EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

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MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

Tooth decay: how genetics and epigenetics could pave the way for a vaccine revolution

Trabalho submetido por

Ines Bouaita

para a obtenção do grau de Mestre em Medicina Dentária

Junho 2025

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Trabalho orientado por

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RESUMO

Introdução: Durante muito tempo, a cárie dentária foi considerada uma doença associada sobretudo a comportamentos individuais e a fatores ambientais modificáveis, como a má higiene oral, o consumo excessivo de açúcares fermentáveis e a exposição insuficiente ao flúor. No entanto, estudos recentes revelam uma realidade muito mais complexa, na qual fatores genéticos, epigenéticos e microbiológicos interagem com determinantes sociais, comportamentais e biológicos, moldando de forma dinâmica a suscetibilidade individual à doença. **Desenvolvimento:** Polimorfismos em genes envolvidos na formação do esmalte dentário (AMELX, ENAM, TUFT1), na função salivar (AQP5, MUC7) e na resposta imunitária local (DEFB1, TLR2) foram associados a uma menor resistência à colonização por *Streptococcus* mutans *(S. mutans),* principal agente etiológico da cárie. Além disso, variantes em genes ligados à perceção do paladar (TAS1R2, TAS1R3, GNAT3) podem aumentar a sensibilidade ao sabor doce e favorecer preferências por alimentos açucarados, intensificando os riscos comportamentais e metabólicos. Contudo, a predisposição genética não basta para explicar o desenvolvimento da doença. Fatores ambientais precoces como desigualdades socioeconómicas, dieta inadequada, baixa ingestão de flúor, tipo de parto, alimentação infantil, uso de antibióticos ou exposição passiva ao tabaco podem modular o risco, muitas vezes através de mecanismos epigenéticos, como a metilação do ADN ou a regulação por microARN. Importa ainda sublinhar que os efeitos genéticos podem variar consoante o sexo e a morfologia dentária, influenciando não só a suscetibilidade, mas também a intensidade e a natureza das respostas imunitárias. **Conclusões:** Neste contexto multifatorial, marcado por vulnerabilidades biológicas herdadas e moduladas pelo ambiente, a vacinação contra *S. mutans* surge como uma via inovadora e promissora. Dados pré-clínicos apoiam o potencial de plataformas recombinantes, baseadas em ADN, proteínas ou plantas, embora persistam desafios ligados à imunidade mucosa, à eficácia e à viabilidade económica.

Palavras-chave: “Cárie dentaria” ; “Vacina anticárie” ; “Fatores genéticos da cárie” ; “Epigenetica”

ABSTRACT

Introduction: Dental caries has long been considered as a disease primarily linked to individual behaviours and modifiable environmental factors, such as poor oral hygiene, excessive sugar consumption or insufficient exposure to fluoride. However, recent research reveals a far more complex reality, in which genetic, epigenetic and microbiological factors interact with social, behavioural and biological determinants to modulate individual susceptibility over time. **Development:** Polymorphisms in genes involved in enamel development (AMELX, ENAM, TUFT1), salivary function (AQP5, MUC7) or immune response (DEFB1, TLR2) have been associated with reduced resistance to cariogenic bacteria such as *Streptococcus* mutans *(S. mutans).* In addition, variants in genes related to taste perception (TAS1R2, TAS1R3, GNAT3) may increase sensitivity to sweet tastes and reinforce sweet food preferences, thereby amplifying behavioural risk factors. It is also important to note that genetic effects may vary depending on gender and dental topography, influencing not only susceptibility to caries but also the strength and nature of immune responses. Nevertheless, genetic predisposition alone is not sufficient to explain the development of the disease. Early environmental exposures such as socioeconomic inequality, unbalanced diet, low fluoride availability, mode of delivery, infant feeding practices, antibiotic use or tobacco smoke can significantly influence risk via epigenetic mechanisms such as DNA methylation and microRNA-mediated regulation of gene expression. **Conclusions:** In this multifactorial context, shaped by both inherited and modifiable biological vulnerabilities, vaccination against *S. mutans* appears to be a promising complementary strategy. Preclinical data support the potential of recombinant, DNA-based and plant-derived platforms. However, important challenges remain, particularly regarding mucosal immunity, long-term safety, population-wide feasibility, and the integration of such vaccines into personalised preventive approaches.

Keywords: “Dental caries” ; “Anticaries vaccine” ; “Genetic factors of caries” ; “Epigenetic”

