Scientific Research Report

Proteome-Wide Mendelian Randomisation Identifies Causal Links of Plasma Proteins With Periodontitis

Chaoning Zhan [a,1], *Yuexin Zhu* [b,1], *Melissa Rachel Fok* [c], *Lijian Jin* [c], *Bing Han* [d#], *Yifan Lin* [a*]

[a] *Division of Paediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Hong Kong, China*
[b] *Beijing Institute of Heart Lung and Blood Vessel Diseases, Beijing Anzhen Hospital, Capital Medical University, Beijing, China*
[c] *Division of Periodontology & Implant Dentistry, Faculty of Dentistry, The University of Hong Kong, Hong Kong, China*
[d] *Department of Orthodontics, Cranial-Facial Growth and Development Center, Peking University School and Hospital of Stomatology, Beijing, China*

ARTICLE INFO

Article history:
Received 1 March 2024
Received in revised form
9 April 2024
Accepted 12 April 2024
Available online 10 May 2024

Key words:
Mendelian randomisation
Periodontitis
Protein
Therapeutic target

ABSTRACT

Objective: Periodontitis is a complex and multifactorial disease and it is challenging to decipher its underlying causes and mechanisms. This study attempted to explore potential circulating proteins in connection to periodontitis through proteome-wide Mendelian randomisation (MR).

Methods: We analysed 1722 circulating proteins to identify prospective drug targets for tackling periodontitis, using the genomic dataset from the FinnGen study. Two-sample MR was conducted to evaluate the bidirectional relationship between circulating proteins and periodontitis risk. A dataset from the UK Biobank was used to validate the findings. Single-cell analysis was performed to assess the cellular expression of the identified proteins within gingival tissues.

Results: MR analyses found that genetically predicted circulating levels of von Willebrand factor A domain-containing 1 (von Willebrand factor A domain containing 1 [VWA1], odds ratios: 0.94, 95% CI 0.92-0.97, $P = 1.28 \times 10^{-5}$) were inversely associated with periodontitis. In contrast, the level of growth differentiation factor 15 (growth differentiation factor 15 [GDF15], odds ratios: 1.05, 95% CI 1.02-1.07, $P = 2.12 \times 10^{-5}$) might be associated with an increased risk of periodontitis. Single-cell analysis indicated that VWA1 was primarily expressed in endothelial cells of healthy gingival tissues, while the main source of GDF15 was not derived from periodontal cells.

Conclusions: The present study suggests that certain plasma proteins like VWA1 and GDF15 may be potentially indicative of the risk and susceptibility to periodontitis. These proteins could possibly be the potential therapeutic targets for treating periodontitis, and further investigation is highly warranted.

Introduction

Periodontitis as a common oral disease is a major global health burden accounting for considerable socio-economic impacts worldwide.[1,2] It results from the dysregulated interactions of dysbiotic biofilms/microbiome and the resultant host immune-inflammatory responses that lead to periodontal destruction and eventually tooth loss.[3] There are great variations in individual susceptibility to gingivitis and periodontitis.[4] Moreover, periodontitis has a close association

* *Corresponding author*. Division of Paediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Hong Kong 999077, China.
# *Corresponding author*. Department of Orthodontics, Cranial-Facial Growth and Development Center, School and Hospital of Stomatology, Peking University, Beijing 100081, China.
E-mail addresses: kqbinghan@bjmu.edu.cn (B. Han), yflin@hku.hk (Y. Lin).
Yifan Lin: http://orcid.org/0000-0002-2614-9159

[1] Chaoning Zhan and Yuexin Zhu contributed equally to this work.

https://doi.org/10.1016/j.identj.2024.04.019

with its systemic comorbidities, such as diabetes, cardiovascular disease, chronic kidney disease, obesity, mental stress, and even poor sleep quality.[5] Indeed, the etiopathogenesis of periodontitis is rather complex and multifaceted, with various mouth-body interactions via underlying infection and immune-inflammatory mechanisms as well as shared risk factors.[6] As such, other than the routine treatment to control supragingival and subgingival plaque biofilms, the proposed host modulatory approaches (eg, probiotics, anti-inflammatory, and pro-resolution agents as well as immunomodulators) in adjunct to conventional periodontal interventions represent an appealing strategy. These approaches are in line with the emerging notion of personalised medicine, offering effective management for susceptible periodontitis patients.[3] For instance, it has previously been shown that systemic tocilizumab may serve as a host modulatory therapy to improve periodontal status by targeting the interleukin-6 (IL-6) receptor.[7]

In some cases, circulating proteins like various pro-inflammatory cytokines appear in the blood because of active cellular secretion or leakage under pathological conditions.[8] Actually, they are the crucial modulators of various molecular pathways and could be potentially the biomarkers for periodontal assessment and diagnosis as well as the pharmaceutical targets for adjunctive therapies.[9] Notably, the serum adiponectin concentration in periodontitis patients is found to be decreased, while the leptin concentration increases in a meta-analysis of 25 cross-sectional studies.[10] Additionally, heat-shock protein-27, functions as an anti-inflammatory, anti-oxidative, and anti-apoptotic molecule, and its level is lower in aggressive periodontitis patients than that in healthy individuals and chronic periodontitis patients.[11] However, the reported links of these circulating proteins with periodontitis are mainly based on observational studies, and there is a possibility of multi-confounders and reverse causality accounting for such interconnections. In addition, randomised control trials are very challenging for unravelling the potential causal link of circulating proteins with periodontitis.

