



Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Salivary lactoferrin levels in Down Syndrome: a case-control study

Desireé Antequera^{a,b,1}, Lucía Sande^{c,1}, Eliane García Mato^c, Deborah Romualdi^{a,b,d}, Laura Carrero^{a,b}, Cristina Muncio^{a,b}, Pedro Diz^c, Eva Carro^{a,b,*} ^a Neurobiology of Alzheimer's Disease Unit, Functional Unit for Research Into Chronic Diseases, Instituto de Salud Carlos III, Madrid, Spain^b Network Centre for Biomedical Research in Neurodegenerative Diseases (CIBERNED), ISCIII, Spain^c Grupo de Investigación en Odontología Médico-Quirúrgica (OMEQUI), Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain^d Programa de Doctorado en Ciencias Biomédicas y Salud Pública, IMIENS, Universidad Nacional de Educación a Distancia (UNED), Spain

ARTICLE INFO

Keywords:

Down Syndrome
Saliva
Lactoferrin
Biomarkers
Alzheimer's disease
Cognition
Infections

ABSTRACT

Individuals with Down Syndrome (DS) have a high age-dependent risk of developing Alzheimer's disease (AD). In addition to genetic causes, this high risk involves dysregulated immune-inflammatory system. Low lactoferrin levels, one of the main antimicrobial proteins present in saliva, has been associated with AD. Here, we evaluated whether salivary lactoferrin levels change across the life span of individuals with DS. The study included 152 participants, 72 subjects with DS and 80 euploid individuals, and were divided into those under and over 45 years of age, accordingly with the age-dependent risk of AD. Median of salivary lactoferrin were higher among DS individual, in parallel to salivary total protein, but there were no differences in the ratio of lactoferrin to total protein in saliva between groups. Only DS individuals had higher median salivary lactoferrin levels in the age group under 45 years. Meanwhile non-significant differences were detected for the ratio salivary lactoferrin levels to total salivary protein between groups under 45 years, those levels were lower in DS subjects over 45 years old compared with the age-matched control group. Furthermore, the ratio of salivary lactoferrin levels to total protein in DS was associated with cognitive decline being lower in demented groups compared with mild and moderate cognitive impairment groups. In summary, this study indicates that salivary lactoferrin was dysregulated in DS, with significant lower ratio of salivary lactoferrin levels to total salivary proteins in individuals with DS over 45 years old, a population with a gradually increasing risk of AD.

1. Introduction

Down syndrome (DS), caused by complete or partial triplication of chromosome 21, is the most common chromosomal condition occurring in humans (Antonarakis et al., 2020). Most adults with DS will develop amyloid and tau pathology consistent with Alzheimer disease (AD) by the age of 40–50 (Ballard et al., 2016; Davidson et al., 2018; Fortea et al., 2020; McCarron et al., 2017; Veteleanu et al., 2023; Wiseman et al., 2015).

This increased risk of AD is presumably conferred through genetic predispositions arising from trisomy 21 and amyloid precursor protein (APP) overexpression, but also other genes on chromosome 21 interacting with genes on other chromosomes leading to metabolic dysfunction and dysregulated pathways, including the immune-inflammatory system (Martini et al., 2022). It is now accepted that

chronic peripheral inflammation and infections may contribute to AD pathogenesis in DS (Kamer et al., 2016). In combination, these alterations can produce a precarious biological environment that favors the development of AD in people with DS (Flores-Aguilar et al., 2020).

Abnormal responses of the immune system in DS have been linked to increased susceptibility to infections (Ram and Chinen, 2011). For example, it is well established that people with DS have increased prevalence and severity of periodontal diseases (Contaldo et al., 2021). Periodontal diseases can initiate or contribute to the AD pathogenesis through multiple pathways (Schwahn et al., 2022; Yang et al., 2023). Oral bacteria can get into the blood stream, invading the brain, crossing a weakened blood-brain barrier, and contribute to AD pathophysiology (Dominy et al., 2019; Lei et al., 2023). Thus, control of these pathogens by antibacterial approaches could be an alternative to reduce AD development (Plascencia-Villa and Perry, 2020). Alterations in

* Corresponding author. Neurobiology of Alzheimer's Disease Unit, Chronic Disease Programme, Instituto de Salud Carlos III, Majadahonda, 28222, Madrid, Spain.
E-mail address: eva.carro@isciii.es (E. Carro).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.bbih.2025.100999>

Received 4 December 2024; Received in revised form 14 March 2025; Accepted 21 April 2025

Available online 21 April 2025

2666-3546/© 2025 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

immunological parameters have been described in saliva of DS subjects (Chaushu et al., 2002b). It has been reported a severe reduction in the bacterial specific salivary antibodies in DS individuals, resulting a worrying immunodeficiency (Chaushu et al., 2007). Moreover, salivary Ig levels may serve as a predictor of the susceptibility of DS individuals to other infections (Chaushu et al., 2002a, 2003). Additionally, levels of the antimicrobial histatin 5 were significantly decreased in saliva of elderly subjects with DS (Komatsu et al., 2021). Because histatin 5 exhibits potent antifungal activity, this reduction may explain why DS individuals are known to be susceptible to fungal infections, such as oral candidiasis (Maranhão et al., 2020). All these findings point to the high risk of infections in people with DS, attributed, at least in part, to defects of the immune system.

Lactoferrin is one of the major antimicrobial proteins present in saliva with several functions, such as antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory and immunomodulatory (Berlutti et al., 2011; Kruzel et al., 2017; Valenti and Antonini, 2005). Lactoferrin plays an important role in the host defense against oral pathogens, and it's involved in the control of oral microbiome (Legrand, 2012; Legrand et al., 2005; Lyngge Pedersen and Belstrøm, 2019). In this context, it was reported that lactoferrin displayed proteinase inhibitory activity against *P. gingivalis*, significantly inhibiting gingipains (Dashper et al., 2012). However, other studies have also supported that oral pathogens could degrade lactoferrin (Alugupalli and Kalfas, 1996; de Lillo et al., 1996). In addition, Olsen and Singhrao proposed that salivary lactoferrin deficiency may act as an unknown trigger of oral microbial dysbiosis, supporting the concept that low levels of lactoferrin might indicate oral dysbiosis (Olsen and Singhrao, 2021). Reduced salivary lactoferrin levels, along with infection and cholinergic hypothesis, provide a new model to explain AD pathogenesis (Nara et al., 2021). Remarkably, salivary lactoferrin levels are significantly reduced in prodromal AD and AD dementia, as they were associated with the amyloid-PET imaging profile (Antequera et al., 2023; Carro et al., 2017; González-Sánchez et al., 2020). New studies reported reduced levels of salivary lactoferrin in AD patients (Zalewska et al., 2021), and positive correlation of memory impairment and lower levels of salivary lactoferrin in older-aged non-Hispanic white and Black Americans at risk for AD due to parental history (Hammerschlag et al., 2024). However, other study by Glerup et al. reported no differences between AD patients and controls (Glerup et al., 2021). Moreover, in the Glerup study, lactoferrin levels across all groups, including controls, were significantly higher than those reported in other studies (Ramenzoni et al., 2021; Rosa et al., 2017; Wu et al., 2018). Potential explanations for this discrepancy include differences between the studied samples, pre-analytical variables (such as fasting time prior to sample collection), or the assays employed (Glerup et al., 2021). Additionally, in the Glerup study, the saliva samples were collected immediately following the lumbar puncture, which raises the possibility that the participants at that time had reduced salivary flow as a consequence of fear, anxiety, or other somatosensory stimuli such as pain (Proctor, 2016). It has been demonstrated that salivary concentrations of lactoferrin and total proteins significantly increase when salivary secretion decreases, as evidenced in patients who have undergone radiotherapy involving the major salivary glands or those with Sjögren's syndrome (Almståhl et al., 2001). Moreover, the association between lactoferrin and A β also correlated with poorer memory (Reseco et al., 2021). All these data support the hypothesis that salivary glands dysfunction may be an early event associated with A β brain accumulation (Antequera et al., 2021; Bermejo-Pareja et al., 2020). According with this theory, alterations in salivary redox balance associated with chronic inflammation, including reduced lactoferrin concentrations, were also described in AD (Zalewska et al., 2021). Additionally, individuals with DS exhibit high levels of oxidative damage biomarkers in saliva such as superoxide dismutase and malondialdehyde (de Sousa et al., 2015). We therefore hypothesized that lower lactoferrin levels can be observed in individuals with DS, predisposing them to infections and the development of AD. Thus, the

aim of this study was to evaluate the potential link between salivary levels of lactoferrin and DS by comparing DS individuals of all ages and non-syndromic age-matched controls.