RÉSUMÉ

Introduction: La carie dentaire a longtemps été perçue comme une maladie principalement liée aux comportements individuels et à des facteurs environnementaux modifiables, tels qu'une mauvaise hygiène bucco-dentaire, une consommation excessive de sucre ou une exposition insuffisante au fluor. Toutefois, des recherches récentes révèlent une réalité bien plus complexe, dans laquelle des facteurs génétiques, épigénétiques et microbiologiques interagissent avec des déterminants sociaux, comportementaux et biologiques pour moduler la susceptibilité individuelle. **Développement:** Des polymorphismes dans des gènes impliqués dans le développement de l'émail (AMELX, ENAM, TUFT1), la fonction salivaire (AQP5, MUC7) ou la réponse immunitaire (DEFB1, TLR2) ont été associés à une résistance réduite aux bactéries cariogènes telles que *Streptococcus mutans (S. mutans)*. De plus, des variants de gènes liés à la perception du goût (TAS1R2, TAS1R3, GNAT3) peuvent accentuer la sensibilité au goût sucré et renforcer les préférences alimentaires sucrées, amplifiant ainsi les risques comportementaux associés. Il convient également de souligner que les effets génétiques peuvent varier selon le sexe et la topographie dentaire, influençant à la fois la susceptibilité à la carie et l'intensité des réponses immunitaires. Cependant, la prédisposition génétique ne suffit pas à expliquer l'apparition de la maladie. Des facteurs environnementaux précoces (inégalités socio-économiques, alimentation déséquilibrée, faible exposition au fluor, mode d'accouchement, alimentation infantile, usage d'antibiotiques ou exposition à la fumée de tabac) peuvent moduler ce risque via des mécanismes épigénétiques, tels que la méthylation de l'ADN et la régulation par microARN. **Conclusions:** Dans ce contexte multifactoriel, marqué par des vulnérabilités biologiques héritées et modulées, la vaccination contre *S. mutans* apparaît comme une voie complémentaire prometteuse. Des données précliniques soutiennent le potentiel des plateformes recombinantes, bien que subsistent des défis liés à l'immunité muqueuse, à la sécurité et à l'applicabilité populationnelle.

Mots-clés: "Carie dentaire" ; "Vaccin anticaries" ; "Facteurs génétiques de la carie" ; "Épigénétique"

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ABBREVIATIONS

3'UTR - 3' Untranslated Region

ABC - ATP-Binding Cassette

ATP - Adenosine Triphosphate

BPA - Bisphenol A

CAIV - Cold-Adapted Influenza Vaccine

CD4+ - T helper cells

CMV – Cytomegalovirus

COVID-19 - Coronavirus Disease 2019

DMFT - Decayed, Missing, and Filled Teeth

DMRs - Differential Methylation Regions

DNA - Deoxyribonucleic Acid

DZ – Dizygotic

ECC - Early Childhood caries

EMT – Epithelial-Mesenchymal Transition

Fabs - Fragment antigen-binding regions of antibodies

FDA – Food and Drug Administration

FDI – World Dental Federation

GALT - Gut-Associated Lymphoid Tissue

GBP - Glucan-binding protein

GBR – Glucan-Binding Region

GlnH Protein – Glutamine-binding periplasmic Protein

GTFs – Glucosyltransferases

GWAS - Genome-Wide Association Study

HATs – Histone Acetyltransferases

HBV – Hepatitis B Virus

HDACs – Histone Deacetylases

HLA – Human Leukocyte Antigen

HOMINGS – Human Oral Microbe Identification using Next Generation Sequencing

HMTs – Histone Methyltransferases

HSPM - Hypomineralisation of the Second Primary Molars

HSV1 - Virus Herpes Simplex 1

IgA / IgG - Immunoglobulin A / G

IL – Interleukin

lncRNAs - Long non-coding RNAs

mRNAs - Messenger RNAs

MHC - Major Histocompatibility Complex

miRNAs - MicroRNAs

micro-CT - micro-Computed Tomography

MREs - MiRNA Response Elements

MZ - Monozygotic

NHANES - National Health and Nutrition Examination Survey

ncRNAs - Non-coding RNAs

NGS - Next-generation sequencing

PAc - Antigen I/II

PCR - Polymerase Chain Reaction

PETS - Peri/postnatal Epigenetic Twin Study

PM2.5 - Particulate Matter <2.5 μm

rPstS - recombinant Phosphate-specific transport system substrate-binding protein

SAM - S-adenosylmethionine

SBR - Saliva-Binding Region

SCCs - Solitary Chemosensory Cells

SIgA - Secretory Immunoglobulin A

SNP - Single Nucleotide Polymorphisms

QALYs - Quality-Adjusted Life Years

TLR - Toll-Like Receptor

TNF-α - Tumour Necrosis Factor alpha

WHO - World Health Organization

ZIF-8 NPs - Zeolite-8 Imidazolate structure Nanoparticles

GLOSSARY

I. Genetics, epigenetics and host susceptibility

DMR (differentially methylated region): Genomic regions with distinct DNA methylation profiles according to tissue, individual or environmental context, often associated with the regulation of gene expression.

Genetic polymorphism: The presence of two or more variants (alleles) at a specific DNA locus in a population. These variations can influence characteristics such as enamel structure, salivary composition, or immune function, affecting an individual's susceptibility to dental caries.

Phenotypic expression VS genetic predisposition: Genetic predisposition refers to the inherited susceptibility to a trait or disease, while phenotypic expression is the observable manifestation shaped by environmental, epigenetic and behavioural factors.

PRC2 (Polycomb repressive complex 2): A chromatin-modifying complex that catalyses trimethylation of histone H3 at lysine 27 (H3K27me3), leading to transcriptional repression of target genes.

SWI/SNF: ATP-dependent chromatin remodelling complex that modifies nucleosome positioning to regulate DNA accessibility and transcriptional activity.

TET enzymes (Ten-Eleven translocation proteins): Family of dioxygenases that actively demethylate DNA by converting 5-methylcytosine into 5-hydroxymethylcytosine, which is crucial for epigenetic reprogramming.

Transcription factor occupancy theory: This theory suggests that the binding of a transcription factor to a promoter or enhancer can protect these sites from DNA methylation or expose them, thereby influencing gene regulation.

