction to the alveolar crest on the radiograph exceeded 2 mm for one or more permanent teeth in the upper or lower jaw (31).

Collection and processing of gingival crevicular fluid samples

Before the clinical examination, gingival crevicular fluid samples were collected from each patient from the mesial surface of teeth 16, 26, 36, 46 and 41 and from the distal surface of tooth 11. Before gingival crevicular fluid collection, supragingival plaque was eliminated using a cotton pellet and a curette, and the tooth surface was gently dried with air. A paper strip (Periopaper; Pro Flow, Amityville, NY, USA) was inserted into each sul-

cus and left for 15 s. Paper strips contaminated with blood during gingival crevicular fluid sampling were discarded. Gingival crevicular fluid volume was determined using a Periotron 8000 (Pro Flow) system, calculated by interpolation from a standard curve and expressed as microliters of gingival crevicular fluid. The Periopaper was placed in 120 μL of assay buffer containing 0.9% NaCl, 0.01 M EDTA, 0.3% bovine-globulin, 0.005% Triton-X-100, 0.05% sodium azide, 0.0255 M NaH_2PO_4 and 0.0245 M Na_2HPO_4 (pH 6.8) and kept frozen at −70°C. The concentrations of MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 and of TIMP-1, TIMP-2 and IMP-3 (expressed as pg/μL adjusted for volume* of gingival crevicular fluid) were determined in gingival crevicular fluid samples using the commercially available human MMP/TIMP multianalyte kit from R&D Systems Inc. (Minneapolis, MN, USA), in accordance with the manufacturer's instructions. Briefly, each well of a 96-well microplate was prewetted with 100 μL of wash buffer and then 50 μL of diluted microparticle mixture was added to each well. The gingival crevicular fluid samples (diluted 1 : 2 for MMP-2, MMP-3 and MMP-13, 1 : 40 for MMP-8 and MMP-9 and 1 : 10 for TIMP-1, TIMP-2 and TIMP-3) were then added to each well and incubated for 2 h at room temperature on a horizontal orbital microplate shaker. After the washing procedure, 50 μL of diluted antibody cocktail was added to each well and incubated for 1 h at room temperature. Finally, 50 μL of diluted streptavidin–phycoerythrin conjugate was added to each well and incubated for 30 min at room temperature. After the last incubation, the concentrations of MMPs and TIMPs were determined using a Luminex analyser (Bio-Rad Laboratories, Hercules, CA, USA). According to the manufacturer, MMPs and TIMPs do not cross-react (less than 0.5% cross-reactivity) with other MMP and TIMP family members. MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 recognize both natural and recombinant human pro- and mature types of TIMP, as well as TIMP-1 complexed with MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13. TIMPs (TIMP-1, TIMP-2, TIMP-3 and TIMP-4) recognize both natural and recombinant human TIMPs.

The concentration of TNF-α in gingival crevicular fluid was only studied among the subjects with gingivitis (DS-G and HC-G) (23), using the commercially available Bio-Plex Cytokine Assay (Bio-Rad Laboratories, Hercules, CA, USA) in accordance with the manufacturer's instructions. In brief, each well of a 96-well microplate was prewetted with 100 μL of wash buffer and 50 μL of coupled magnetic beads was added to each well. Then, a 50-μL sample of gingival crevicular fluid was added to each well and incubated for 30 min at room temperature. After the washing procedure, 25 μL of detection antibodies was added to each well and incubated for 30 min at room temperature. Finally, 50 μL of diluted streptavidin–phycoerythrin conjugate was added to each well and incubated for 10 min at room temperature and the level of TNF-α was determined using a Luminex analyser (Bio-Rad Laboratories).

Statistical analysis

The Mann–Whitney *U*-test (two-tailed) was used to compare the medians of the variables, and the chi-square exact test was used to compare the categorical variables of the groups. Pearson's correlation was used to calculate the correlations between groups. Fisher's *Z* transformation was used when testing the difference in correlation coefficients between subjects with Down's syndrome and controls. To adjust for multiple testing, the Bonferroni analysis was performed. The Statistical Package for the Social Sciences (Release 2011, IBM SPSS STATISTICS FOR WINDOWS, Version 20.0; IBM Corp., Armonk, NY, USA) was used as the statistical program.