2. Materials and methods

2.1. Participants

In this case-control study, we included 72 participants with DS (40 females, 32 males, age range 3–66 years, as case group), and 80 age-matched subjects without DS (56 females, 24 males, age range 3–56 years, as control group) (Table 1). These participants were recruited among all individuals with DS who regularly attended special educational or occupational therapy centers in Santiago de Compostela, Lugo, and Madrid (Spain), between December 2022 and November 2023. All participants satisfied the following inclusion criteria: genetically confirmed diagnosis of DS, sufficient degree of collaboration to perform a saliva sampling and availability of an informed consent signed by the participants or their legal guardians. The exclusion criteria were subjects who are taking acetylcholinesterase inhibitors, presence of harmful habits (e.g., smoking), having done moderate physical activity in the last 3 h.

The DS and control subjects were divided into four groups according to age: two groups consist of DS and control subjects under 45 years of age, and the other two groups consisting of DS and control subjects over 45 years of age. This classification was performed accordingly with the age-dependent risk of AD (Ballard et al., 2016; Davidson et al., 2018; Fortea et al., 2020; McCarron et al., 2017; Veteleanu et al., 2023; Wiseman et al., 2015).

The severity of intellectual disability was scored at a consensus meeting between the physician, the neuropsychologist, and the occupational therapist of the center where the participants attended, based on cognitive status, detailed medical history, intellectual disability profiling, and recent life events, as previously described (Handen et al., 2020). During consensus determinations, changes in personality, behavior, and activities of daily living were also considered, following previous recommendations (Smith, 2001). Intellectual disability was

Table 1
Characteristics of participants.

Variable	control	Down Syndrome	Total	Test	P value
N	80	72	152	Chi-sq χ^2 (1)	0.708
<45 yearsold	61 (76.25%)	53 (73.61%)	114 (75%)	= 0.141	
≥45 yearsold	19 (23.75%)	19 (26.39%)	38 (25%)		
Age, Mean (DS)	30.30 (15.74)	32.68 (15.77)	31.43 (15.75)	Mann-Whitney	0.346
<45 years old	24.42 (12.03)	25.60 (12.12)	25.01 (12.07)	W = 2624.500	
≥45 years old	51.21 (4.23)	52.16 (5.42)	51.68 (4.82)		
Gender (F/M)	56/24	40/32	96/56		
<45 years old	43/18	30/23	73/41		
≥45 years old	14/5	10/9	24/14		
Cognitive decline					
Mild		32 (44.4%)			
Moderate		24 (33.3%)			
Severe		10 (13.9%)			
Dementia		6 (8.3%)			

Abbreviations: F, female; M, male; ns, not significant.

categorized as mild, moderate, severe, or profound according to the Diagnostic and Statistical Manual of Mental Disorders, which considers intellectual functioning (IQ), the individual's adaptive functioning, the level of support required for daily activities, communication skills, social abilities, as well as other factors such as health conditions and environmental influences (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition).

Severe/profound participants were evaluated with the modified cued recall test (mCRT) Spanish version, which has demonstrated excellent accuracy in detecting AD-related cognitive decline in DS people (Videla et al., 2022). Participants have been classified as demented if there is a history of progressive memory loss, disorientation, and functional decline over a period of at least 1 year (Handen et al., 2020). Classification was based on the individuals' best-ever level of functioning. The information was obtained through family interviews and review of medical or educational records for past assessment results.

The study protocol was approved by the Research Ethics Committee of Santiago-Lugo University (Xunta de Galicia; reference 2018/510), following the standards for medical research in humans recommended by the Declaration of Helsinki. All participants or their legally authorized representative gave written informed consent before enrolment.

This report follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

2.2. Saliva collection

Unstimulated saliva samples were collected and processed from all subjects as described previously (Carro et al., 2017). All individuals were asked to avoid eating, drinking, or performing oral hygiene measures for at least 3 h before the sample collection. Saliva samples (~0.5 ml) were kept on ice throughout the collection and stored frozen until processing. Then, saliva samples were centrifuged at 1000 rpm for 10 min at 4 °C and the supernatants were aliquoted in polypropylene tubes with Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany) and kept on -80 °C until further analytical processing.

2.3. Biochemical analysis

Total protein concentration of saliva samples was analyzed using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

Levels of lactoferrin in saliva samples were determined by enzyme-linked immunosorbent assays (ELISA) using the Human Lactoferrin ELISA kit (FineTest, Wuhan, China) according to manufacturer's instructions. Saliva samples were first diluted 1:1000 and then run in duplicate. The intra-assay coefficients of variation (CVs) ranged from 4.7 % to 6.0 %, the inter-assay CVs ranged from 4.6 % to 5.3 %, and the lower limit of detection was 0.3 ng/mL according to the manufacturer.

2.4. Statistical analysis

All statistical analyses were performed using R software version 3.4.1 (R Development Core Team, 2017; Vienna, Austria). Baseline characteristics were summarized using standard descriptive statistics. Continuous variables were presented as median [IQR] when normality assumptions were not met, and as mean [SD] for normally distributed data. The chi-squared test was used to assess differences between categorical variables, while the Mann-Whitney *U* test was applied for comparisons of non-normally distributed continuous variables. One-way analysis of variance (ANOVA) was used for multigroup comparisons of normally distributed data, and the Kruskal-Wallis test was used for non-normally distributed data, followed by the Bonferroni test for pairwise comparisons. Differences in the median scores of salivary markers among age groups were analyzed using the Scheirer-Ray-Hare test. To examine changes in salivary markers across age, we fitted linear regression models and applied locally estimated scatterplot smoothing

(LOESS) regression for each study group. The correlation between concentrations of salivary total protein and lactoferrin was assessed using the Pearson correlation test. A *p*-value <0.05 was considered statistically significant for all tests.

3. Results

3.1. Study population

The Table 1 displays baseline demographic and cognitive score data for the study. The results confirm that DS cases and controls have similar sex and age distribution. Of the 72 DS subjects, 32 individuals (44.4 %) presented mild cognitive decline, 24 (33.3 %) moderate, 10 (13.9 %) severe, and 6 (8.3 %) suffered from dementia.

3.2. Concentrations of lactoferrin and total protein in the saliva of DS and control subjects

We first analyzed salivary levels of lactoferrin measured in the whole study groups. The median lactoferrin levels were higher in DS cases (7.43 µg/ml [95 % CI; IQR, 4.72–10.86 µg/ml]) compared to those observed in controls (4.44 µg/ml [95 % CI; IQR, 2.98–7.10 µg/ml]) (*p* = 0.001; Fig. 1A and Table S1). Statistical analysis also showed that DS group had significantly higher median salivary protein concentration (265.68 µg/ml [95 % CI; IQR, 169.61–410.05 µg/ml]) than the control group (183.43 µg/ml [95 % CI; IQR, 90.61–356.39 µg/ml]) (*p* = 0.0021; Fig. 1B and Table S1). Furthermore, there was a positive correlation between salivary lactoferrin levels and total salivary protein concentration only in the DS group (Pearson correlation *r* = 0.34; *p* = 0.003; Fig. 1C). It is relevant to note that we didn't find significant differences in the mean total salivary protein concentration between mild cognitive impairment, AD patients and control subjects (Carro et al., 2017).

Thus, when the ratio of salivary lactoferrin content to total protein was compared between DS and control groups, no significant differences were observed in the whole cohort (Fig. 1D and Table 1).

We also analyzed the association between each of the main variables (salivary lactoferrin levels, total salivary protein levels, and ratio of salivary lactoferrin levels to total salivary protein) with respect to the study groups and sex, as well as the interaction between both. The results showed no significant differences between males and females (Table S2), despite of the differences in prevalence of AD in both sex.

3.3. Age differences in lactoferrin and total protein levels in the saliva of DS and control subjects

We next analyzed the association between each of the main variables with respect to the study groups and age with a threshold of 45 years. Statistical analysis showed that, in subjects under 45 years of age, the mean salivary concentration of lactoferrin was significantly higher in DS subjects than in the age-matched control group (*p* < 0.001; Fig. 2A and Table S3). Additionally, salivary lactoferrin levels also rose with age with significant differences in the control group between under and over 45 years of age (*p* < 0.001; Fig. 2A and Table S3). Lineal regression analysis also revealed these differences between study groups under 45 years of age, meanwhile the LOESS regression shows a downward trend in lactoferrin values from the age of 45 years in subjects with DS compared to the continued rise in controls (Fig. 2B, and Table S4).

The total salivary protein concentration was significantly higher in DS subjects under 45 years of age compared to the age-matched control group, meanwhile there were no significant differences between study groups over 45 years of age (*p* < 0.001; Fig. 2C and Table S5). The regression curves of the linear model also revealed these differences between study groups under 45 years of age. In the LOESS curve, a similar upward trend was observed in total salivary protein values in both groups until 45 years of age, and from then on, the trends became similar (Fig. 2D, and Table S6).