Mendelian randomisation (MR) is a rapid, inexpensive, and powerful tool to search for causal inference in large datasets.[12-14] In MR studies, genetic variants (single-nucleotide polymorphisms), such as the circulating protein variants used in the current study, are used as instrumental variables (IVs) for exposure to assess the causal associations of exposure with outcome. In essence, genetic variants are assigned randomly and they are not affected by self-adopted and environmental factors. Hence, MR is less prone to confounding. Researchers have recently used MR to investigate the causal connections of circulating molecules with oral disease traits. For instance, vitamin D and 25-hydroxyvitamin D have no causal role in periodontitis,[12,13] and there is a genetic association of between circulating IL-9/IL-17 with periodontitis after evaluating 41 circulating cytokines.[14] However, there have been no proteome-wide MR analyses exploring potential therapeutic targets for periodontitis. Recently, a proteome-wide MR study identifies certain proteins (eg, MST1, CXCL5, and STAT3) associated with the risk of inflammatory bowel disease that represent potential therapeutic targets.[15] Another study shows that GCNT4, RAB14, ABO, CD207, and C1GALT1C1 to have causal relations with the increased risk of COVID-19, the resultant hospitalisation and mortality.[16] To accelerate drug research and development for tackling periodontitis, we used the proteome-wide MR analysis to identify the potential causal relationships between circulating proteins and periodontitis. Furthermore, single-cell-type expression analysis was carried out on gingival tissues to identify the cell types responsible for the gene expression enrichment. Lastly, the druggability assessment was employed to evaluate the potential of the identified proteins as the alternative therapeutic targets amenable for periodontal interventions.

Methods

Study design

In MR studies, genetic variants used as IVs must meet the following 3 main assumptions (Figure 1): (1) genetic instruments need be correlated with the exposure (ie, the levels of circulating proteins in this study); (2) genetic instruments are uncorrelated with any confounders between exposure and outcome (ie, periodontitis in this study); and (3) the effects of genetic instruments should be limited to their effects on the exposure of interest. Assumptions 2 and 3 are known collectively as no horizontal pleiotropy. This protein-wide Mendelian randomisation study identified the potential therapeutic targets for periodontitis. All original studies involved in this study were ethically approved and all data sets were publicly available. The present study is presented following the checklist of strengthening the reporting of observational studies in epidemiology using MR. The protocol and details of the study were not pre-registered.

Periodontitis GWAS data

The genome-wide association study (GWAS) summarised data for periodontitis were provided by FinnGen consortium R9 release.[17] This study included 346,900 controls and 30,377 clinical periodontitis individuals (ICD-10 K05.30, K05.31, K05.4). Periodontitis, as defined by the database (https://r9.risteys.finngen.fi/endpoints/K11_PARODON_OPER), includes individuals who have been diagnosed with the condition from hospitals, exhibiting both pronounced and complex forms characterised by clinical manifestations such as attachment loss, deep periodontal pockets, and alveolar bone loss. Control are those individuals that reported not having periodontitis.

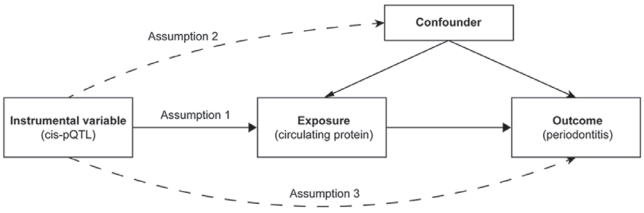

Fig. 1 – Schematic representation of the Mendelian randomisation principle and its 3 main assumptions. Solid lines indicate desirable associations, while dashed lines indicate undesirable associations.

Circulating protein GWAS data

In the primary MR analysis, the summarised GWAS data on circulating proteins were provided by a GWAS of 4719 proteins from 35,559 Icelanders.[18] The 4719 unique circulating proteins were obtained from 4907 aptamers by a SomaScan version 4 assay (SomaLogic). The detailed GWAS information is shown in the original paper.[18] For external validation, data on protein Quantitative Trait Loci (pQTLs) were obtained from the UK Biobank.[19]

Validation of MR assumptions

To satisfy the first assumption, pQTLs were selected as genetic instruments based on their genome-wide significance. A genetic instrument with an F statistic greater than 10 was considered as unlikely to have significant weak-instrument bias. To fulfill the second and third assumptions, only cis-pQLTs were selected. In this study, cis-pQLTs were considered to be those located within 250 kb either upstream or downstream of the transcription start site. Additionally, the MR-Egger regression for proteins was conducted with at least 3 genetic instruments, and a significant regression intercept ($P < .05$) also indicated horizontal pleiotropy. Finally, 1722 proteins were included in the following analysis.