Results

The characteristics of the subjects with respect to gender, age, general health, sociodemographic factors and oral hygiene habits, as well as the clinical conditions in terms of BOP%, probing depth and ABL are presented in Table 1. The DS-G group comprised 21 subjects with a mean age of 16.1 years, the DS-P group comprised 12 subjects with a mean age of 15.0 years, the HC-G group comprised 26 subjects with a mean age of 16.5 and the HC-P group comprised eight subjects with a mean age of 15.6 years. There were no significant differences between the DS-G group and the HC-G group regarding the clinical variables BOP%, probing depth and the occurrence of ABL. The DS-P group showed significantly higher ($p < 0.05$) BOP% compared with the HC-P group. No significant differences were found regarding probing depth and the occurrence of ABL between the DS-P group and the HC-P group (Table 1).

Biochemical analysis

The levels of MMPs and TIMPs in gingival crevicular fluid from subjects with Down's syndrome and matched controls are presented in Tables 2 and 3. Significantly higher volumes of gingival crevicular fluid (μL) were obtained from the DS-G group compared with the HC-G group ($p < 0.005$). The median values of MMP-2, MMP-3, MMP-8, MMP-9, TIMP-2 and TNF-α, expressed as pg/μL adjusted for volume* of gingival crevicular fluid, were significantly higher in the DS-G group compared with the HC-G group ($p < 0.005$) (Table 2). The DS-P group exhibited a significantly lower ($p < 0.005$) concentration of TIMP-2 (pg/μL adjusted for volume* of gingival crevicular fluid) compared with the HC-P group (Table 3).

Correlation analysis

The relationship between MMPs and TIMPs was studied by Pearson correlation analysis. In contrast to the HC-G group, there was a significant, positive correlation between TIMP-2 and both MMP-8 ($r = 0.703$; $p < 0.001$) and MMP-9 ($r = 0.651$; $p = 0.001$) in the DS-G group

Table 2. The median value of MMPs and TIMPs, expressed as pg/μL adjusted for volume* of gingival crevicular fluid, in subjects with Down's syndrome and gingivitis and in controls with gingivitis

Variables	Down's syndrome with gingivitis (n = 21)	controls with gingivitis (n = 26)	p-Value
Gingival crevicular fluid (μL)	0.25 (0.20–0.28)	0.16 (0.11–0.21)	0.001
MMP-2	0.14 (0.07–0.17)	0.05 (0.03–0.08)	0.003
MMP-3	0.02 (0.01–0.04)	0.003 (0.001–0.007)	< 0.001
MMP-8	24 (14–51)	6.4 (2.1–13.8)	< 0.001
MMP-9	31 (21–63)	7.1 (4.1–15.4)	< 0.001
MMP-13	0.09 (0.05–0.15)	0.10 (0.05–0.20)	0.920
TIMP-1	0.23 (0.15–0.61)	0.18 (0.09–0.33)	0.312
TIMP-2	1.8 (1.2–2.4)	0.88 (0.51–1.35)	0.005
TIMP-3	0.05 (0.04–0.07)	0.05 (0.03–0.06)	0.312

Results are given as median (interquartile range). The interquartile range = 25%–75% percentiles.
Significant at the level of 0.0055 after the Bonferroni adjustment.

(Fig. 1). In the HC-G group, however, significant, positive correlations were found between TIMP-3 and MMP-2 (r = 0.619; p = 0.001), MMP-8 (r = 0.497; p = 0.01) and MMP-9 (r = 0.435; p = 0.026), respectively, which were not demonstrated in the DS-G group (Fig. 2). When analysing the relationship between TNF-α and TIMPs, there was a significant, positive correlation between TNF-α and TIMP-3 (r = 0.501; p = 0.009) in the HC-G group, which was not seen in the DS-G group (Fig. 3).

We also studied whether the relationship between MMPs and TIMPs differed between subjects with Down's syndrome and controls. The slope of the regression line between MMP-8 and TIMP-2 differed significantly between the DS-G and HC-G groups (p = 0.006). However, there were no statistical differences between the DS-P and HC-P groups regarding the relationships between MMPs and TIMPs.

Discussion

Novel findings in the present study is that enhanced levels of MMPs can be detected in gingival crevicular fluid and an altered relationship between MMP-8 and TIMP-2 exists in subjects with Down's syndrome exhibiting gingivitis compared with matched controls. Regarding periodontitis, there were no significant differences between subjects with Down's syndrome and controls concerning the relationship between MMPs and TIMPs in gingival crevicular fluid.