Xist (X-inactive specific transcript): A long non-coding RNA that spans one of the X chromosomes in female mammals and recruits PRC2 to trigger chromosome-wide gene silencing

II. Research into the microbiome and host-environment interaction

Alpha microbial diversity: Measure of species richness and homogeneity within a microbial community, reflecting the ecological complexity of environments such as the oral cavity.

Genetic predisposition and phenotypic expression: The contrast between an individual's genetic risk and the actual occurrence of a disease, which depends on exposure to the environment and gene-environment interactions.

HOMINGS technology (Human Oral Microbe Identification using Next Generation Sequencing): High-throughput sequencing method using species-specific probes to identify and quantify the bacteria of the oral microbiome.

Pyrosequencing: DNA sequencing method that detects the release of pyrophosphate during nucleotide incorporation, allowing quantitative analysis of short regions of DNA.

III. Genetics, immunology and biotechnology

ABC transporter systems: ATP-binding cassette transporters in bacteria such as *Streptococcus mutans*, which ensure nutrient uptake and toxin efflux, thereby contributing to virulence.

Active immunisation: Stimulation of the immune system by the introduction of an antigen (e.g. by vaccination) to induce long-term memory and protection.

BALB/c mouse: Inbred mouse strain frequently used in immunological studies because of its well-characterised Th2 immune response.

Dual promoter system (nirB + CMV): A construct combining a bacterial promoter (nirB) and a mammalian promoter (CMV) to enhance gene expression in bacterial vectors and host cells.

LTK63/LTK4R: Non-toxic heat-labile E. coli enterotoxin derivatives used as adjuvants in mucosal vaccines to boost local immune responses.

Passive immunisation: The administration of pre-formulated antibodies to provide immediate, short-term protection without the need to activate the host's immune system.

Phage Display: Molecular technique using bacteriophages to display peptides or antibody fragments on their surface in order to identify high-affinity ligands.

QALY (quality-adjusted life year): A measure of the overall burden of disease, combining life expectancy and quality of life, used in health economics to evaluate medical interventions.

Secretory immunoglobulin A (SIgA): Antibody predominant in mucosal secretions (e.g. saliva), essential for neutralising pathogens and maintaining oral homeostasis.

Vertical/horizontal transmission of *Streptococcus mutans*: Vertical transmission refers to the transfer of *S. mutans* from mother to child, whereas horizontal transmission occurs between individuals (e.g. siblings and carers).

I) INTRODUCTION

Dental caries is one of the most widespread diseases worldwide, affecting people of all ages[1,2]. It represents a significant public health concern with notable functional, social, and economic implications. Long seen as simply the result of poor hygiene or excessive sugar consumption, dental caries is now recognised as a multifactorial disease resulting from complex interactions between behavioural, environmental, microbiological, genetic, and epigenetic factors. This complexity explains why traditional prevention campaigns often have limited long-term effectiveness, highlighting the need for a more comprehensive and personalised approach[2].

Biologically, dental caries develops from the formation of bacterial biofilm on tooth surfaces, especially in the regular presence of fermentable sugars. This biofilm produces organic acids that lower the pH, of the mouth, gradually leading to the demineralisation of the enamel and then the dentine[2,3].The frequency of sugar consumption, the quality of saliva and the ability of the local immune system to defend itself strongly influence this process. This explains why some people develop dental caries quickly while others do not, even with similar habits. Differences in diet, the composition of the oral microbiota or the resistance of the body play a crucial role in this fragile balance[2].

Part of this variability between individuals can be explained by genetics. Some people are naturally more susceptible to dental caries due to variations in their genes, which influence tooth quality, saliva composition, taste perception or the oral immune response. Slight differences in the expression or regulation of specific genes can be enough to make someone more vulnerable to acid attacks or bacterial colonisation[4,5,6]. However, these genetic factors do not work alone: they constantly interact with the environment.

In this context, epigenetics emerges as a pivotal field of study. This phenomenon enables us to comprehend how the environment can modulate the expression of specific genes without altering the deoxyribonucleic (DNA) sequence itself. A multitude of factors influence these epigenetic mechanisms, which in turn modify gene activity. Such factors include diet, stress, toxins and the microbiota. Recent studies have identified certain epigenetic signatures associated with increased vulnerability to dental caries. The potential for these markers to facilitate early identification of at-risk children is a

promising avenue for future research. It can thus be concluded that epigenetics establishes a correlation between an individual's genetic makeup and the environmental factors that shape their existence[7,8].

All these discoveries are paving the way for a new approach to prevention and treatment: personalised dentistry[8]. We are no longer limited to general advice on brushing or reducing sugar intake. We can now anticipate a patient's vulnerability by considering their biological profile and lifestyle. Targeted strategies can be put in place, such as adapting diet, stimulating saliva production or strengthening local immune defences[9]. Among the emerging therapeutic strategies under investigation, the development of anti-caries vaccines stands out as an auspicious promising and actively explored approach[10]. By targeting the bacteria responsible for the disease, these vaccines could prevent *Streptococcus mutans* from adhering to teeth or reduce its virulence, providing long-lasting protection, particularly for the most exposed populations[11,12,13,14].

In short, dental caries should no longer be seen as a simple consequence of poor habits or excessive sugar consumption. It is the result of a continuous interplay between genetic predisposition, environmental exposures, and epigenetic regulation that orchestrate their interaction. It is in this complexity that we find the keys to better understanding, preventing and treating this ancient yet ever-present disease. By embracing this more integrated vision, dentistry is progressively embracing a more preventive and personalised paradigm informed by emerging molecular evidence.