MR analysis

In this study, circulating proteins were set as exposure, and the periodontitis was used as the outcome. Two-sample MR analysis was performed using index single-nucleotide polymorphisms for proteins to explore the potential causal effects on periodontitis among the circulating proteins profiled in the proteomic study. Inverse variance weighted or Wald's ratio MR analyses were conducted using the TwoSampleMR R package for proteins with ≥2 IVs or only 1 IV, respectively.[20] Weighted mode and median as well as MR-Egger were employed to account for horizontal pleiotropy. Note that cML-MA was conducted to control for correlated and uncorrelated pleiotropic effects.[21] The results are shown as odds ratios (ORs) per standard deviation (SD) increase in genetically determined circulating proteins. We applied Bonferroni correction to address the issue of multiple comparisons in the primary analysis. A threshold *P*-value of .05 divided by the number of circulating proteins was set (0.05/1722), and we obtained a final significance threshold of $P < 2.90 \times 10^{-5}$. Based on this threshold, we selected the most significant findings for further external validation. The *P*-value threshold of external validation was set as .05.

Steiger filtering and bidirectional MR analysis

Steiger filtering was implemented to sort the captured proteins. Then, the instruments in the primary analysis were used for bidirectional MR analysis to investigate potential reverse causality occurrences. For statistical significance, a *P*-value of .05 was set as the threshold. We deliberately omitted any circulating proteins if any indications of reverse causality were found.

Single-cell-type expression analysis

Single-cell RNA-sequencing data from human gingival mucosa was used to further evaluate the cell type-specific expression of target genes linked to periodontitis. The dataset GSE164241 was downloaded from the GEO database, which included 13 healthy samples and 8 periodontitis samples. Data preprocessing and transformation were conducted on the basis of the raw single-cell RNA-seq data with the help of the 'Seurat' package (ver. 3.2.2, https://github.com/satijalab/seurat). The RNA-seq data of normal gingival tissues from healthy individuals and inflammatory gingival tissues from patients with untreated severe periodontitis included 29,409 cells and 23,496 cells respectively. Genes with more than 5000 features per cell, cells with fewer than 200 unique features, and cells with a mito percent of more than 15% were removed. Cell types were predicted through manual annotation. To determine whether the identified periodontitis causal protein-coding genes were highly expressed in a specific cell type in gingival tissue, a differential expression analysis using the Wilcoxon Rank Sum test was performed to compare gene expression levels between a cell type and others. The R package 'Nebulosa' was used to conduct (joint) weighted kernel density estimation of gene expression through the utilisation of the plot_density function with default parameters, in order to determine the targeted gene-enriched cell populations.[22]

Druggable protein identification

To determine the development status of the druggability of the identified circulating proteins, a search of the DrugBank, Therapeutic Target Database, and Pharmacogenomics Knowledge Base was conducted. The names of the drugs, their status, and other relevant information were documented. These proteins were classified into 5 categories to assess their potential druggability: (1) Successful target with at least one drug-targeting protein approved; (2) Clinical trial target (the drugs tested in various trials); (3) Preclinical target (the drugs in the preclinical stage of development); (4) Druggable target (the protein already documented in the literature but not included in the above database); (5) Not currently listed as druggable (no studies undertaken as a drug-targeting protein).

Results

Primary proteome-wide and bidirectional MR analyses

In this study, MR analysis was used to examine the causal relationships of 1722 circulating proteins with periodontitis. Each genetic instrument had an F-statistic higher than 10, indicating a good level of strength. Primary 2-sample MR analysis revealed associations between 4 circulating proteins and periodontitis at the Bonferroni significance level ($P < 2.90 \times 10^{-5}$). Specifically, for each 1-SD increase in genetically predicted protein levels, the ORs for periodontitis were as follows: tripeptidyl peptidase 1 (TPP1) at 0.79 (95% CI 0.72-0.87), von Willebrand factor A domain containing 1 (VWA1) at 0.94 (95% CI 0.92-0.97), growth differentiation factor 15 (GDF15) at