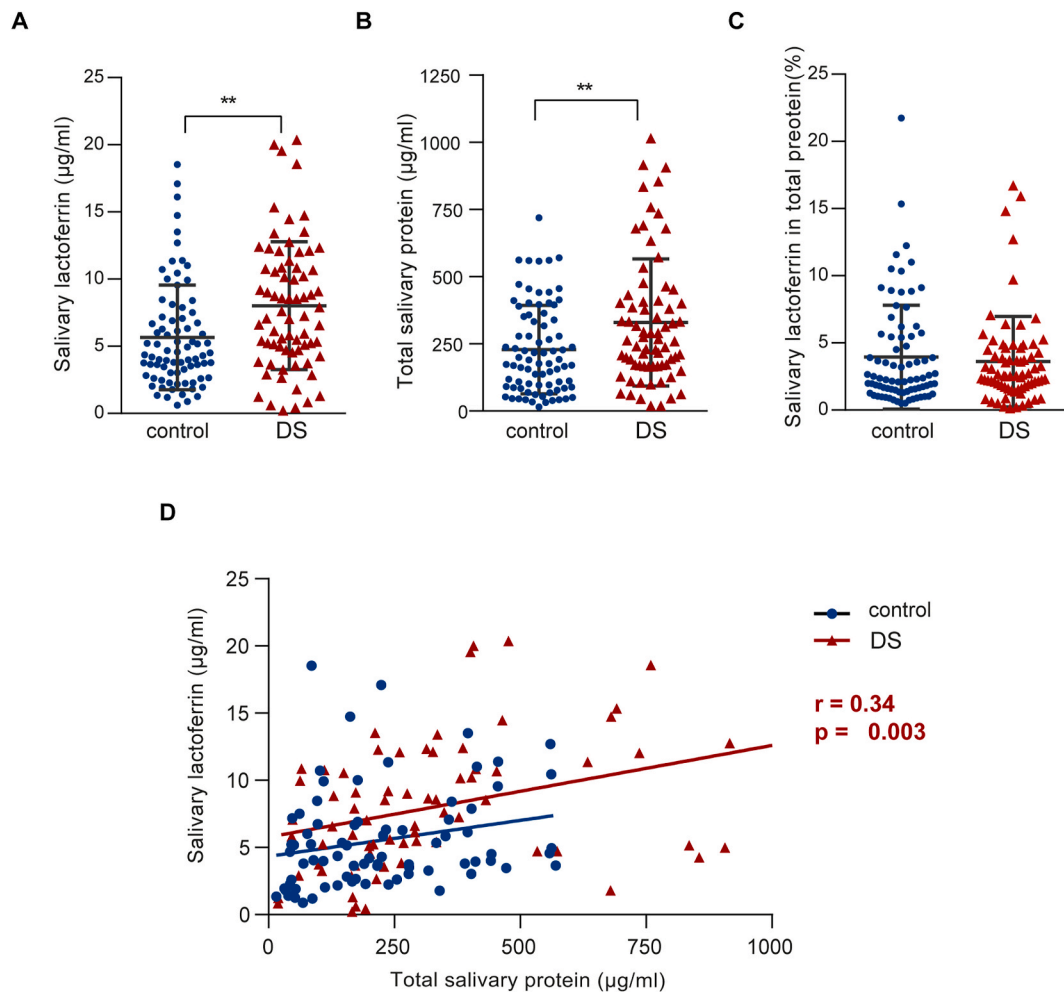


Fig. 1. Concentrations of lactoferrin and total protein in the saliva of Down Syndrome (DS) individuals compared with control subjects. Comparison of salivary lactoferrin (A), and total protein (B) concentrations, and salivary lactoferrin levels in total salivary proteins (D) between the DS and normal groups. (C) Correlation between salivary lactoferrin and total salivary proteins in DS and control groups. (n = 72 DS, 80 control). ** $p < 0.01$, *** $p < 0.001$.

As salivary levels of lactoferrin were correlated with the total salivary protein concentration, lactoferrin levels were normalized to the total protein concentration in saliva. Statistical analysis revealed that in subjects over 45 years of age, the ratio of salivary lactoferrin levels to total protein in DS subjects was lower than in controls ($p = 0.013$; Fig. 2E and Table S7). The regression curves of the linear model LOESS curves only tended to diverge between DS and control groups over 45 years of age (Fig. 2F).

3.4. Association of cognitive decline with age and lactoferrin and total protein levels in the saliva of DS subjects

We also analyzed the prevalence of cognitive decline with age in DS subjects based on the severity of intellectual disability of each participant. The changes in the cognitive decline performance with age in DS subjects is shown in Fig. 3A. Statistical analysis suggests that there was a significant difference in ages between the four cognitive deficit groups ($\chi^2 = 18.700$; $p < 0.001$; Fig. 3A). As expected, the cognitive decline scores increased with age, and DS individuals with severe cognitive decline and dementia were significantly older than those with moderate cognitive decline (Fig. 3A and Table S8).

We next compared salivary lactoferrin and total protein concentrations across cognitive decline scores. The means of salivary lactoferrin concentration or salivary protein concentration did not differ by cognitive decline (Fig. 3B and C). However, the ratio of salivary

lactoferrin levels to total protein in DS subjects varied depending on the degree of cognitive decline being lower in demented group comparing with moderate cognitive impairment group ($\chi^2 = 9.958$; $p = 0.019$; Fig. 3D and Table S9).

An analysis was carried out using a linear model to determine the joint influence of age and cognitive deficit. Linear regression model fitting revealed that the joint influence of age and cognitive deficit in the demented group significantly affected the ratio of salivary lactoferrin levels to total protein in DS subjects ($p = 0.008$; Fig. 3E). This ratio decreases exponentially with age in the DS demented group, approximately 2.5 % for each additional year of age.

4. Discussion

Reduced salivary levels of lactoferrin have been reported and suggested as potential biomarker of AD (Antequera et al., 2023; Carro et al., 2017; González-Sánchez et al., 2020). The essential roles played by lactoferrin point to their levels as a key target of vulnerability to neurodegeneration (Abdelhamid et al., 2020; Eker et al., 2023; Liu et al., 2020; Xu et al., 2019; Yong et al., 2023). In the present study, we have analyzed the salivary lactoferrin levels of DS and control individuals at different ages. To our knowledge, this is the first population-based cohort study exploring age-related changes of salivary lactoferrin levels in people with DS.