Objectives: In this context**,** the main objective of this narrative review is to summarise the available scientific data on the genetic and epigenetic factors associated with individual susceptibility to dental caries. Considering recent advances in molecular biology, genomics, and epigenetics, this work aims to clarify the mechanisms through which these hereditary factors interact with environmental exposures particularly during critical developmental periods such as the prenatal, perinatal, and early childhood phases. Special emphasis is placed on the role of epigenetic mechanisms, including DNA methylation, microRNAs, and histone modifications, in modulating this susceptibility in response to factors such as nutrition, antibiotic use, or maternal smoking. By synthesising these findings, this review explores how such a modifiable yet inherited vulnerability could inform the development of more targeted preventive approaches. Particular

attention is given to the prospects of anti-caries vaccines, especially those based on recombinant platforms, as a complementary tool for protecting high-risk population.

II) DEVELOPMENT

In order to consider in detail these interdependent dimensions, it is first necessary to clarify the methodological approach used in this narrative analysis. This literature review was developed as part of a final-year project in accordance with the official regulations of the Bachelor's and Master's programmes at the Instituto Universitário Egas Moniz. The methodology used in this narrative review was based on an extensive search of major scientific databases, including PubMed, Google Scholar and Embase. These platforms were selected for their broad coverage of scientific publications in the medical and dental fields, allowing access to relevant studies on dental caries, oral microbiology, associated genetic and epigenetic factors, and advances in vaccination.

Searches were conducted out using specific keywords related to the topic, such as “dental caries”**,** “anticaries vaccine”**,** “genetic factors of caries”**,** and “epigenetic”. Priority was given to recent publications, particularly those from the last five to ten years, to ensure the information was up to date and reflected current developments in the field. However, earlier studies were also considered when they provided essential historical context or were referenced by more recent research.

The selection process followed defined inclusion criteria: articles had to be published in peer-reviewed journals, in English, French or Portuguese, and provide full access to results, data, and conclusions. This approach ensured scientific rigour and the relevance of the data analysed. Non-scientific content, non-peer-reviewed publications, and studies deemed outdated were systematically excluded.

The analysis of selected publications focused particularly on those exploring the potential of a vaccine against dental caries. Studies targeting microbial agents such as *Streptococcus mutans* were considered especially relevant. Special attention was given to identifying innovative immunisation approaches with the potential to contribute to a paradigm shift in dental caries prevention.

1. Genes Involved In Oral Health

Genetics is the science that studies the organisation, functioning, regulation and modification of genes, whether by their transmission from one generation to another or through their expression in the same individual. The objective is to understand how this information influences our appearance, our health and even some of our behaviours. Every human being has a unique genetic heritage encoded in a molecule called DNA. This DNA is present in all our cells and functions as a set of instructions: it contains instructions organised into small segments called genes. These genes are responsible for the production of proteins, which are essential for the proper functioning of our bodies (Figs.1-2)[15].

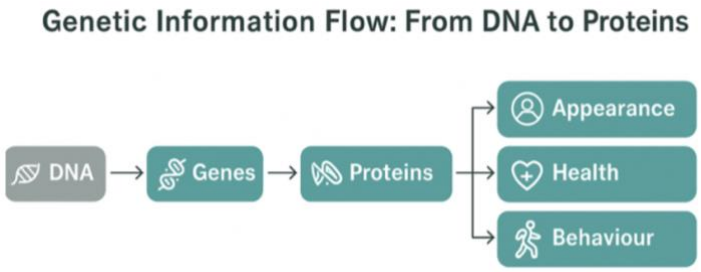

Figure 1: Conceptual diagram of genetics. Developed using Napkin software.

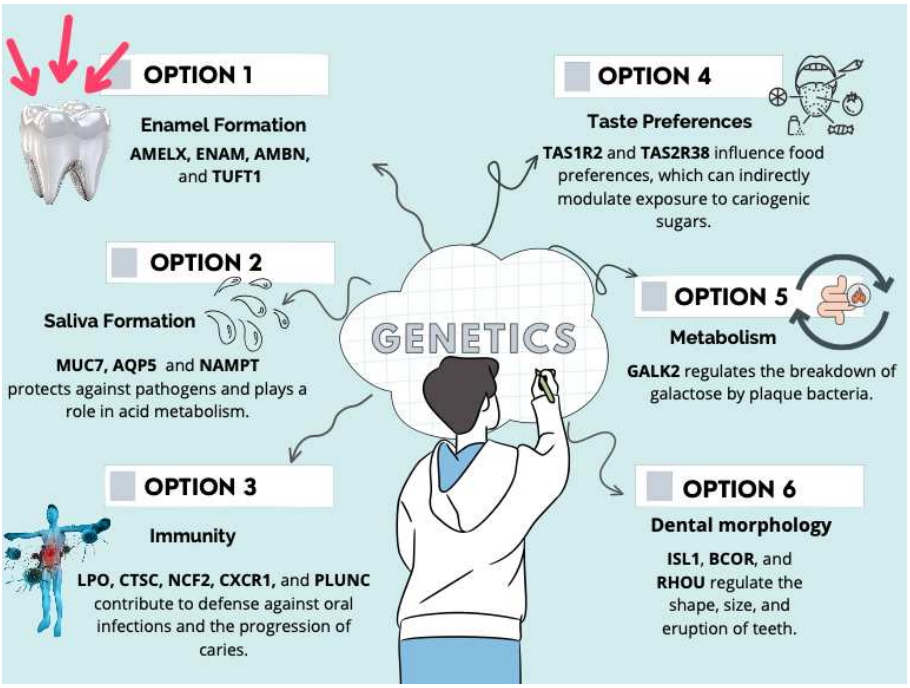

Figure 2: Genetic factors involved in dental caries susceptibility: from enamel formation to taste preferences[4,5]. Figure developed by Ines Bouaita using Canva software.