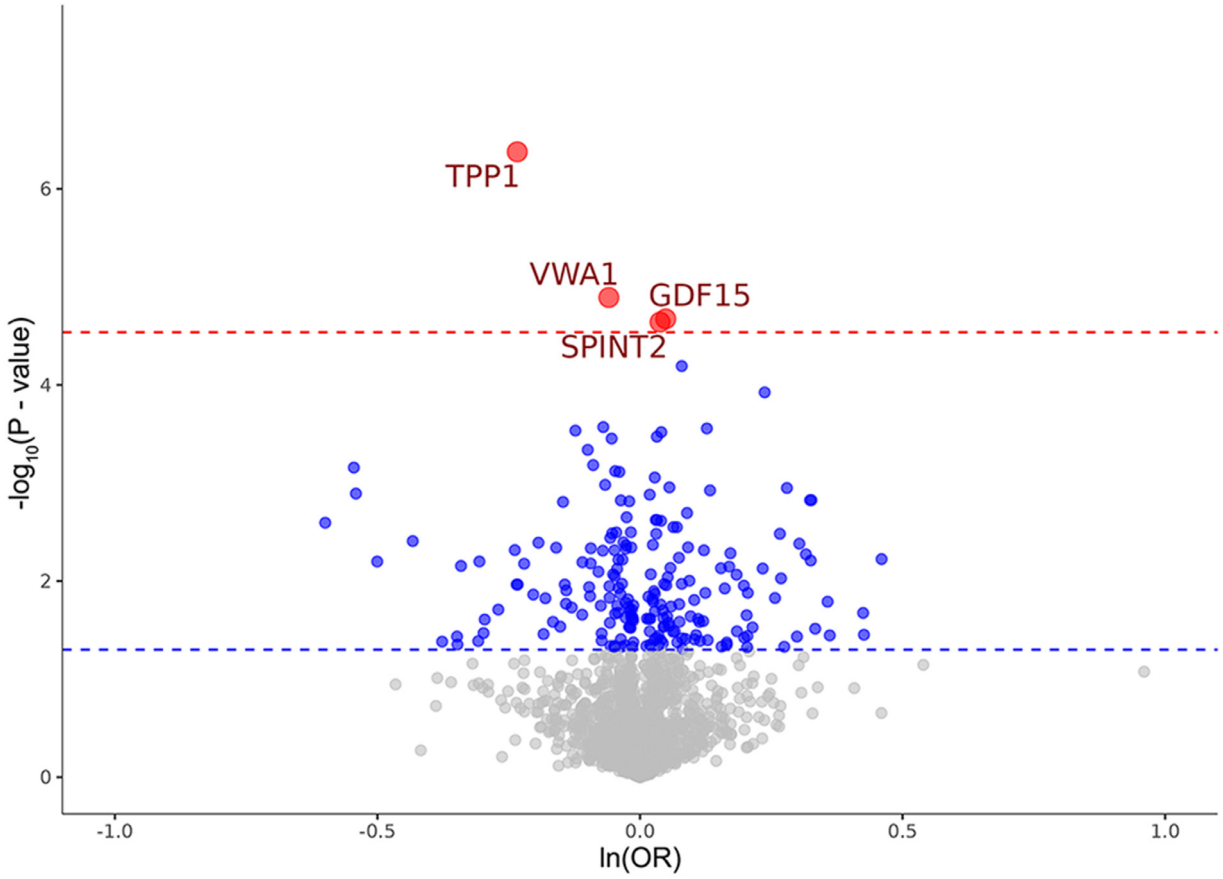

Fig. 2 – Volcano plots of the Mendelian randomisation results illustrating the relationship between 1722 circulating proteins and periodontitis risk. Blue dots indicate the P-values are more 2.90×10^{-5}, but less than .05, and red dots indicate the P-values are less than 2.90×10^{-5}.

1.05 (95% CI 1.02-1.07), and serine peptidase inhibitor, Kunitz type 2 (SPINT2) at 1.04 (95% CI 1.02-1.06) (Figure 2 and Table 1). These results suggest that lower levels of TPP1 and VWA1 are linked to a higher risk of periodontitis, while higher levels of GDF15 and SPINT2 are linked to a higher risk of periodontitis. These associations are generally supported by the results of additional analyses, including weighted mode and median as well as MR-Egger (Table S1). The Steiger filtering and bidirectional MR confirmed the directionality of the causal associations (Table S2).

External validation of causal proteins for periodontitis

The causal relationships between VWA1, GDF15, and periodontitis were successfully replicated using the UK Biobank during the external validation process ($P < .05$). Unfortunately, we failed to replicate the relationships of TPP1 and SPINT2 in the external validation stage (Table 1).

Expression of cell-type specificity in inflammatory gingival tissues

To determine whether the coding genes for the VWA1 and GDF15 were cell-type-specifically enriched in gingival tissues from patients with periodontitis, the single-cell-type expression analysis was conducted on the basis of the single-cell RNA-seq data for human gingival tissues. Cells were classified into 5 types (endothelial cells, fibroblasts, immune cells, epithelial cells, and other types of cells) (Figure 3A). In gingival tissues samples of healthy controls and periodontitis

Table 1 – Mendelian randomisation results for plasma proteins associated with periodontitis after Bonferroni correction.

Protein	Unipoint	SNP (*n*)	OR (95% CI)	*P*-value (discovery)	*P*-value* (replication)	PVE (%)	F statistics
TPP1	O14773	8	0.79 (0.72, 0.87)	4.19×10^{-7}	1.71×10^{-1}	3.08	51.26
VWA1	Q6PCB0	22	0.94 (0.92, 0.97)	1.28×10^{-5}	1.64×10^{-2}	30.99	105.89
GDF15	Q6UXV0	46	1.05 (1.02, 1.07)	2.12×10^{-5}	6.73×10^{-3}	29.95	101.58
SPINT2	O43291	45	1.04 (1.02, 1.06)	2.29×10^{-5}	1.16×10^{-1}	40.19	89.20

OR, odds ratio; PVE, proportion of variance explained; SNP, single-nucleotide polymorphism.

* Only the most significant *P*-value is shown.