Our results showed that there are differences in the salivary protein

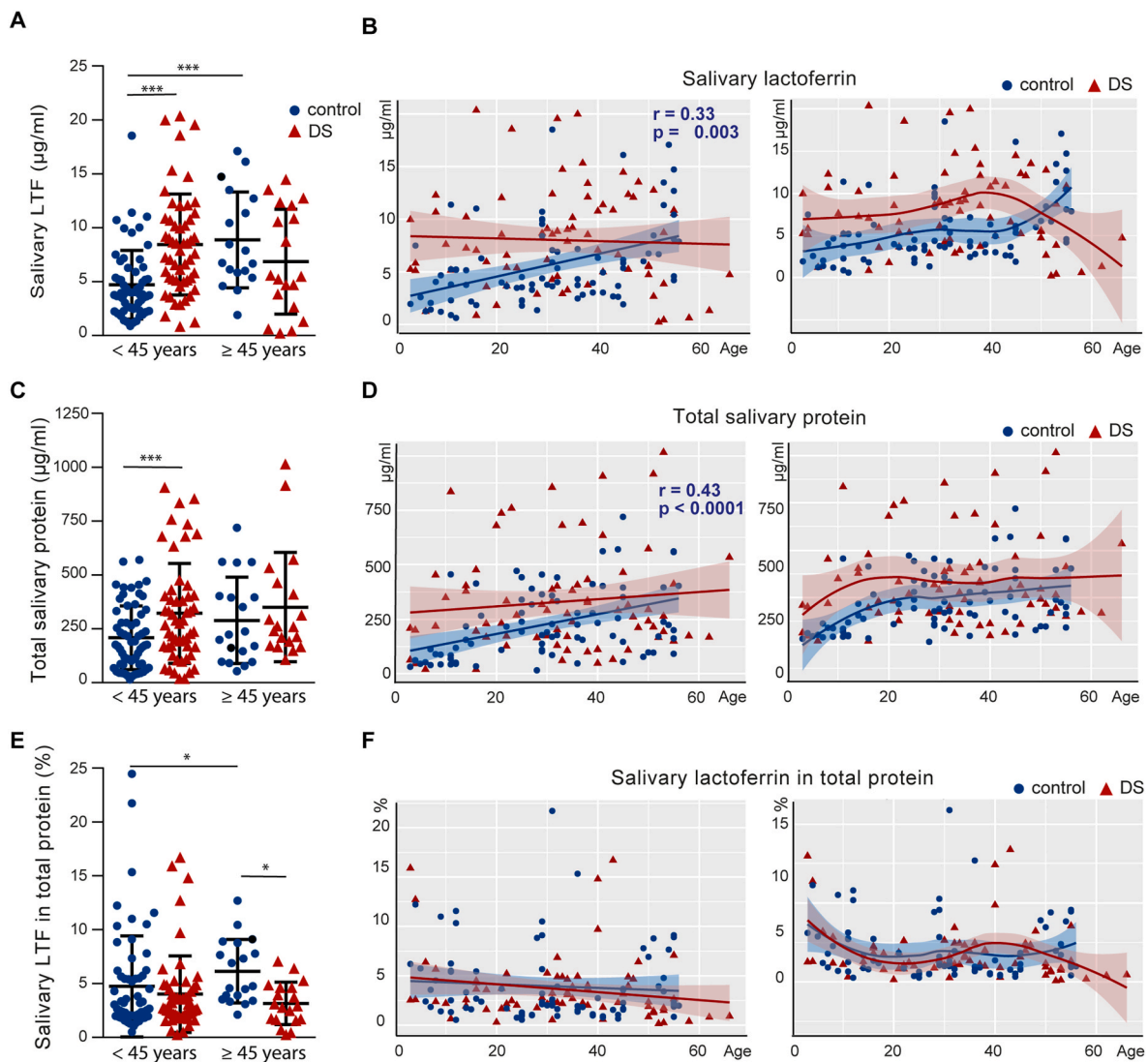


Fig. 2. Changes with age in salivary concentration levels by study groups. Scatter plots represent the median concentrations of salivary lactoferrin (A), total salivary protein (C), and the ratio of salivary lactoferrin levels in total salivary proteins (E) in DS and control groups subdivided according to age (under and over 45 years of age). Longitudinal changes with age in salivary lactoferrin (B), total salivary protein (D), and the ratio of salivary lactoferrin levels in total salivary proteins (F) using regression curves of the linear (left panels) and LOESS (right panels) models for each group and 95 % CI, representing the evolution of age-related changes. LTF: lactoferrin. (n = 72 DS, 80 control). * $p < 0.05$, *** $p < 0.001$.

concentration between people with and without DS. In the whole cohort, we found that salivary lactoferrin levels were significantly higher (~30 %) among people with DS than those observed in the control group. Moreover, total protein concentration was more than 68 % higher in the saliva of DS group compared to the control group. This is in line with previous data showing that total protein concentration in DS saliva was significantly higher compared to that observed in the saliva of control subjects (Komatsu et al., 2021; Siqueira and Nicolau, 2002; Yarat et al., 1999). In the present study, we found a correlation between salivary lactoferrin and total protein concentrations in the entire cohort but also in each of the groups studied. Therefore, when lactoferrin levels were adjusted to the total protein concentration in saliva, no statistically significant differences were seen between the groups.

We found a clear age dependency pattern of salivary lactoferrin levels, different between DS and control subjects. In saliva samples from children and young adults under 45 years, the lactoferrin concentrations were significantly higher in the DS group than in the control group, meanwhile it was similar in both study groups over 45 years. If we analyze the effect of age in each of the study groups, lactoferrin levels increased with age among healthy individuals while remaining stable in

the DS group. Under 45 years of age, total salivary protein concentrations were also higher in DS individuals compared to age-matched healthy subjects. Taken together, our findings indicate that DS individuals over 45 years have lower salivary lactoferrin levels, related to the amount of total protein, compared to age-matched controls. These results are consistent with findings reporting reduced salivary levels of histatin 5 in elderly individuals with DS (Komatsu et al., 2021). We propose that deficits in the salivary levels of these antimicrobial proteins would indicate that immune response is attenuated in DS individual aged over 45 years, a population group at higher risk to AD developing (Ballard et al., 2016; Davidson et al., 2018; Fortea et al., 2020; McCarron et al., 2017; Veteleanu et al., 2023; Wiseman et al., 2015).

Healthy infants have an adaptive immune system that matures with age, to establish defense mechanisms against foreign structures such as viral or bacterial pathogens (Pieren et al., 2022; Simon et al., 2015). Notably, in the general population, salivary lactoferrin levels are lower in children than in young adults (Tenovuo et al., 1986), reaching the highest levels in the middle adult stage to end up descending in advanced age adults and elderly (Bartolome et al., 2021; Shugars et al., 2001). This is in line with our data, where control individuals under 20

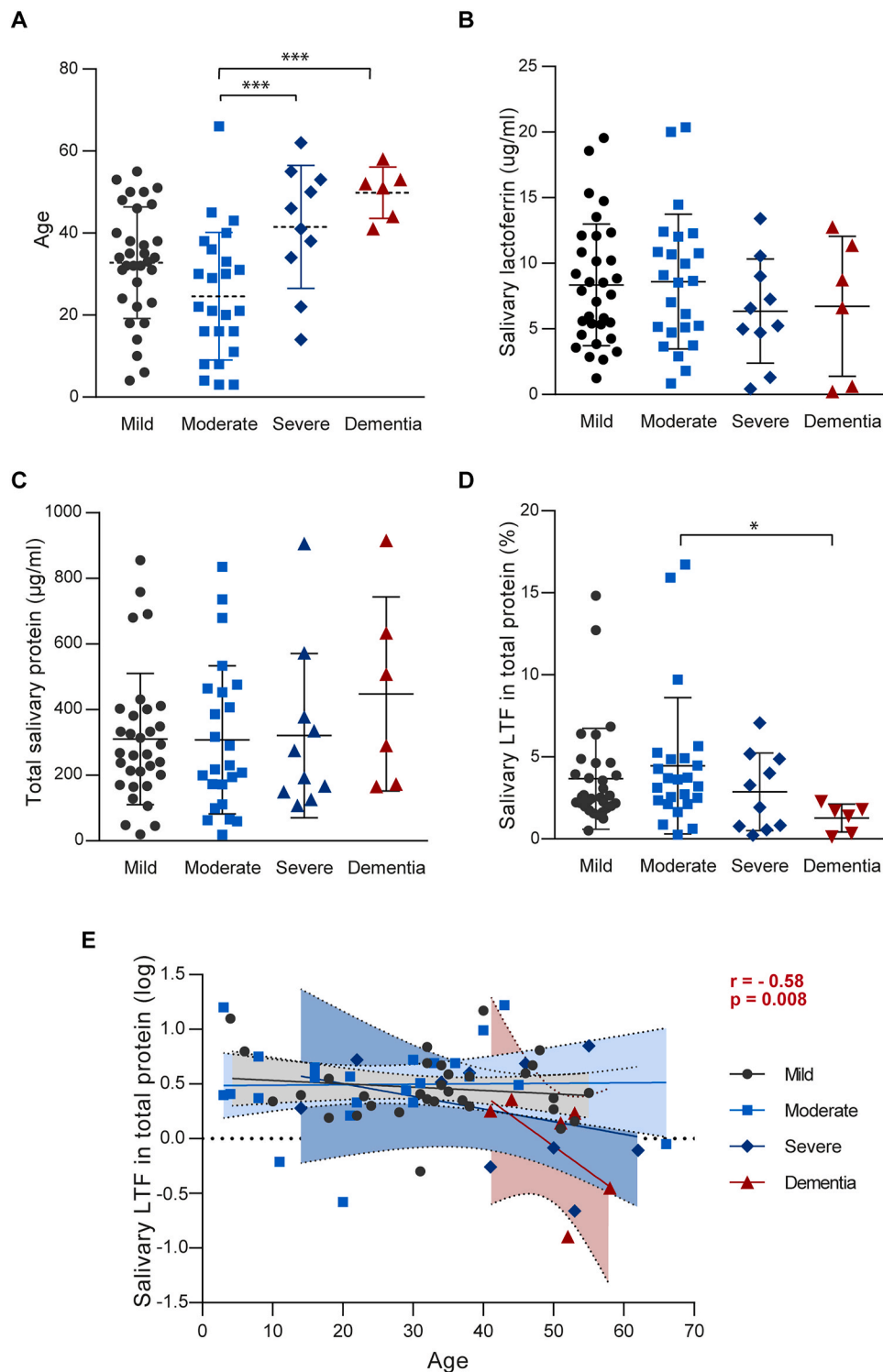


Fig. 3. Association of salivary concentrations with cognitive decline in DS subjects. (A) Scatter plots of the median age in the DS group by cognitive decline. (B–D) Scatter plots of the median concentrations of salivary lactoferrin (B), total salivary protein (C), and lactoferrin in total protein (D) in the DS groups according with their cognitive decline score. (E) Longitudinal changes in the ratio of salivary lactoferrin in total protein according to cognitive decline in DS subjects. Colored lines and bands denote the lineal regression and its 95 % CI. (n = 72 DS [32 with mild cognitive impairment, 24 with moderate, 10 with severe, 6 with dementia]). * $p < 0.05$, *** $p < 0.001$.

years old had an average of lactoferrin levels in saliva lower than younger individuals (20–45 years old). In our present study, DS subjects under 45 years of age had increased salivary lactoferrin levels than control subjects at the same age, however when they were normalized to the total protein concentration in saliva, there was no differences in the

resulting ratio. Moreover, while the ratio of salivary lactoferrin levels to total protein increased with age in control population, it remained unchanged in DS group, suggesting that some immunological mechanisms could be altered. In fact, in DS subjects over 45 years of age this ratio is lower than in age-matched controls. Our findings suggest that salivary

defenses against microbial infections may not have been fully developed throughout their lifetime. Reduced ratio of salivary lactoferrin levels to total protein in DS subjects at older ages can facilitate proliferation of oral pathogens, acting as a trigger for oral dysbiosis (Kruzel et al., 2017), and the development of chronic infection (Olsen and Singhrao, 2021).