Children with Down's syndrome are more susceptible to periodontitis compared with healthy subjects as well as with other groups of mentally disabled patients (1,5,32). However, the mechanism(s) responsible for this high susceptibility is still unclear, although we recently reported a difference in the balance between pro-inflammatory and anti-inflammatory cytokines in gingival crevicular fluid, indicating an altered host response in Down syndrome (33,34). To our knowledge, this is the first study evaluating both MMPs and TIMPs in subjects with Down's syndrome with various degrees of severity of periodontal disease.

In the present study, we demonstrated significantly higher levels of MMP-2, MMP-3, MMP-8, MMP-9 and TIMP-2 in the gingival crevicular fluid of subjects with Down's syndrome exhibiting gingivitis compared with a healthy matched control group. Enhanced levels of MMP-2, MMP-8 and MMP-9 in the gingival crevicular fluid of subjects with Down's syndrome has previously been reported (26,27). In the DS-P group,

Table 3. The median value of MMPs and TIMPs, expressed as pg/μL adjusted for volume* of gingival crevicular fluid, in subjects with Down's syndrome and periodontitis and in controls with periodontitis

Variables	Down's syndrome with periodontitis (n = 12)	Controls with periodontitis (n = 8)	p-Value
Gingival crevicular fluid (μL)	0.30 (0.21–0.37)	0.41 (0.22–0.54)	0.650
MMP-2	0.37 (0.14–0.54)	0.09 (0.02–0.32)	0.170
MMP-3	0.06 (0.02–0.09)	0.07 (0.01–0.12)	1.0
MMP-8	30 (17–94)	79 (59–445)	0.170
MMP-9	36 (15–78)	105 (56–248)	0.170
MMP-13	0.3 (0.1–1.0)	0.3 (0.2–0.8)	1.0
TIMP-1	0.4 (0.2–0.7)	1.1 (0.7–1.5)	0.020
TIMP-2	2.0 (1.6–5.1)	8.2 (6.1–12.6)	0.001
TIMP-3	0.06 (0.04–0.09)	0.09 (0.07–0.12)	0.170

Results are given as median (interquartile range). The interquartile range = 25%–75% percentiles.
Significant at the level of 0.0055 after the Bonferroni adjustment.