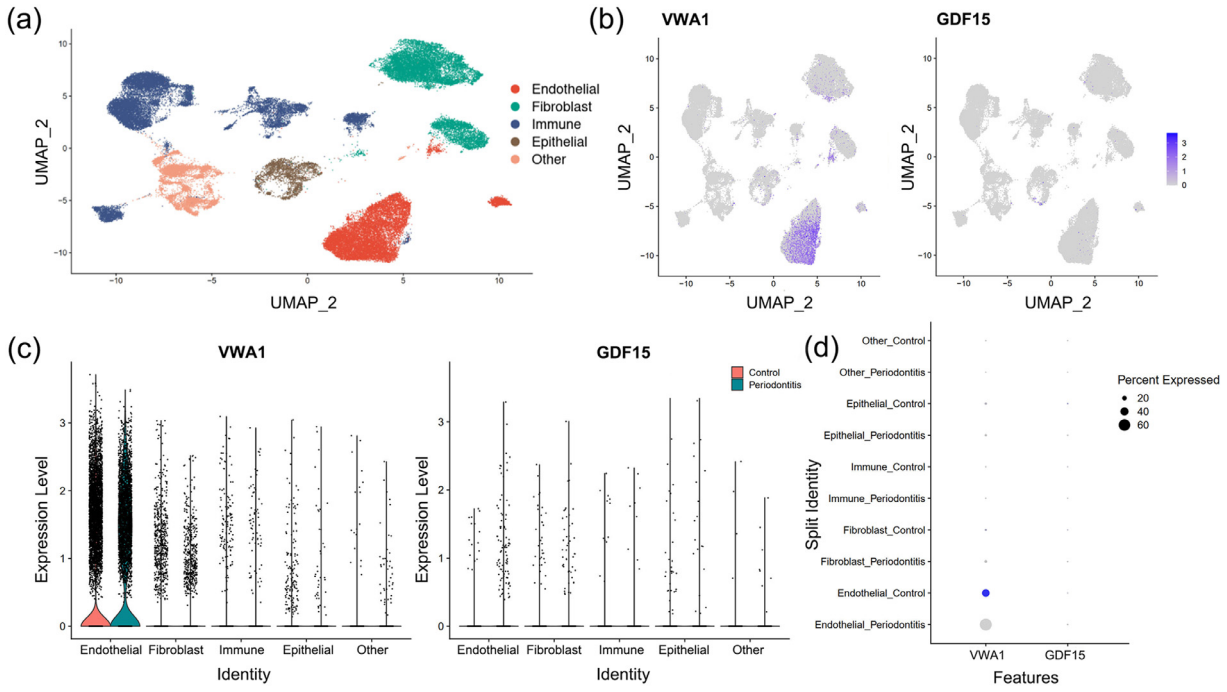

Fig. 3 – Single-cell-type expression of the genes coding VWA1 and GDF15 in gingival tissues. A, A total of 5 cell types were scrutinised. B-D, The expression of VWA1 and GDF15 in each type of cell within gingival tissues.

patients, both protein-coding genes were expressed. Only VWA1 had cell-type-specific enrichment in endothelial cells in normal gingival tissues, whereas the expression of GDF15 was low in both normal and inflamed gingival tissues. We obtained the tissue-specific expression profiles of GDF15 from the GTEx Portal database and found that it was mainly expressed in the kidneys.

Druggability of identified proteins

The circulating VWA1 and GDF15 identified in the MR analysis were searched as candidate protein targets in drug databases. The target categories, corresponding drug names, and other information on the drugs are shown in Table 2. They were not found to be drug targets for periodontitis. Although there was no information available on VWA1 and GDF15 in the drug databases, several studies have used GDF15 as a drug target. Sulindac sulfide, resveratrol, indole-3-carbinol, 3,3′-diindolylmethane, and conjugated linoleic acids have been used to induce GDF15 expression to promote the apoptosis of colorectal carcinoma cells.[23-26] In addition, AF957 and other GDF15 inhibitory antibodies have been designed to treat cancer cachexia.[27]

Discussion

This study was the first to evaluate the potential causal relationships of certain plasma proteins with periodontitis, via employing the proteome-wide MR analysis. We conducted a 2-sample MR analysis to assess the 1-way causal roles of 1722 circulating proteins in periodontitis for drug development, exploring 2 protein-periodontitis associations. We discovered that genetically predicted higher levels of VWA1 were inversely related to periodontitis risk, while GDF15 was positively associated with periodontitis risk. A druggability evaluation prioritised the GDF15 biomarker, which has been mainly employed as a drug target in gastrointestinal cancer.[24-26] Drugs have also been used to promote GDF15

Table 2 – Summary of drugs targeting significant identified proteins.

Gene	Target category	Drug name	Drug group	Additional information
VWA1	Not currently listed as druggable	NA	NA	NA
GDF15	Druggable	Sulindac sulfide	LR	Inducing GDF15 upregulation in the colon and liver of mice
		Resveratrol	LR	Inducing GDF15 expression in a p53-dependent manner
		Indole-3-carbinol	LR	Inducing GDF15 expression in a p53-independent manner
		3,3′-diindolylmethane	LR	Inducing expression of GDF15 in a p53-independent manner
		Conjugated linoleic acids	LR	Activating GDF15 expression by the overexpression of ATF3
		AF957	LR	Goat anti-human GDF15 polyclonal antibody

ATF3, activating transcription factor 3; LR, literature-reported.

expression to induce cancer cell apoptosis. Additionally, GDF15-specific antibodies have been recommended as agents to treat cachexia-associated weight loss. Hence, GDF15 antibodies have the potential to be repurposed as a treatment strategy for periodontitis. Despite the lack of information on drugs targeting VWA1-expressed protein, this protein still deserves attention as a promising new therapeutic target for periodontitis.