1.1 Genes Involved In Tooth Development Of Dental Hard Tissues

Tooth development is a step-by-step process that begins early in life and involves the interaction between two main types of tissues: the oral epithelium and the underlying neural crest-derived mesenchyme. It progresses through distinct stages, initiation, bud, cap, bell, and apposition, each regulated by complex genetic and molecular signals. As the tooth forms, specialised cell types emerge: ameloblasts, responsible for enamel formation, and odontoblasts, which form the underlying dentin layer. Enamel is produced during the apposition and maturation phases, after dentinogenesis has begun, reflecting the tightly coordinated and sequential nature of dental tissue development[16,17].

Once formed, tooth enamel serves as the primary barrier against acid erosion and bacterial invasion. Its structural integrity is essential to protect the underlying dentin and pulp tissues. The formation and mineralisation of enamel rely on a complex network of genes that regulate the synthesis, organisation and degradation of proteins within the enamel matrix. Disruptions in these genes can lead to defective enamel, reduced resistance to acid attack, and ultimately an increased risk of dental caries[16,17].

Among the most critical genes involved are those encoding proteins secreted by ameloblasts, the specialised cells responsible for enamel formation[16,17].

- AMELX (Amelogenin X-linked) encodes amelogenin, a key structural protein of the enamel matrix. Mutations in AMELX can lead to enamel hypoplasia, resulting in thin, porous enamel that is more susceptible to demineralisation[4,5,7,18,19,20,21].
- ENAM (Enamelin) codes for enamelin, another essential protein that contributes to enamel thickness and stabilises the matrix during the early stages of mineralisation. Altered ENAM expression has been associated with impaired enamel formation and defective repair following acid exposure[4,5,7,18,19,20,21,22].
- AMBN (Ameloblastin) plays a comparable role in matrix organisation and in the adhesion of ameloblasts. Defects in this gene may compromise enamel integrity[4,5,18,19,20,21,22].
- TUFT1 (Tuftelin 1) is also implicated in the initiation of mineralisation. Genetic variants have been associated with increased susceptibility to dental caries, especially in children, indicating possible gene–environment interactions[4,5,18,19,20,21,22].

Additional proteins and enzymes are involved in the maturation phase of enamel development.

- TFIP11 (Tuftelin Interacting Protein 11) and KLK4 (Kallikrein-Related Peptidase 4) contribute to the removal of residual organic matrix components, a process essential for final enamel hardening[4,7,19,20,21,22].
- KLK4 acts in complement to MMP20 (Matrix Metallopeptidase 20), an enzyme that degrades excess matrix proteins during enamel development. If MMP20 is deficient or defective, enamel remains improperly mineralised and porous[4,7,19,20,21,22,23].
- Other metalloproteinases, such as MMP13 and MMP16, may participate in general extracellular matrix remodelling. Their abnormal expression can further compromise enamel structure and mechanical resilience[4,19,23].

Complementary protective roles are performed by other genes that support enamel development through signalling or transport functions:

- PKD2 (Polycystin-2) is involved in intracellular signalling and matrix–cell interactions during tooth development[4].
- ABCG2 (ATP-Binding Cassette Subfamily G Member 2) may contribute to the transport and clearance of toxic substances from the enamel organ, thereby maintaining the homeostasis of the developing enamel[4].

Beyond enamel-specific mechanisms, other genetic factors contribute to the integrity of the entire dental organ. Genes involved in dentin development can indirectly affect enamel resistance by altering tooth architecture and mechanical support. The SCPP (Secretory Calcium-Binding Phosphoprotein) gene family is plays a central role in the mineralisation of both enamel and dentin, contributing to the mechanical strength and chemical stability of dental tissues. Although dentin is less mineralised than enamel, it provides a crucial supportive role[4].

- DSPP (Dentine Sialophosphoprotein) encodes a major non-collagenous protein of the dentin matrix and is essential for proper dentin mineralisation. Mutations in DSPP are associated with dentinogenesis imperfecta, leading to structural

weaknesses that may indirectly compromise enamel by undermining overall tooth stability, and increasing the risk of caries progression[21,24,25,26].

In addition to matrix proteins and enzymes directly involved in mineralisation**,** regulatory genes also influence the cellular processes that underpin tooth formation.

- ESRRB (Estrogen-Related Receptor Beta) has been implicated in the regulation of ameloblast differentiation and enamel maturation. Although some studies suggest a potential involvement of ESRRB in shaping enamel structure and influencing caries susceptibility, findings remain inconclusive, highlighting the need for further investigation[20,21,22,24].
- NEDD9 (Neural Precursor Cell Expressed, Developmentally Downregulated 9) may indirectly affect tooth development by modulating the migration of cranial neural crest cells, the embryonic precursors of odontoblasts and ameloblasts[4].

Together, these genetic factors illustrate the complexity of dental hard tissue formation, in which enamel and dentin development are intricately coordinated. Genetic variations affecting any stage of these pathways may compromise tooth resilience and elevate the risk of demineralisation and dental caries.