It is well known that individuals with DS exhibit a higher risk of suffering infectious diseases compared to the general population, including periodontal diseases (Abanto et al., 2011; Contaldo et al., 2021). Among the oldest known immune defense molecules, antimicrobial proteins/peptides control oral microorganisms (Gorr and Abdolhosseini, 2011; Johnstone and Herzberg, 2022). Specifically, lactoferrin is a first line defense protein for protection against microbial infections, contributing to the maintenance of oral eubiosis (Kruzel et al., 2017; Lyng Pedersen and Belström, 2019). We and others propose that low salivary lactoferrin content might facilitate for oral dysbiosis to proceed freely, and the expansion of pathogens or their inflammatory products to the brain (Bermejo-Pareja et al., 2020; Municio and Carro, 2023; Olsen and Singhrao, 2021). In humans, dysbiosis of the oral subgingival microbiome has been associated with cerebrospinal fluid (CSF) evidence of the AD-signature pathology, which includes A β and tauopathy (Kamer et al., 2021). It is remarkable that a special vulnerability to infections affects individuals with DS after the age of 50 years (Guffroy et al., 2019). From that age, rates of dementia reach up 55–70 % (Ballard et al., 2016). This is consistent with the effect of dysregulated immune system, affecting both innate and adaptive immunity, in the increased risk of AD associated with DS (Martini et al., 2022). Taken together, this is in line with our previous hypothesis that low salivary lactoferrin concentrations might represent a decline in the oral defensive protection, exacerbating the risk of AD (Bermejo-Pareja et al., 2020). And this theory might be also applied in DS, as the ratio of salivary lactoferrin levels to total protein is particularly lower in DS subjects over 45 years of age.

Recently, disturbances in iron homeostasis linked to increased cytokine expression and hepcidin, a hormone that regulates systemic iron homeostasis, were described in people with DS and AD, suggesting shared mechanisms between increased susceptibility to infections and neurodegeneration (Raha-Chowdhury et al., 2021; Raha et al., 2021). Iron dyshomeostasis and decreased levels of transferrin in DS, lead to upregulation of neurotoxicity mechanisms (Barone et al., 2018). Lactoferrin plays a key role in iron homeostasis, with an iron sequestration

mechanism, resulting in a decrease in free iron availability that can limit the growth and pathogenicity of invasive microbial pathogens, providing an important means of host defense (Bartolomé et al., 2022; Rosa et al., 2017; Vellyagounder et al., 2018). We cannot exclude the role of lactoferrin in the iron dyshomeostasis in DS, as iron overload facilitating the growth of microbial pathogens (Fig. 4).

Dysfunction of salivary glands in DS has been also reported, including changes in saliva composition or even absence of salivary glands (Chaushu et al., 2002b, 2007; Komatsu et al., 2021; Odeh et al., 2013). Since lactoferrin in saliva is mainly secreted by salivary glands, the decrease in lactoferrin levels in DS saliva may be explained by salivary gland dysfunction. Moreover, hypothalamic abnormalities in DS, such as neuronal loss (Wisniewski and Bobinski, 1991), or circadian-related disturbances (Bassell et al., 2015; Fernandez et al., 2017; Leng et al., 2019) were reported. As salivary gland secretion is under hypothalamic control (Proctor and Carpenter, 2007, 2014), hypothalamic alterations in DS could lead to salivary glands deregulation, similarly to that described in AD models (Antequera et al., 2021).

Our study also showed that salivary lactoferrin levels are strongly associated with cognitive decline in DS. These findings may have important implications for differentiating dementia-related cognitive decline from intellectual disability in people with DS. This is in line with recent studies highlighting the need for accessible and non-invasive biomarkers for detecting AD pathophysiological processes in DS individuals and predicting the onset of neurodegenerative cognitive decline (Carmona-Iragui et al., 2021; Grasso et al., 2024; Pentz et al., 2021; Snyder et al., 2020). Taken together, our results suggest that reduced salivary lactoferrin levels to total protein might be a potential biomarker able to predict the following cognitive decline in DS subjects.

This study is not exempt from certain limitations and future research should include improvements. The sample size is limited, to include a larger cohort would allow to study more potential association with clinical and demographic parameters, including more neurological and neuropsychological assessments to perfectly discriminate between impairment due to AD dementia and intellectual disability because of DS. Increase the sub-analysis by age. Lastly, longitudinal studies will allow longitudinal cognitive assessments, including prodromal, AD dementia and accuracy value of salivary lactoferrin in people with DS. Moreover, additional studies using larger cohorts would allow us to explore whether lactoferrin levels in other fluids, such as blood, vary in

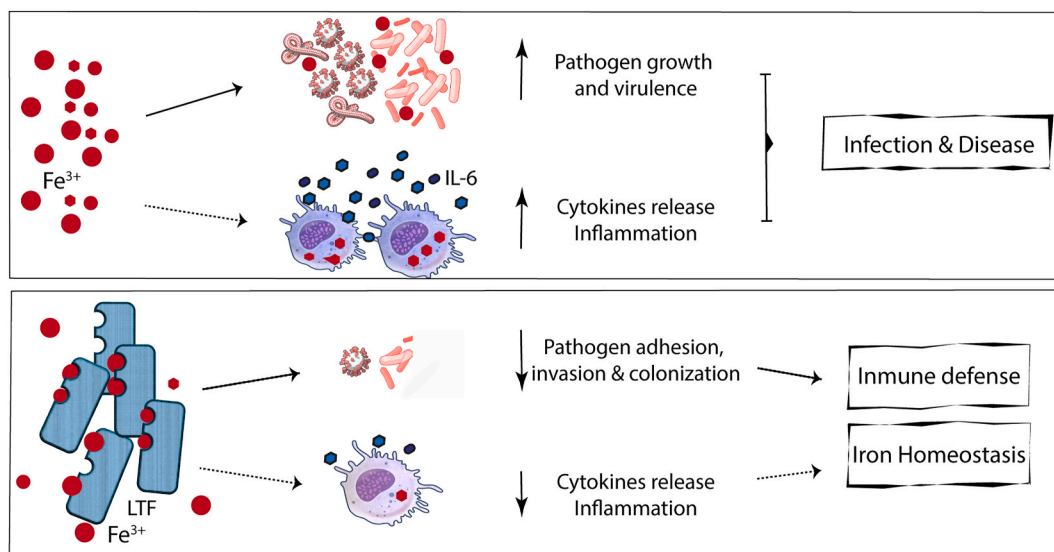


Fig. 4. Diagram on the role of the iron dyshomeostasis. Upper panel: The availability of free iron (Fe^{3+}) favors the growth of pathogens and increases their virulence. It also affects immune system cells, which release more pro-inflammatory cytokines, triggering an inflammatory process. Lower panel: Each molecule of lactoferrin is capable of capturing two Fe^{3+} , this decreases its availability to pathogenic microorganisms, limiting their growth, adhesion, invasion, and colonization. It also reduces the inflammatory process.

patients with DS.

5. Conclusions

In summary, this study found that salivary lactoferrin was dysregulated in DS, with significant lower ratio of salivary lactoferrin content to total protein in individuals with DS over 45 years old, a population with a gradually increasing risk of AD, suggesting that salivary lactoferrin can reflect an immune dysregulation state. These findings support the need for assisting DS individuals to prevent or delay AD-onset in future clinical trials.