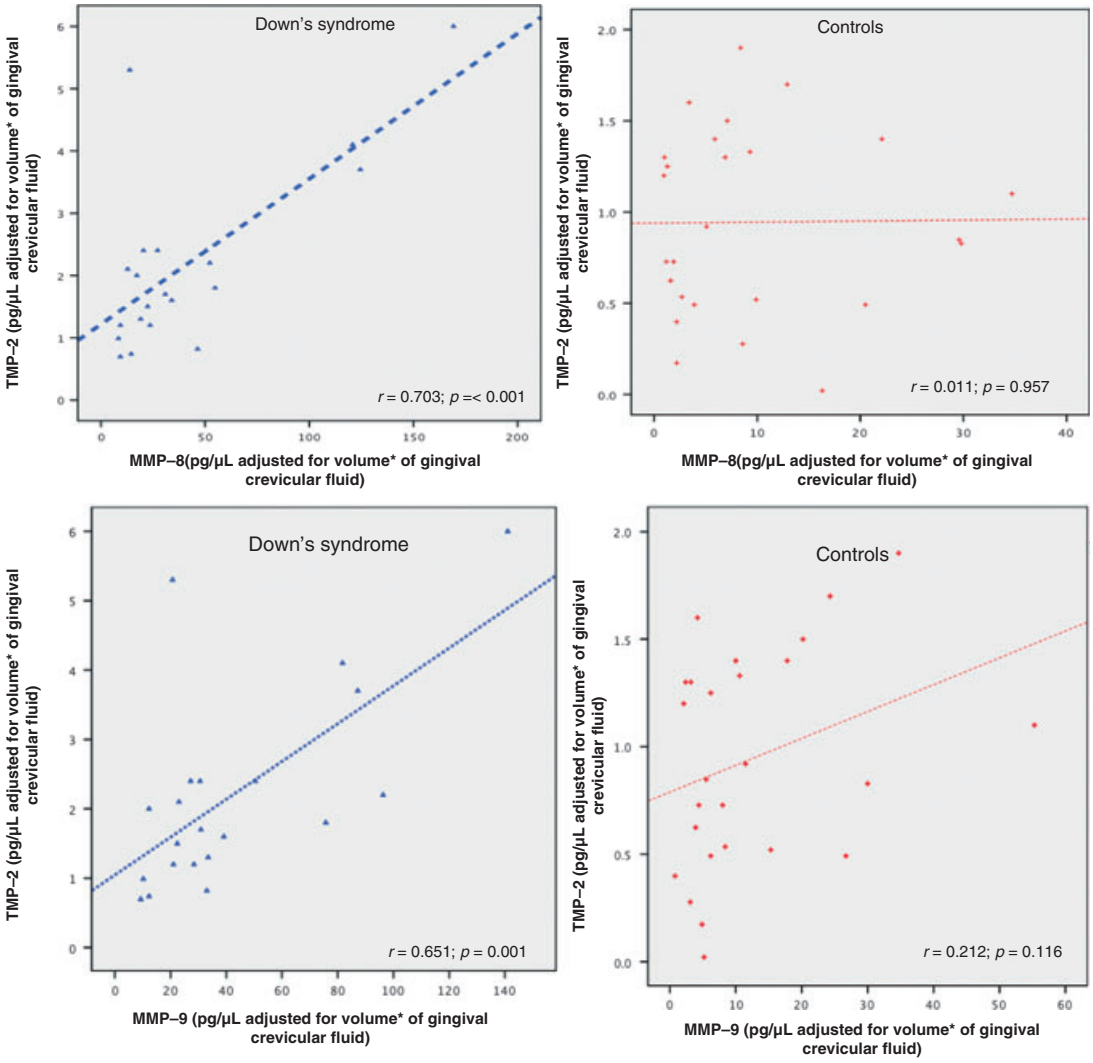

Fig. 1. Correlation between TIMP-2/MMP-8 (expressed as pg/µL adjusted for volume* of gingival crevicular fluid) and TIMP-2/MMP-9 (expressed as pg/µL adjusted for volume* of gingival crevicular fluid) in subjects with Down's syndrome and gingivitis and in control patients with gingivitis.

there were no differences, compared with the HC-P group, regarding MMPs in gingival crevicular fluid. However, higher concentrations of MMP-3, MMP-8, MMP-9, MMP-13, TIMP-1, TIMP-2 and TIMP-3 were demonstrated in the HC-P group compared with the HC-G group, which is in agreement with previous findings (35), indicating an up-regulation of the inflammatory response among controls from gingivitis to periodontitis, which was not evident among subjects with Down's syndrome. This difference between subjects with Down's syndrome and controls is interesting and might indicate that the inflammatory response in subjects with Down's syndrome is already up-regulated during gingivitis.

We also studied whether the relationship between MMPs and TIMPs in gingival crevicular fluid was different between controls and subjects with Down's syndrome. In the DS-G group, there was a positive relationship between the concentrations of MMP-8 and TIMP-2, whereas in the HC-G group, such a positive correlation was not demonstrated. The slope of the regression line concerning the relationship between MMP-8 and TIMP-2 differed significantly between subjects with Down's syndrome exhibiting gingivitis and controls with gingivitis, indicating an altered balance between MMP-8 and TIMP-2 in subjects with Down's syndrome. It should be noted that subjects with Down's syndrome exhibited higher levels of both MMP-8 and TIMP-2 in gingival crevicular fluid compared with the controls. However, the altered balance between MMP-8 and TIMP-2 in gingival crevicular fluid was mainly caused by a comparatively greater increase in MMP-8 (four-fold) compared with TIMP-2 (two-fold), which may result in enhanced tissue breakdown during the early stage of gingival inflammation (36). However, taking previous findings concerning the function of MMP-8 into consider-