1.2 Genes Involved In Saliva Composition And Function

Saliva is a complex biological fluid composed mainly of water (≈99%), along with electrolytes (such as bicarbonate, calcium and phosphate), proteins and enzymes (including mucins, amylase, lysozyme, lactoferrin), antimicrobial peptides, and antioxidants. This unique composition allows saliva to fulfil several essential functions in oral health[2,5].

Each of these functions relies on specific components of the salivary fluid, whose production and activity are under genetic control. Disruption of the genes encoding these components may compromise oral homeostasis and increase vulnerability to dental caries and other oral diseases.

<u>Regulation of pH and neutralisation of acids</u>

- Components involved: bicarbonate ions and the enzyme carbonic anhydrase VI.
- Function: maintains a stable salivary pH and limits oral acidity.

- Associated gene:
    - CA6 encodes carbonic anhydrase VI, which converts CO_2 into bicarbonate. Variations in this gene can reduce the buffering capacity of saliva and promote demineralisation[4,5,21,28].

Remineralisation of enamel and formation of the pellicle

- Components involved: Calcium, phosphate and proline-rich proteins.
- Function: Strengthen enamel and prevent bacterial adhesion.
- Associated genes:
    - PRH1 and PRH2 code for salivary proteins integrated into the acquired pellicle. They promote remineralisation and form a protective barrier against bacteria[4,5,7,21].

Lubrication and protection of mucous membranes

- Components involved: Mucins, in particular MUC7
- Function: Hydrate oral tissues and facilitate movement and protect the mucosa against friction and microorganisms.
- Associated gene:
    - MUC7 codes for a mucin that prevents bacteria from adhering to oral surfaces. Low expression promotes the formation of dental plaque[4,21,29].

Antimicrobial defence and protection against oxidative stress

- Components involved: Lysozyme, lactoferrin, antimicrobial peptides and nicotinamide.
- Function: Limit bacterial growth and protect tissues.
- Associated gene:
    - NAMPT produces nicotinamide, an antioxidant that protects against bacterial stress. Its deficiency weakens local defences[4,29].

Mucosal integrity and epithelial repair

- Component involved: Trefoil factor 2 (TFF2).
- Function: Strengthen the mucosal barrier and support wound healing.
- Associated gene:
    - TFF2 is involved in the formation of the mucosal protective film. Alteration of the gene increases sensitivity to irritation[27].

Salivary hydration and volume

- Component involved: Aquaporin 5.
- Function: Ensure sufficient salivary flow to clean the mouth.
- Associated gene:
    - AQP5 codes for a channel that allows the transport of water in the salivary glands. Mutations can cause dry mouth and reduce the elimination of bacteria[4,21,28,29].

1.3 Genes Involved In Oral Immune Response

The oral cavity is constantly exposed to microbial challenges, making the immune system a key player in maintaining oral health. Local immune defences, supported by both innate and adaptive mechanisms, are finely regulated to protect the tissues without triggering excessive inflammation. Salivary components such as antimicrobial peptides, chemokines, and cytokines contribute to the first line of defence, while immune cells and receptors ensure effective microbial recognition and clearance.
The genes involved in these responses encode proteins that act in the saliva, mucosa or immune cells. Genetic variations affecting these genes can weaken host defences, favour bacterial overgrowth, and increase the risk of dental caries and oral infections[4,29].

Antimicrobial defence in saliva

- Components involved: Lactoperoxidase (LPO), β-defensin 1 (DEFB1), Lactoferrin (LTF).
- Function: Destroy or inhibit oral pathogens
- Associated genes:
    - LPO catalyses the production of peroxides that damage bacterial membranes. Mutations reduce antimicrobial activity[4,29].
    - DEFB1 encodes a peptide with antibacterial, antiviral and anti-inflammatory actions. Underexpression is associated with increased caries risk[4,21,29].
    - LTF sequesters iron and regulates both innate and adaptative responses. Certain variants are linked to higher susceptibility to caries[4,5,20,21,29].

Control of inflammation and microbial recognition

- Components involved: Cytokines, TLR4 receptor.

- Function: Detect pathogens and regulate inflammation.
- Associated genes:
    - TLR4 identifies *S. mutans* and triggers immune response. Defects in this gene delay pathogen detection and favour dysbiosis[21].
    - IL-1 (Interleukin-1) promotes inflammation. Certain polymorphisms increase risk of periodontal inflammation and root caries[4,21].

Neutrophil recruitment and activation

- Components involved: Chemokine receptors CXCR1, CXCR2, CTSC, NCF2
- Function: Guide and activate neutrophils to sites of infection
- Associated genes:
    - CXCR1/CXCR2 guide neutrophils. Mutations can impair immune cell recruitment[4].
    - CTSC activates enzymes that degrade microbes. Deficiencies reduce pathogen destruction[4].
    - NCF2 helps produce reactive oxygen species. Anomalies limit microbial killing[4].

Prevention of bacterial adhesion

- Components involved: PRH1, PRH2.
- Function: Block *S. mutans* from binding to enamel.
- Associated genes:
    - PRH1 / PRH2 encode salivary proteins that occupy adhesion sites. Variants such as Db increase risk of caries[4,5,7,29].