CRedit authorship contribution statement

Desireé Antequera: Methodology, Investigation, Formal analysis. **Lucía Sande:** Methodology, Investigation, Formal analysis. **Eliane García Mato:** Methodology, Investigation, Formal analysis. **Deborah Romualdi:** Methodology, Investigation. **Laura Carrero:** Methodology, Investigation. **Cristina Muncio:** Methodology, Investigation. **Pedro Diz:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Eva Carro:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Funding

This work was funded by Grants from Instituto de Salud Carlos III (PI22CIII/00042), CIBERNED (CB05/06/0022, PI2021/03), and the Spanish Ministry of Science and Innovation (PID2022-139195OB-I00).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eva Carro reports administrative support was provided by Carlos III Health Institute. Reports a relationship with that includes: Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank all the participants with Down syndrome and their families and/or caregivers for their support and dedication to this research. We also acknowledge Asociación Down Compostela, Asociación Down Lugo y Fundación Síndrome de Down de Madrid. We also thank Biostattech for their support in analyzing data reported in this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbih.2025.100999>.

Data availability

Data will be made available on request.

References

- Abanto, J., Ciamponi, A.L., Francischini, E., Murakami, C., de Rezende, N.P., Gallottini, M., 2011. Medical problems and oral care of patients with Down syndrome: a literature review. *Spec. Care Dent.* 31, 197–203.
- Abdelhamid, M., Jung, C.G., Zhou, C., Abdullah, M., Nakano, M., Wakabayashi, H., Abe, F., Michikawa, M., 2020. Dietary lactoferrin supplementation prevents memory impairment and reduces amyloid- β generation in J20 mice. *J. Alzheimers Dis.* 74, 245–259.
- Almståhl, A., Wikström, M., Groenink, J., 2001. Lactoferrin, amylase and mucin MUC5B and their relation to the oral microflora in hyposalivation of different origins. *Oral Microbiol. Immunol.* 16, 345–352.
- Alugupalli, K.R., Kalfas, S., 1996. Degradation of lactoferrin by periodontitis-associated bacteria. *FEMS Microbiol. Lett.* 145, 209–214.
- Antequera, D., Carrero, L., Gonzalez-Sanchez, M., Cantero, J.L., Orive, G., Muncio, C., Carro, E., 2023. Reduced salivary lactoferrin levels in early-onset Alzheimer's disease. *Aging Dis.*
- Antequera, D., Moneo, D., Carrero, L., Bartolome, F., Ferrer, I., Proctor, G., Carro, E., 2021. Salivary lactoferrin expression in a mouse model of Alzheimer's disease. *Front. Immunol.* 12, 749468.
- Antonarakis, S.E., Skotko, B.G., Raffi, M.S., Strydom, A., Pape, S.E., Bianchi, D.W., Sherman, S.L., Reeves, R.H., 2020. Down syndrome. *Nat. Rev. Dis. Primers* 6, 9.
- Ballard, C., Mobley, W., Hardy, J., Williams, G., Corbett, A., 2016. Dementia in Down's syndrome. *Lancet Neurol.* 15, 622–636.
- Barone, E., Arena, A., Head, E., Butterfield, D.A., Perluigi, M., 2018. Disturbance of redox homeostasis in Down Syndrome: role of iron dysmetabolism. *Free Radic. Biol. Med.* 114, 84–93.
- Bartolome, F., Orive, G., Carro, E., 2021. Standardizing salivary lactoferrin measurements to obtain a robust diagnostic biomarker for Alzheimer's disease. *Alzheimers Dement (Amst)* 13, e12173.
- Bartolomé, F., Rosa, L., Valenti, P., Lopera, F., Hernández-Gallego, J., Cantero, J.L., Orive, G., Carro, E., 2022. Lactoferrin as immune-enhancement strategy for SARS-CoV-2 infection in Alzheimer's disease patients. *Front. Immunol.* 13, 878201.
- Bassell, J.L., Phan, H., Leu, R., Kronk, R., Visootsak, J., 2015. Sleep profiles in children with Down syndrome. *Am J Med Genet A* 167a, 1830–1835.
- Berluti, F., Pantanella, F., Natalizi, T., Frioni, A., Paesano, R., Polimeni, A., Valenti, P., 2011. Antiviral properties of lactoferrin—a natural immunity molecule. *Molecules* 16, 6992–7018.
- Bermejo-Pareja, F., Del Ser, T., Valentí, M., de la Fuente, M., Bartolome, F., Carro, E., 2020. Salivary lactoferrin as biomarker for Alzheimer's disease: brain-immunity interactions. *Alzheimers Dement* 16, 1196–1204.
- Carmona-Iragui, M., Alcolea, D., Barroeta, I., Videla, L., Muñoz, L., Van Pelt, K.L., Schmitt, F.A., Lightner, D.D., Koehl, L.M., Jicha, G., Sacco, S., Mircher, C., Pape, S.E., Hithersay, R., Clare, I.C.H., Holland, A.J., Nübling, G., Levin, J., Zaman, S.H., Strydom, A., Rebillat, A.S., Head, E., Blesa, R., Lleó, A., Fortea, J., 2021. Diagnostic and prognostic performance and longitudinal changes in plasma neurofilament light chain concentrations in adults with Down syndrome: a cohort study. *Lancet Neurol.* 20, 605–614.
- Carro, E., Bartolomé, F., Bermejo-Pareja, F., Villarejo-Galende, A., Molina, J.A., Ortiz, P., Calero, M., Rabano, A., Cantero, J.L., Orive, G., 2017. Early diagnosis of mild cognitive impairment and Alzheimer's disease based on salivary lactoferrin. *Alzheimers Dement (Amst)* 8, 131–138.
- Chausu, S., Chausu, G., Zigmund, M., Yefenof, E., Stabholz, A., Shapira, J., Merrick, J., Bachrach, G., 2007. Age-dependent deficiency in saliva and salivary antibodies secretion in Down's syndrome. *Arch. Oral Biol.* 52, 1088–1096.
- Chausu, S., Yefe Nof, E., Becker, A., Shapira, J., Chausu, G., 2003. Parotid salivary immunoglobulins, recurrent respiratory tract infections and gingival health in institutionalized and non-institutionalized subjects with Down's syndrome. *J. Intellect. Disabil. Res.* 47, 101–107.
- Chausu, S., Yefenof, E., Becker, A., Shapira, J., Chausu, G., 2002a. A link between parotid salivary Ig level and recurrent respiratory infections in young Down's syndrome patients. *Oral Microbiol. Immunol.* 17, 172–176.
- Chausu, S., Yefenof, E., Becker, A., Shapira, J., Chausu, G., 2002b. Severe impairment of secretory Ig production in parotid saliva of Down Syndrome individuals. *J. Dent. Res.* 81, 308–312.
- Contaldo, M., Lucchese, A., Romano, A., Della Vella, F., Di Stasio, D., Serpico, R., Petruzzi, M., 2021. Oral microbiota features in subjects with down syndrome and periodontal diseases: a systematic review. *Int. J. Mol. Sci.* 22.
- Dashper, S.G., Pan, Y., Veith, P.D., Chen, Y.Y., Toh, E.C., Liu, S.W., Cross, K.J., Reynolds, E.C., 2012. Lactoferrin inhibits *Porphyromonas gingivalis* proteinases and has sustained biofilm inhibitory activity. *Antimicrob. Agents Chemother.* 56, 1548–1556.
- Davidson, Y.S., Robinson, A., Prasher, V.P., Mann, D.M.A., 2018. The age of onset and evolution of Braak tangle stage and Thal amyloid pathology of Alzheimer's disease in individuals with Down syndrome. *Acta Neuropathol. Commun.* 6, 56.
- de Lillo, A., Teanpaisan, R., Fierro, J.F., Douglas, C.W., 1996. Binding and degradation of lactoferrin by *Porphyromonas gingivalis*, *Prevotella intermedia* and *Prevotella nigrescens*. *FEMS Immunol. Med. Microbiol.* 14, 135–143.
- de Sousa, M.C., Vieira, R.B., Dos Santos, D.S., Carvalho, C.A., Camargo, S.E., Mancini, M. N., de Oliveira, L.D., 2015. Antioxidants and biomarkers of oxidative damage in the saliva of patients with Down's syndrome. *Arch. Oral Biol.* 60, 600–605.
- Dominy, S.S., Lynch, C., Ermini, F., Benedyk, M., Marczyk, A., Konradi, A., Nguyen, M., Haditsch, U., Raha, D., Griffin, C., Holsinger, L.J., Arastu-Kapur, S., Kaba, S., Lee, A., Ryder, M.I., Potempa, B., Mydel, P., Hellvard, A., Adamowicz, K., Hasturk, H., Walker, G.D., Reynolds, E.C., Faull, R.L.M., Curtis, M.A., Dragunow, M., Potempa, J., 2019. *Porphyromonas gingivalis* in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv.* 5, eaau3333.
- Eker, F., Bolat, E., Pekdemir, B., Duman, H., Karav, S., 2023. Lactoferrin: neuroprotection against Parkinson's disease and secondary molecule for potential treatment. *Front. Aging Neurosci.* 15, 1204149.
- Fernandez, F., Nyhuis, C.C., Anand, P., Demara, B.I., Ruby, N.F., Spanò, G., Clark, C., Edgin, J.O., 2017. Young children with Down syndrome show normal development of circadian rhythms, but poor sleep efficiency: a cross-sectional study across the first 60 months of life. *Sleep Med.* 33, 134–144.