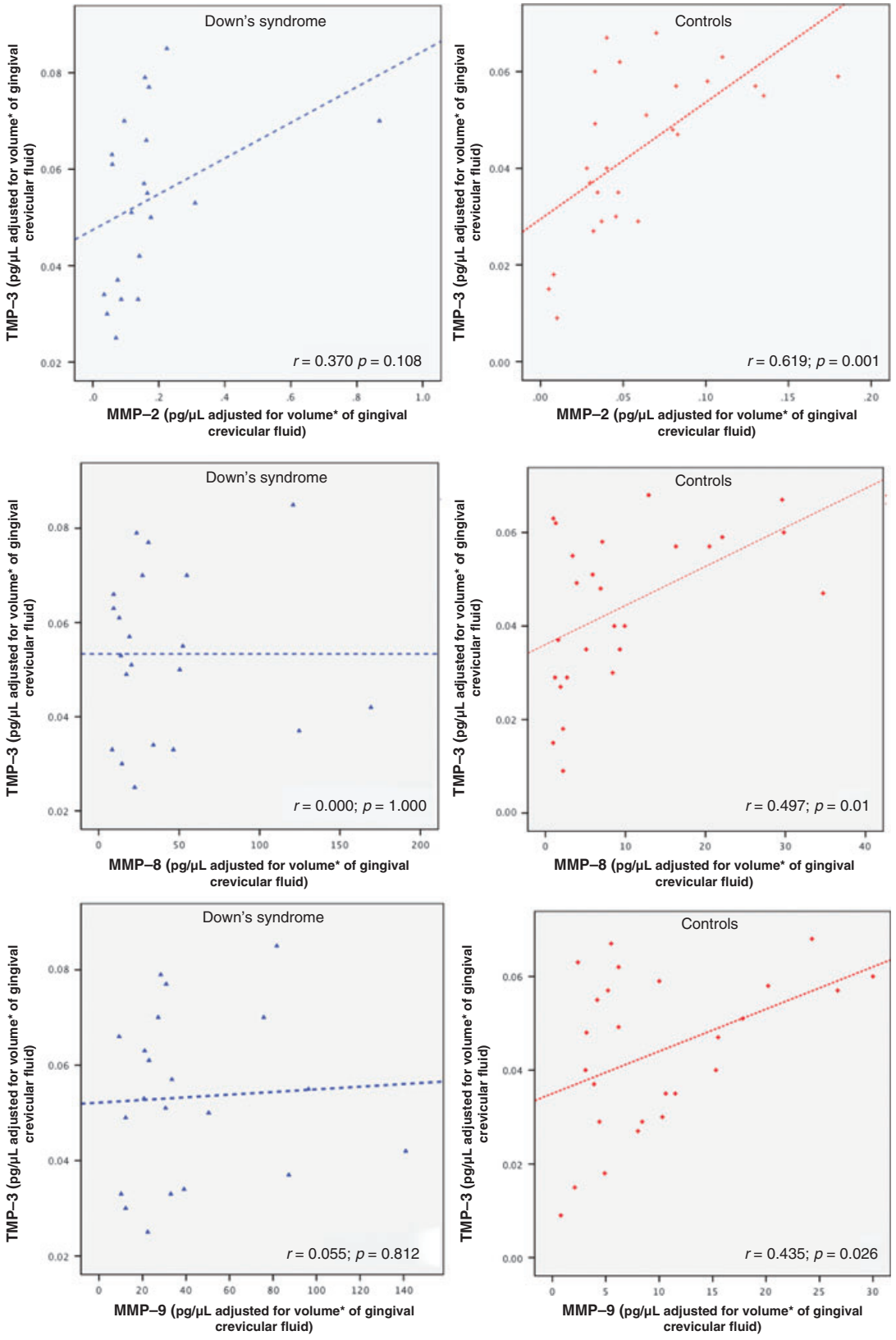

Fig. 2. Correlation among TIMP-3/MMP-2 (expressed as pg/µL adjusted for volume* of gingival crevicular fluid), TIMP-3/MMP-8 (expressed as pg/µL adjusted for volume* of gingival crevicular fluid) and TIMP-3/MMP-9 (expressed as pg/µL adjusted for volume* of gingival crevicular fluid) in subjects with Down's syndrome and gingivitis and in control patients with gingivitis.