Mucosal immunity and antigen presentation

- Components involved: HLA-DRB1, HLA-DQB1 (MHC class II).
- Function: Present microbial antigens to immune cells.
- Associated genes:
    - HLA-DRB1 variants (e.g. DR3, DR4) associated with higher colonisation by *S. mutans*[4,5,7,21,29].
    - HLA-DQB1 may have protective effects in some variants[4,5,7,21,29].

Complement system activation

- Components involved: MBL2, MASP2.

- Function: Recognise pathogens and trigger lysis.
- Associated genes:
    - MBL2 / MASP2 encode components of the lectin pathway. Mutations reduce effectiveness of microbial clearance[4,5,7,21,29,30].

Auxiliary defence and mucosal repair

- Components involved: PLUNC, LYZL2, ALOX15.
- Function: Reinforce epithelial barriers and support antimicrobial action.
- Associated genes:
    - PLUNC slows growth of oral pathogens[4,5].
    - LYZL2 works with LPO and SLPI to degrade bacterial walls. Mutations compromise this synergy[4,5].
    - ALOX15 involved in lipid mediators of inflammation. Its role in oral immunity still under investigation[22,31].

1.4 Genes Related To Taste Preferences And Their Impact On Oral Health

The perception of flavours (sweet, bitter, salty, sour, and umami) plays a fundamental role in how we choose and enjoy our food. These preferences, shaped by physiological, cultural, and economic factors, are also partially influenced by genetic predisposition. Historically, humans have naturally developed a preference for sweet tastes, as sweet foods were once rare and rich in energy. Today, with the widespread availability of these foods, this attraction to sweetness can quickly pose problems for our oral health[32].

Conversely, some people, depending on their genetic heritage, are more sensitive to bitter tastes. Research has shown that genetic variations can make certain flavours more or less appetising, thus affecting our food preferences[33,34].

Two large families of receptors are involved in flavour detection: the T1R (Taste receptor 1) family, associated with sweet and umami tastes, and the T2R (Taste receptor 2) family, involved in the perception of bitter tastes. The T1R family includes three receptors: T1R1, T1R2, and T1R3 (Taste receptor 3), which combine in heterodimers to detect different molecules. The T1R2–T1R3 complex is specifically responsible for the perception of sweet taste, while T1R1–T1R3 is sensitive to umami taste[29]. The TAS1R2 (Taste receptor

type 1 member 2) gene, which codes for one of the subunits of the sweet taste receptor, is associated with varying degrees of sensitivity to this flavour. When a person has a more sensitive version of this gene, they may perceive sweet foods more intensely, which may enhance their preference for sugar-rich foods[4,5,29,33,34,35].

The TAS2R38 gene plays a crucial role in detecting bitter compounds. It codes for a specific receptor of the T2R family, expressed in the taste cells of the tongue. This receptor enables the perception of bitterness in certain foods, such as cruciferous vegetables (broccoli, cabbage, and turnips), coffee, and certain medications. Two main versions of this gene exist in the human population: the PAV (Proline-Alanine-Valine) allele, which codes for a highly sensitive receptor, and the AVI (Alanine-Valine-Isoleucine) allele, which is much less functional. Individuals carrying two PAV alleles (PAV/PAV genotype) are called “tasters” and experience bitterness very intensely. Conversely, AVI/AVI homozygous individuals, known as “non-tasters”, perceive this flavour very weakly, if at all. This polymorphism not only affects taste perception but also influences eating behaviour: “non-tasters” tend to avoid bitter foods that are beneficial to health, such as certain antioxidant-rich vegetables, and to eat more sweet or fatty foods to compensate for this low taste stimulation[5,29,35,36,37]. This increased preference for sweetness among “non-tasters” was associated with more frequent and higher consumption of simple sugars. A diet rich in fermentable sugars is a well-established risk factor for dental caries. Several studies have shown that children and adults with the AVI/AVI genotype have a higher prevalence of caries than “tasters” due to their more cariogenic eating habits[36,37]. Conversely, homozygous carriers of the PAV allele (known as “tasters” or PAV/PAV) show increased sensitivity to bitter compounds, particularly to 6-n-propylthiouracil (PROP), a commonly used phenotypic marker (PROP test) for assessing individual differences in bitter taste sensitivity. This taste hypersensitivity leads these individuals to avoid bitter foods but also to limit their consumption of very sweet or fatty products, resulting in a diet that is generally less cariogenic. “Tasters” are thus distinguished by a lower consumption of fermentable sugars, which could explain the lower prevalence of caries observed in this group. In addition, this heightened sensitivity to bitter taste also appears to influence other aspects of eating behaviour, such as the pleasure of eating, the feeling of satiety and the control of food intake, thereby indirectly contributing to this protective effect against the risk of caries[32,38].

Considering this, individuals identified as having “non tasters” (AVI/AVI genotype) may benefit from personalised nutritional advice that considers their genetic taste profile. Practical strategies could include encouraging the use of non-cariogenic sweeteners (such as xylitol), promoting regular exposure to fluoride, increasing consumption of fibre-rich foods to stimulate saliva and implementing early education programmes aimed at moderating sugar consumption. This type of personalised preventive approach can help reduce the impact of genetically determined taste preferences on oral health outcomes.