- Flores-Aguilar, L., Iulita, M.F., Kovacs, O., Torres, M.D., Levi, S.M., Zhang, Y., Askenazi, M., Wisniewski, T., Busciglio, J., Cuello, A.C., 2020. Evolution of neuroinflammation across the lifespan of individuals with Down syndrome. *Brain* 143, 3653–3671.
- Fortea, J., Vilaplana, E., Carmona-Iragui, M., Benejam, B., Videla, L., Barroeta, I., Fernández, S., Altuna, M., Pegueroles, J., Montal, V., Valldeu, S., Giménez, S., González-Ortiz, S., Muñoz, L., Estellés, T., Illán-Gala, L., Belbin, O., Camacho, V., Wilson, L.R., Annus, T., Osorio, R.S., Videla, S., Lehmann, S., Holland, A.J., Alcolea, D., Clarimón, J., Zaman, S.H., Blesa, R., Lleó, A., 2020. Clinical and biomarker changes of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. *Lancet* 395, 1988–1997.
- Gleerup, H.S., Jensen, C.S., Høgh, P., Hasselbalch, S.G., Simonsen, A.H., 2021. Lactoferrin in cerebrospinal fluid and saliva is not a diagnostic biomarker for Alzheimer's disease in a mixed memory clinic population. *EBioMedicine* 67, 103361.
- González-Sánchez, M., Bartolome, F., Antequera, D., Puertas-Martín, V., González, P., González-Grande, A., Llamas-Velasco, S., Herrero-San Martín, A., Pérez-Martínez, D., Villarejo-Galende, A., Atienza, M., Palomar-Bonet, M., Cantero, J.L., Perry, G., Orive, G., Ibañez, B., Bueno, H., Fuster, V., Carro, E., 2020. Decreased salivary lactoferrin levels are specific to Alzheimer's disease. *EBioMedicine* 57, 102834.
- Gorr, S.U., Abdolhosseini, M., 2011. Antimicrobial peptides and periodontal disease. *J. Clin. Periodontol.* 38 (Suppl. 1), 126–141.
- Grasso, M., Fidiolo, A., L'Episcopo, F., Recupero, M., Barone, C., Bacalini, M.G., Benatti, C., Giambirone, M.C., Caruso, G., Greco, D., Di Nuovo, S., Romano, C., Ferri, R., Buono, S., Cuello, A.C., Blom, J.M.C., Tascetta, F., Piazza, P.V., De La Torre, R., Caraci, F., 2024. Low TGF- β 1 plasma levels are associated with cognitive decline in Down syndrome. *Front. Pharmacol.* 15, 1379965.
- Guffroy, A., Dieudonné, Y., Uring-Lambert, B., Goetz, J., Alembik, Y., Korganow, A.S., 2019. Infection risk among adults with down syndrome: a two group series of 101 patients in a tertiary center. *Orphanet J. Rare Dis.* 14, 15.
- Hammerschlag, B.L., Butts, B., Likos, K., Verble, D.D., Nimmagadda, N., Virani, R., Ramanathan, S., Wharton, W., 2024. Pilot: salivary lactoferrin as a biomarker of Alzheimer's disease. *medRxiv*.
- Handen, B.L., Lott, I.T., Christian, B.T., Schupf, N., S., O.B., Mapstone, M., Fagan, A.M., Lee, J.H., Tudorascu, D., Wang, M.C., Head, E., Klunk, W., Ances, B., Lai, F., Zaman, S., Krinsky-McHale, S., Brickman, A.M., Rosas, H.D., Cohen, A., Andrews, H., Hartley, S., Silverman, W., 2020. The Alzheimer's biomarker consortium-down syndrome: rationale and methodology. *Alzheimers Dement (Amst)* 12, e12065.
- Johnstone, K.F., Herzberg, M.C., 2022. Antimicrobial peptides: defending the mucosal epithelial barrier. *Front. Oral Health* 3, 958480.
- Kamer, A.R., Fortea, J.O., Videla, S., Mayoral, A., Janal, M., Carmona-Iragui, M., Benejam, B., Craig, R.G., Saxena, D., Corby, P., Glodzik, L., Annam, K.R., Robbins, M., de Leon, M.J., 2016. Periodontal disease's contribution to Alzheimer's disease progression in Down syndrome. *Alzheimers Dement (Amst)* 2, 49–57.
- Kamer, A.R., Pushalkar, S., Gulivindala, D., Butler, T., Li, Y., Annam, K.R.C., Glodzik, L., Ballman, K.V., Corby, P.M., Blennow, K., Zetterberg, H., Saxena, D., de Leon, M.J., 2021. Periodontal dysbiosis associates with reduced CSF A β 42 in cognitively normal elderly. *Alzheimers Dement (Amst)* 13, e12172.
- Komatsu, T., Watanabe, K., Hamada, N., Helmerhorst, E., Oppenheim, F., Lee, M.C., 2021. Association between antimicrobial peptide histatin 5 levels and prevalence of Candida in saliva of patients with down syndrome. *Antibiotics (Basel)* 10.
- Kruzel, M.L., Zimecki, M., Actor, J.K., 2017. Lactoferrin in a context of inflammation-induced pathology. *Front. Immunol.* 8, 1438.
- Legrand, D., 2012. Lactoferrin, a key molecule in immune and inflammatory processes. *Biochem. Cell. Biol.* 90, 252–268.
- Legrand, D., Ellass, E., Carpentier, M., Mazurier, J., 2005. Lactoferrin: a modulator of immune and inflammatory responses. *Cell. Mol. Life Sci.* 62, 2549–2559.
- Lei, S., Li, J., Yu, J., Li, F., Pan, Y., Chen, X., Ma, C., Zhao, W., Tang, X., 2023. Porphyromonas gingivalis bacteremia increases the permeability of the blood-brain barrier via the Mfsd2a/Caveolin-1 mediated transcytosis pathway. *Int. J. Oral Sci.* 15, 3.
- Leng, Y., Musiek, E.S., Hu, K., Cappuccio, F.P., Yaffe, K., 2019. Association between circadian rhythms and neurodegenerative diseases. *Lancet Neurol.* 18, 307–318.
- Liu, H., Wu, H., Zhu, N., Xu, Z., Wang, Y., Qu, Y., Wang, J., 2020. Lactoferrin protects against iron dysregulation, oxidative stress, and apoptosis in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease in mice. *J. Neurochem.* 152, 397–415.
- Lynge Pedersen, A.M., Belstrøm, D., 2019. The role of natural salivary defences in maintaining a healthy oral microbiota. *J. Dent.* 80 (Suppl. 1), S3–s12.
- Maranhão, F.C.A., Mendonça, N.M., Teixeira, T.C., Lages, G., de Melo, J.A., Porciuncula, C.G.G., da Silva Filho, E.A., Silva, D.M.W., 2020. Molecular identification of Candida species in the oral microbiota of individuals with down syndrome: a case-control study. *Mycopathologia* 185, 537–543.
- Martini, A.C., Gross, T.J., Head, E., Mapstone, M., 2022. Beyond amyloid: immune, cerebrovascular, and metabolic contributions to Alzheimer disease in people with Down syndrome. *Neuron* 110, 2063–2079.
- McCarron, M., McCallion, P., Reilly, E., Dunne, P., Carroll, R., Mulryan, N., 2017. A prospective 20-year longitudinal follow-up of dementia in persons with Down syndrome. *J. Intellect. Disabil. Res.* 61, 843–852.
- Municio, C., Carro, E., 2023. Implication of salivary lactoferrin and periodontal-mediated infections in Alzheimer's disease. *Neural Regen Res.* 18, 2691–2692.
- Nara, P.L., Sindelar, D., Penn, M.S., Potempa, J., Griffin, W.S.T., 2021. Porphyromonas gingivalis outer membrane vesicles as the major driver of and explanation for neuropathogenesis, the cholinergic hypothesis, iron dyshomeostasis, and salivary lactoferrin in Alzheimer's disease. *J. Alzheimers Dis.* 82, 1417–1450.
- Odeh, M., Hershkovits, M., Bornstein, J., Loberant, N., Blumenthal, M., Ophir, E., 2013. Congenital absence of salivary glands in Down syndrome. *Arch. Dis. Child.* 98, 781–783.
- Olsen, I., Singhrao, S.K., 2021. Low levels of salivary lactoferrin may affect oral dysbiosis and contribute to Alzheimer's disease: a hypothesis. *Med. Hypotheses* 146, 110393.