The VWA1 gene encoding von Willebrand factor A domain-related protein (WARP) is mainly expressed in muscle, cartilage, and endothelial cell basement membranes.[28] Our single-cell analysis also revealed the high expression of VWA1 in endothelial cells within periodontal tissues. WARP is able to form multimeric structures and connect to other extracellular matrix components, including perlecan and collagen VI.[29] There have been few studies published on VWA1 and periodontitis. The protective effect of VWA1 may be achieved through the promotion of periodontal healing, as increased VWA1 expression is positively correlated with post-infarct healing, and WARP-knockout mice tend to die from cardiac rupture after myocardial infarction.[30] Furthermore, WARP plays a role in fracture healing.[31] Although no studies have determined the involvement of WARP in periodontal tissue healing, the function of type VI collagen in controlling biomineralisation in alveolar bone, especially during periodontitis, has been confirmed.[32] Because of the high-affinity associations between WARP and type VI collagen in vivo, WARP probably participates as an adapter protein in periodontal tissue healing during inflammation.[29]

Another possible explanation for WARP's protective function in periodontium is its neuromodulation ability. It has been shown that mice lacking WARP have impaired peripheral nerve structure and function and, for example, show a delayed response to painful stimuli.[33] Notably, functional sensory peripheral nerves not only contribute to nociception but also mediate periodontitis. Specifically, calcitonin gene-related peptide (CGRP) and substance P (SP), the 2 primary sensory neuropeptides that mediate nociception, also play crucial roles in modulating inflammation and alveolar bone resorption during periodontitis.[34,35] Several studies have shown that SP aggravates periodontitis by upregulating the RANKL/OPG ratio and HIF-1α expression in gingival fibroblasts.[35] In contrast, CGRP is more likely to inhibit inflammation and promote periodontal healing via the cAMP/PKA signalling pathway.[36,37] In diabetic rats, CGRP also has the ability to enhance peri-implant angiogenesis and osseointegration.[38] Notably, CGRP has a more significant impact than SP in periodontitis. Firstly, CGRP-positive neurons are more than SP-positive neurons in trigeminal ganglion;[39] second, sensory denervation leads to increased TNF-α and IL-1β and severe alveolar bone loss.[40] Therefore, decreased WARP levels may be linked to an increased risk of periodontitis, as WARP deficiency impairs peripheral neural function and neuropeptide signalling, especially in CGRP-relevant pathways.

It is known that macrophage inhibitory cytokine-1, known as GDF15, belongs to a divergent superfamily of the transforming growth factor β.[41] While human GDF15 is barely detectable in physiological conditions, it can be secreted by cardiac and blood vessel wall tissues in response to abnormal pressure load or heart volume expansion to maintain cell and tissue homeostasis.[42] Therefore, it is regarded as a biomarker that can be used to anticipate unfavourable outcomes in individuals suffering from cardiovascular disease and heart failure.[43] In addition, GDF15 was discovered to be the predominant soluble cytokine across various cancer types in large-scale screening,[44] and it has been further proposed as a predictive and prognostic biomarker in several cancers, including oral squamous cell carcinoma.[45] Current pharmaceutical drugs targeting GDF15 are mainly used to inhibit cancers in laboratory studies.[23,24]

In our study, GFD15 was the only protein identified that has been explored as a circulating biomarker for periodontitis prognosis in an randomised control trial.[46] In line with this finding, our study expanded on the evidence and confirmed that the association between GDF15 and periodontitis is causal. Considering the pro-inflammatory function of GDF15 in periodontal ligament cells, GDF15 may aggravate periodontitis by promoting the release of pro-inflammatory cytokines (TNFα, IL6, IL8, COX2/PGE2,), the stimulation of THP1 monocytic cells, and the M1-like polarisation of RAW264.7 cells,[47] leading to an excessive inflammatory response. In addition, the GDF15 protein can promote the expression ratio of RANKL/OPG in periodontal ligament cells and the osteoclast differentiation of RAW264.7 cells to exacerbate periodontal hard tissue destruction.[48] In contrast, GDF15 has also been shown to inhibit immune responses by inhibiting the recruitment of immune cells, the function of antigen-presenting cells, and the polarisation of M1 macrophages in certain studies.[49] Therefore, high expression of GDF15 may lead to an increased number of periodontal pathogenic bacteria because of its anti-inflammatory function. Because GDF15 acts as both a pro-inflammatory and an anti-inflammatory cytokine, the specific role of GDF15 in periodontitis needs further in vivo study.

In this study, MR analysis reduced the bias caused by reverse causality and confounding and improved the reliability of the causal inference. Another strength of this investigation was the utilisation of GWASs with substantial sample sizes, which enhanced the power of the analysis to identify mild-to-moderate connections. We also conducted single-cell analysis to detect VWA1- and GDF15-enriched cell types in healthy and inflamed gingiva.

Although this study identified 2 promising drug targets, some limitations of this study need to be considered. Firstly, we only evaluated the role of circulating proteins in periodontitis while could not assess the protein levels in other tissues. In the single-cell analysis, we could not identify any GDF15-enriched cell types, indicating that the circulating GDF15 was secreted by other tissues and organs. The data from the GTEx database show that GDF15 was mainly expressed in human kidneys, and GDF15 is upregulated in kidney fibrosis and in toxic acute kidney injury.[50] Considering the close association of periodontitis with kidney diseases, GDF15 is probably released from the kidneys into the circulation to reach periodontal tissues and eventually exacerbate periodontal inflammation. Secondly, the single-cell data were limited to gingiva and did not include alveolar bone. As increased VWA1 expression in bone is correlated with fracture healing in mice,[31] assessing the genes expressed in the alveolar bone may offer a deeper understanding of periodontal pathogenesis and provide additional insights into the role of WARP in periodontitis.