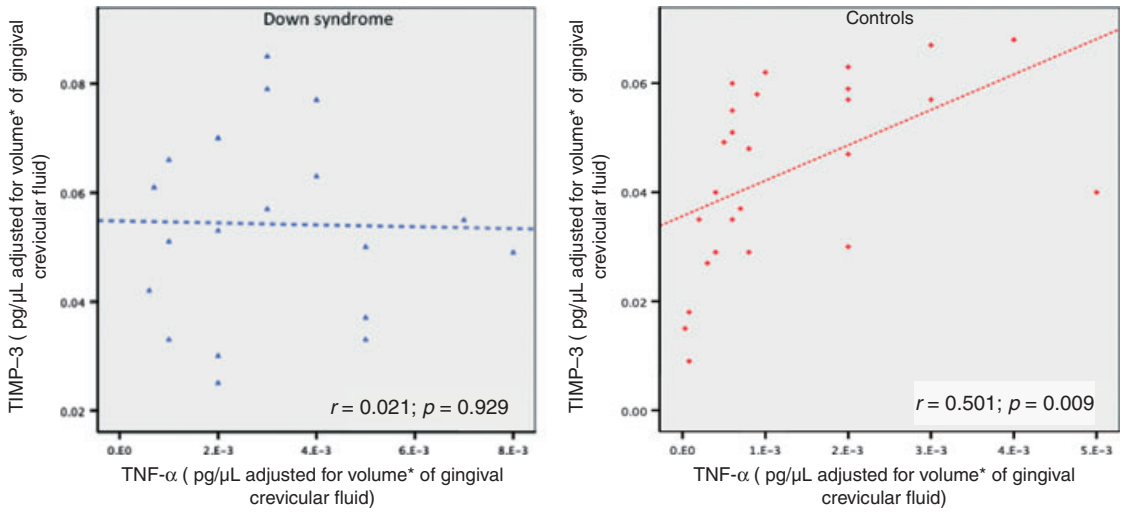

Fig. 3. Correlation between TIMP-3/tumor necrosis factor alpha (TNF-α) (expressed as pg/μL adjusted for volume* of gingival crevicular fluid) in subjects with Down's syndrome and gingivitis and in control patients with gingivitis.

ation, the altered balance between MMP-8/TIMP-2 seen in subjects with Down's syndrome might also result in an impaired resolution of the chronic inflammation (17–20).

There are data demonstrating an increased expression of TIMPs in diseased periodontal tissues of adults, which presumably reflect an attempt to maintain tissue homeostasis (37–39). In light of these findings, our results indicate that the increase in TIMP-2 in subjects with Down's syndrome and gingivitis may not be enough to compensate for the enhanced production of MMP-8. Together with our previous findings, demonstrating an altered balance between pro-inflammatory and anti-inflammatory cytokines in Down's syndrome (33), the enhanced chronic inflammation in Down's syndrome (27,40) will probably result in greater matrix degradation and thereby partly explain the enhanced risk for the development of periodontitis.

It is not possible to determine which form of MMP is present in gingival crevicular fluid because all forms of MMP-8 (pro, active and complexes) are measured by the ELISA method used in this study. However, it has been shown that the active form of MMP-8 is mainly found in sites with periodontitis, whereas a latent form of MMP-8 is associated with gingivitis (41,42). One therefore has to consider that it is not possible to identify subjects with Down's syndrome at risk for developing periodontitis by using MMP-8 as a marker and analysis with commercially ELISA systems (41,43–45).

The lack of positive correlations between TIMP-3 and the concentrations of MMP-8 and MMP-9 in the Down's syndrome group with gingivitis also indicate an altered host response in Down's syndrome. The lack of correlation between TIMP-3 and TNF-α is interesting in light of the fact that TIMP-3 has been reported to be a regulator of inflammation (25). The authors demonstrated that knockout mice lacking the gene for TIMP-3 developed more inflammation. TIMP-3 inhibits TNF-α-converting enzyme, a protease that generates soluble TNF from the cell-surface-bound form of the cytokine (46,47). The cytokine TNF-α is a key inflammatory mediator in periodontal disease (48,49) through its role in the activation of MMPs (10, 11). Interestingly, we previously reported enhanced levels of TNF-α in the gingival crevicular fluid of subjects with Down's syndrome (33).

There are a couple of limitations of the study. The small numbers of subjects included, as well as the cross-sectional design, do not allow any causal relationships to be determined. Furthermore, there is also a need to control for confounding factors, such as chronic disease, sociodemographic factors and oral-hygiene habits, regarding susceptibility to periodontitis. However, because of the small number of subjects, it is not possible to perform a multivariate analysis. In order to elaborate the reasons behind the increased susceptibility for periodontitis often seen in subjects with Down's syndrome, there is need for a larger cohort and more functional cellular studies.

The prostanoid prostaglandin E2, which we previously reported to be enhanced in gingival crevicular fluid in subjects with Down's syndrome (27,40), has also been reported to be involved in the regulation of MMPs (50,51). In a recent study by Lee *et al.* (52), it was indicated that inhibition of prostaglandin E2 suppresses the expression and/or activation of MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 and increases the expression of TIMP-1, TIMP-2, TIMP-3 and TIMP-4. Whether the altered proportion of MMP-8 and TIMP-2 levels in gingival crevicular fluid in subjects with Down's syndrome is directly or indirectly related to the arachidonic metabolite prostaglandin E2 is an important point for investigation in future studies.

In conclusion, subjects with Down's syndrome and gingivitis exhibit higher concentrations of MMPs in gingival crevicular fluid and an altered relationship between MMP-8 and TIMP-2,

which might impair the periodontal tissue turnover.

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