- Pentz, R., Iulita, M.F., Ducatenzeiler, A., Videla, L., Benejam, B., Carmona-Iragui, M., Blesa, R., Lleó, A., Fortea, J., Cuello, A.C., 2021. Nerve growth factor (NGF) pathway biomarkers in Down syndrome prior to and after the onset of clinical Alzheimer's disease: a paired CSF and plasma study. *Alzheimers Dement* 17, 605–617.
- Pieren, D.K.J., Boer, M.C., de Wit, J., 2022. The adaptive immune system in early life: the shift makes it count. *Front. Immunol.* 13, 1031924.
- Plascencia-Villa, G., Perry, G., 2020. Status and future directions of clinical trials in Alzheimer's disease. *Int. Rev. Neurobiol.* 154, 3–50.
- Proctor, G.B., 2016. The physiology of salivary secretion. *Periodontol* 70, 11–25, 2000.
- Proctor, G.B., Carpenter, G.H., 2007. Regulation of salivary gland function by autonomic nerves. *Auton. Neurosci.* 133, 3–18.
- Proctor, G.B., Carpenter, G.H., 2014. Salivary secretion: mechanism and neural regulation. *Monogr. Oral Sci.* 24, 14–29.
- Raha-Chowdhury, R., Raha, A.A., Henderson, J., Ghaffari, S.D., Grigoriou, M., Beresford-Webb, J., Allinson, K., Chakraborty, S., Holland, A., Zaman, S.H., 2021. Impaired iron homeostasis and haematopoiesis impacts inflammation in the ageing process in down syndrome dementia. *J. Clin. Med.* 10.
- Raha, A.A., Ghaffari, S.D., Henderson, J., Chakraborty, S., Allinson, K., Friedland, R.P., Holland, A., Zaman, S.H., Mukaetova-Ladinska, E.B., Raha-Chowdhury, R., 2021. Hepcidin increases cytokines in Alzheimer's disease and down's syndrome dementia: implication of impaired iron homeostasis in neuroinflammation. *Front. Aging Neurosci.* 13, 653591.
- Ram, G., Chinen, J., 2011. Infections and immunodeficiency in Down syndrome. *Clin. Exp. Immunol.* 164, 9–16.
- Ramenzoni, L.L., Hofer, D., Solderer, A., Wiedemeier, D., Attin, T., Schmidlin, P.R., 2021. Origin of MMP-8 and Lactoferrin levels from gingival crevicular fluid, salivary glands and whole saliva. *BMC Oral Health* 21, 385.
- Reseco, L., Atienza, M., Fernandez-Alvarez, M., Carro, E., Cantero, J.L., 2021. Salivary lactoferrin is associated with cortical amyloid-beta load, cortical integrity, and memory in aging. *Alzheimers Res. Ther.* 13, 150.
- Rosa, L., Cutone, A., Lepanto, M.S., Paesano, R., Valenti, P., 2017. Lactoferrin: a natural glycoprotein involved in iron and inflammatory homeostasis. *Int. J. Mol. Sci.* 18.
- Schwahn, C., Frenzel, S., Holtfreter, B., Van der Auwera, S., Pink, C., Bülow, R., Friedrich, N., Völzke, H., Biffar, R., Kocher, T., Grabe, H.J., 2022. Effect of periodontal treatment on preclinical Alzheimer's disease-Results of a trial emulation approach. *Alzheimers Dement* 18, 127–141.
- Shugars, D.C., Watkins, C.A., Cowen, H.J., 2001. Salivary concentration of secretory leukocyte protease inhibitor, an antimicrobial protein, is decreased with advanced age. *Gerontology* 47, 246–253.
- Simon, A.K., Hollander, G.A., McMichael, A., 2015. Evolution of the immune system in humans from infancy to old age. *Proc. Biol. Sci.* 282, 20143085.
- Siqueira, W.L., Nicolau, J., 2002. Stimulated whole saliva components in children with Down syndrome. *Spec. Care Dent.* 22, 226–230.
- Smith, D.S., 2001. Health care management of adults with Down syndrome. *Am. Fam. Physician* 64, 1031–1038.
- Snyder, H.M., Bain, L.J., Brickman, A.M., Carrillo, M.C., Esbensen, A.J., Espinosa, J.M., Fernandez, F., Fortea, J., Hartley, S.L., Head, E., Hendrix, J., Kishnani, P.S., Lai, F., Lao, P., Lemere, C., Mobley, W., Mufson, E.J., Potter, H., Zaman, S.H., Granholm, A.C., Rosas, H.D., Strydom, A., Whitten, M.S., Rafii, M.S., 2020. Further understanding the connection between Alzheimer's disease and Down syndrome. *Alzheimers Dement* 16, 1065–1077.
- Tenovuo, J., Lehtonen, O.P., Aaltonen, A.S., Vilja, P., Tuohimaa, P., 1986. Antimicrobial factors in whole saliva of human infants. *Infect. Immun.* 51, 49–53.
- Valenti, P., Antonini, G., 2005. Lactoferrin: an important host defence against microbial and viral attack. *Cell. Mol. Life Sci.* 62, 2576–2587.
- Vellyyagounder, K., Bahdila, D., Pawar, S., Fine, D.H., 2018. Role of lactoferrin and lactoferrin-derived peptides in oral and maxillofacial diseases. *Oral Dis.*
- Veteleanu, A., Pape, S., Davies, K., Kodosaki, E., Hye, A., Zelek, W.M., Strydom, A., Morgan, B.P., 2023. Complement dysregulation and Alzheimer's disease in Down syndrome. *Alzheimers Dement* 19, 1383–1392.
- Videla, L., Benejam, B., Pegueroles, J., Carmona-Iragui, M., Padilla, C., Fernández, S., Barroeta, I., Altuna, M., Valldeu, S., Garzón, D., Ribas, L., Montal, V., Arranz Martínez, J., Rozalem Aranha, M., Alcolea, D., Bejanin, A., Iulita, M.F., Videla Cés, S., Blesa, R., Lleó, A., Fortea, J., 2022. Longitudinal clinical and cognitive changes along the Alzheimer disease continuum in down syndrome. *JAMA Netw. Open* 5, e2225573.
- Wiseman, F.K., Al-Janabi, T., Hardy, J., Karmiloff-Smith, A., Nizetic, D., Tybulewicz, V. L., Fisher, E.M., Strydom, A., 2015. A genetic cause of Alzheimer disease: mechanistic insights from Down syndrome. *Nat. Rev. Neurosci.* 16, 564–574.
- Wisniewski, K.E., Bobinski, M., 1991. Hypothalamic abnormalities in Down syndrome. *Prog. Clin. Biol. Res.* 373, 153–167.
- Wu, Y.C., Ning, L., Tu, Y.K., Huang, C.P., Huang, N.T., Chen, Y.F., Chang, P.C., 2018. Salivary biomarker combination prediction model for the diagnosis of periodontitis in a Taiwanese population. *J. Formos. Med. Assoc.* 117, 841–848.
- Xu, S.F., Zhang, Y.H., Wang, S., Pang, Z.Q., Fan, Y.G., Li, J.Y., Wang, Z.Y., Guo, C., 2019. Lactoferrin ameliorates dopaminergic neurodegeneration and motor deficits in MPTP-treated mice. *Redox Biol.* 21, 101090.
- Yang, Y., Lv, J., Bai, H., Ren, L., Yang, J., Ding, Y., Liu, C., Chen, X., 2023. Periodontal status and saliva metabolic signature in patients with Alzheimer's disease. *J. Alzheimers Dis.* 95, 603–613.

- Yarat, A., Akyüz, S., Koç, L., Erdem, H., Emekli, N., 1999. Salivary sialic acid, protein, salivary flow rate, pH, buffering capacity and caries indices in subjects with Down's syndrome. *J. Dent.* 27, 115–118.
- Yong, S.J., Veerakumarasivam, A., Lim, W.L., Chew, J., 2023. Neuroprotective effects of lactoferrin in Alzheimer's and Parkinson's diseases: a narrative review. *ACS Chem. Neurosci.*
- Zalewska, A., Klimiuk, A., Zięba, S., Wnorowska, O., Rusak, M., Waszkiewicz, N., Szarmach, I., Dzierżanowski, K., Maciejczyk, M., 2021. Salivary gland dysfunction and salivary redox imbalance in patients with Alzheimer's disease. *Sci. Rep.* 11, 23904.