Conclusions

The present MR study has discovered the causal links of 2 circulating proteins with periodontitis, providing new perspectives on the etiopathogenesis of periodontitis and potential therapeutic approaches. Further basic, translational and clinical studies on the biological plausibility of certain plasma proteins like VWA1 and GDF15 in the onset and development of periodontitis are required for validating the current findings and developing novel therapeutic approaches.

Data availability statement

All datasets used in this study are publicly available. All analyses used publicly available data (UKB, decode), including previously published GWAS (https://r9.risteys.finngen.fi/endpoints/K11_PARODON_OPER), Decode 2021, and Decode 2023 which have been cited in the article. The scRNA-seq dataset is available at [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE164241].

Conflict of interest

None disclosed.

Acknowledgement

Publication was made possible in part by support from the HKU Libraries Open Access Author Fund sponsored by the HKU Libraries.

Author contributions

Chaoning Zhan, Yuexin Zhu, Bing Han, and Yifan Lin conceived the study design and acquired the publicly available data. Chaoning Zhan, Yuexin Zhu, Melissa Rachel Fok, Bing Han, and Yifan Lin contributed to the statistical analyses and interpretation of the data. Chaoning Zhan wrote the initial draft. Lijian Jin, Bing Han, and Yifan Lin critically revised the manuscript.

Funding

This research was supported by the Oral Health Research and Innovation Fund from the University of Hong Kong.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2024.04.019.

REFERENCES

1. Jin L J, Lamster I B, Greenspan J S, Pitts N B, Scully C, Warnakulasuriya S. Global burden of oral diseases: emerging concepts, management and interplay with systemic health. Oral Dis 2016;22:609–19.
2. Tonetti M S, Jepsen S, Jin L, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: a call for global action. J Clin Periodontol 2017;44:456–62.
3. Hajishengallis G, Chavakis T, Lambris J D. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000 2020;84:14–34.
4. Nashef A, Qabaja R, Salaymeh Y, et al. Integration of murine and human studies for mapping periodontitis susceptibility. J Dent Res 2018;97:537–46.
5. Parsegian K, Randall D, Curtis M, Ioannidou E. Association between periodontitis and chronic kidney disease. Periodontol 2000 2022;89:114–24.
6. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat Rev Immunol 2021;21:426–40.
7. Kobayashi T, Ito S, Kobayashi D, et al. Interleukin-6 receptor inhibitor tocilizumab ameliorates periodontal inflammation in patients with rheumatoid arthritis and periodontitis as well as tumor necrosis factor inhibitors. Clin Exp Dent Res 2015;1:63–73.
8. Anderson N L, Anderson N G. The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics 2002;1:845–67.
9. Suhre K, McCarthy M I, Schwenk J M. Genetics meets proteomics: perspectives for large population-based studies. Nat Rev Genet 2021;22:19–37.
10. Zhu J, Guo B, Gan X, et al. Association of circulating leptin and adiponectin with periodontitis: a systematic review and meta-analysis. BMC Oral Health 2017;17:104.
11. Kaiser F, Donos N, Henderson B, et al. Association between circulating levels of heat-shock protein 27 and aggressive periodontitis. Cell Stress Chaperones 2018;23:847–56.
12. Baumeister S E, Reckelkamm S L, Baurecht H, et al. A Mendelian randomization study on the effect of 25-hydroxyvitamin D levels on periodontitis. J Periodontol 2022;93:1243–9.
13. Hu Z, Zhou F, Xu H. Circulating vitamin C and D concentrations and risk of dental caries and periodontitis: a Mendelian randomization study. J Clin Periodontol 2022;49:335–44.
14. Huang S J, Li R, Xu S, Liu Y, Li S H, Duan S Z. Assessment of bidirectional relationships between circulating cytokines and periodontitis: insights from a Mendelian randomization analysis. Front Genet 2023;14:1124638.
15. Chen J, Xu F, Ruan X, et al. Therapeutic targets for inflammatory bowel disease: proteome-wide Mendelian randomization and colocalization analyses. EBioMedicine 2023;89:104494.
16. Palmos A B, Millischer V, Menon D K, et al. Proteome-wide Mendelian randomization identifies causal links between blood proteins and severe COVID-19. PLoS Genet 2022;18:e1010042.
17. Kurki M I, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. Nature 2023;613:508–18.
18. Ferkingstad E, Sulem P, Atlason B A, et al. Large-scale integration of the plasma proteome with genetics and disease. Nat Genet 2021;53:1712–21.
19. Eldjarn G H, Ferkingstad E, Lund S H, et al. Large-scale plasma proteomics comparisons through genetics and disease associations. Nature 2023;622:348–58.
20. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife 2018;7:e34408.
21. Xue H, Shen X, Pan W. Constrained maximum likelihood-based Mendelian randomization robust to both correlated and uncorrelated pleiotropic effects. Am J Hum Genet 2021;108:1251–69.

22. Alquicira-Hernandez J, Powell J E. Nebulosa recovers single-cell gene expression signals by kernel density estimation. Bioinformatics 2021;37:2485–7.
23. Lee S H, Kim J S, Yamaguchi K, Eling T E, Baek S J. Indole-3-carbinol and 3,3′-diindolylmethane induce expression of NAG-1 in a p53-independent manner. Biochem Biophys Res Commun 2005;328:63–9.
24. Lee S H, Yamaguchi K, Kim J S, et al. Conjugated linoleic acid stimulates an anti-tumorigenic protein NAG-1 in an isomer specific manner. Carcinogenesis 2006;27(5):972–81.
25. Baek S J, Wilson L C, Eling T E. Resveratrol enhances the expression of non-steroidal anti-inflammatory drug-activated gene (NAG-1) by increasing the expression of p53. Carcinogenesis 2002;23:425–34.
26. Kim K S, Baek S J, Flake G P, Loftin C D, Calvo B F, Eling T E. Expression and regulation of nonsteroidal anti-inflammatory drug-activated gene (NAG-1) in human and mouse tissue. Gastroenterology 2002;122:1388–98.
27. Lerner L, Tao J, Liu Q, et al. MAP3K11/GDF15 axis is a critical driver of cancer cachexia. J Cachexia Sarcopenia Muscle 2016;7:467–82.
28. Fitzgerald J. WARP: a unique extracellular matrix component of cartilage, muscle, and endothelial cell basement membranes. Anat Rec (Hoboken) 2020;303:1619–23.
29. Hansen U, Allen J M, White R, et al. WARP interacts with collagen VI-containing microfibrils in the pericellular matrix of human chondrocytes. PloS One 2012;7:e52793.
30. Deckx S, Carai P, Bateman J, Heymans S, Papageorgiou A P. Breeding strategy determines rupture incidence in post-infarct healing WARPing cardiovascular research. PloS One 2015;10:e0139199.
31. Yuan C, Cai J. Time-series expression profile analysis of fracture healing in young and old mice. Mol Med Rep 2017;16:4529–36.
32. Komori T, Pham H, Jani P, et al. The role of type VI collagen in alveolar bone. Int J Mol Sci 2022;23:14347.
33. Allen J M, Zamurs L, Brachvogel B, et al. Mice lacking the extracellular matrix protein WARP develop normally but have compromised peripheral nerve structure and function. J Biol Chem 2009;284:12020–30.
34. Takahashi N, Matsuda Y, Sato K, et al. Neuronal TRPV1 activation regulates alveolar bone resorption by suppressing osteoclastogenesis via CGRP. Sci Rep 2016;6:29294.
35. Siddiqui Y D, Nie X, Wang S, et al. Substance P aggravates ligature-induced periodontitis in mice. Front Immunol 2023;14:1099017.
36. Luo J, Chen H, Wang G, et al. CGRP-loaded porous microspheres protect BMSCs for alveolar bone regeneration in the periodontitis microenvironment. Adv Healthc Mater 2023;12:e2301366.
37. Holzmann B. Modulation of immune responses by the neuropeptide CGRP. Amino Acids 2013;45:1–7.
38. Guo Y, Chen H, Jiang Y, et al. CGRP regulates the dysfunction of peri-implant angiogenesis and osseointegration in streptozotocin-induced diabetic rats. Bone 2020;139:115464.
39. Gaspersic R, Kovacic U, Cör A, Skaleric U. Unilateral ligature-induced periodontitis influences the expression of neuropeptides in the ipsilateral and contralateral trigeminal ganglion in rats. Arch Oral Biol 2008;53:659–65.
40. Yu X, Gong Z, Lin Q, Wang W, Liu S, Li S. Denervation effectively aggravates rat experimental periodontitis. J Periodontal Res 2017;52:1011–20.
41. Bootcov M R, Bauskin A R, Valenzuela S M, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proc Natl Acad Sci 1997;94:11514–9.
42. Tsai V W W, Husaini Y, Sainsbury A, Brown D A, Breit S N. The MIC-1/GDF15-GFRAL pathway in energy homeostasis: implications for obesity, cachexia, and other associated diseases. Cell Metab 2018;28:353–68.
43. Vedin O, Hagström E, Östlund O, et al. Associations between tooth loss and prognostic biomarkers and the risk for cardiovascular events in patients with stable coronary heart disease. Int J Cardiol 2017;245:271–6.
44. Welsh J B, Sapinoso L M, Kern S G, et al. Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. Proc Natl Acad Sci 2003;100:3410–5.
45. Zhang M, Chen X, Chen H, et al. Identification and validation of potential novel biomarkers for oral squamous cell carcinoma. Bioengineered 2021;12:8845–62.
46. Isola G, Tartaglia G M, Santonocito S, Chaurasia A, Marya A, Lo Giudice A. Growth differentiation factor-15 and circulating biomarkers as predictors of periodontal treatment effects in patients with periodontitis: a randomized-controlled clinical trial. BMC Oral Health 2023;23:582.
47. Stemmler A, Symmank J, Steinmetz J, von Brandenstein K, Hennig CL. Jacobs C GDF15 supports the inflammatory response of PdL fibroblasts stimulated by P. gingivalis LPS and concurrent compression. Int J Mol Sci 2021;22:13608.
48. Li S, Li Q, Zhu Y, Hu W. GDF15 induced by compressive force contributes to osteoclast differentiation in human periodontal ligament cells. Exp Cell Res 2020;387:111745.
49. Zhang Y, Zhang G, Liu Y, et al. GDF15 regulates Malat-1 circular RNA and inactivates NFκB signaling leading to immune tolerogenic DCs for preventing alloimmune rejection in heart transplantation. Front Immunol 2018;9:2407.
50. Valiño-Rivas L, Cuarental L, Ceballos M I, et al. Growth differentiation factor-15 preserves Klotho expression in acute kidney injury and kidney fibrosis. Kidney Int 2022;101:1200–15.