The GNAT3 (Guanine Nucleotide-binding protein, alpha transducing 3) gene is a central factor in taste physiology. It codes for the α subunit of gustducin, a G protein expressed in specialised taste cells. This subunit plays a crucial role in the transduction of sweet, bitter, and umami signals. It acts downstream of taste receptors, notably TAS1R2 (sweet) and TAS2R38 (bitter), converting the chemical signal into a neuronal response. This mechanism makes it a major candidate for explaining inter-individual variability in taste sensitivity and indirectly in eating behaviour towards sugar. Some studies have shown that polymorphisms in the GNAT3 gene can influence the perception of sweet taste. For example, the single nucleotide polymorphisms (SNPs) rs7792845 and rs6962693 have been associated with an increased preference for very sweet solutions, as well as a higher sugar detection threshold, reflecting lower taste sensitivity[39]. Notably, these same variants were also correlated with a lower prevalence of dental caries, suggesting a potential link between taste signalling, eating behaviour and oral health. The protective effect observed could be explained by either lower actual consumption linked to faster sensory saturation or by immune or salivary interactions modulated by GNAT3.

However, these results have not been systematically reproduced. A study conducted on a large Swedish cohort included GNAT3 in the analysis of 101 SNPs linked to sugar consumption. No significant link was found between GNAT3 variants and actual sugar consumption (whether total, added or sweet tasting), even after sensitivity analyses. These findings diverge substantially from previous studies conducted on smaller cohorts, suggesting either an absolute absence of effect or an influence too modest to be detected on a population scale[4,40]. This discrepancy between measured taste perception (in the laboratory) and actual eating behaviour (assessed by questionnaires or food diaries) is an essential methodological point. Indeed, “taste preference” does not necessarily predict

actual consumption, which is strongly influenced by cognitive, social, economic or environmental factors, which may offset or mask individual biological preference[40].

Thus, although GNAT3 remains a gene of major biological interest, its direct behavioural and clinical impact on sugar intake and the risk of dental caries still needs to be clarified. Future studies integrating physiological (taste sensitivity), behavioural (eating habits), and clinical (caries status) data will be necessary to elucidate its role in oral pathophysiology fully.

In contrast to GNAT3, which acts directly in the transduction of taste signals, the SLC2A2 gene, which codes for the GLUT2 transporter, is involved indirectly in the modulation of food preferences. GLUT2 is expressed in key organs of energy metabolism (liver, pancreas, intestine) but also taste cells. Well-known for its role in regulating blood sugar levels, it may also be involved in post-ingestive glucose detection and modulation of brain reward circuits. Specific polymorphisms, particularly the rs5400 SNP, have been associated with a greater appetite for sweet foods, suggesting a potential link with caries risk via increased sweet eating behaviour[4,33,34,36]. However, in some studies, no significant association was found between SLC2A2 variants and actual sugar consumption (total, added, or sweet-ing)[30,40]. Only an inverse correlation with overall carbohydrate intake was observed for rs5400. This discrepancy between the physiological hypothesis and the behavioural data suggests that GLUT2 may be influenced by still poorly understood metabolic or central mechanisms rather than by a direct action on taste perception.

Another important pathway in the regulation of sweet taste involves the FGF21 gene (Fibroblast Growth Factor 21), a growth factor produced by the liver. Unlike taste genes such as TAS1R2 or GNAT3, FGF21 acts after the sugar has been digested, sending a signal to the brain to reduce the desire to consume sugar again. In a large study of more than 22,000 Swedish adults, certain variants of the gene (rs838145, rs838133 and rs8103840) were associated with higher sugar consumption[40]. These results suggest that FGF21 plays a central role in the control of sweet preferences via metabolic and cerebral mechanisms independently of classic taste receptors. Although its direct link with dental caries remains to be demonstrated, FGF21 is a promising gene to include in models seeking to understand sweet-eating behaviours and their impact on oral health.

Other genes, such as FTO (Fat mass and obesity-associated gene) and MC4R (Melanocortin 4 receptor), modulate our eating behaviour in a more general way. FTO, best known for its link to obesity, is also associated with an increased preference for sweet foods. MC4R regulates appetite, and some of its variants are correlated with a stronger attraction to sugar and fatty foods. These preferences can increase the risk of dental caries, particularly by promoting repeated sugar intake (Table 1)[39,40].

Table 1: Genetic variants in taste perception and their potential impact on dietary habits and oral health[39]. Table developed by Ines Bouaita using Canva Software. The data used to construct this table were extracted from the study by Eriksson et al. (2019)[39], which examines the association between allelic variation in taste genes, dietary preferences, and dental caries". This table presents the key genes involved in taste perception (sweet, bitter, fat) and their impact on dietary behaviours. Correlation indicates the relationship between genetic variations and the consumption of specific types of food. A positive correlation suggests that individuals with the genetic variation tend to consume more of the food (e.g., sweeter foods) than those without. In contrast, a negative correlation means they consume less (e.g., bitterness is less liked). These preferences can have direct implications for oral health, including increased risks of dental issues, such as cavities or dietary imbalances.

GENE	TASTE OR SIGNAL INVOLVED	CORRELATION WITH CONSUMPTION	ASSOCIATED ORAL HEALTH RISKS
TAS1R2	Sweet taste	+0.3 to +0.5	Increased sugar consumption → risk of cavities and obesity.
TAS2R38	Bitter taste	–0.3 to –0.5	Low consumption of bitter vegetables → dietary imbalance, possible reduction in oral protection.
GNAT3	Gustatory signaling	Limited data	Possible alteration in taste perception and salivation, influencing the risk of cavities.
FTO	Appetite, preference for sugar	+0.2 to +0.4	Increased consumption of sugary foods → risk of cavities and overweight.
MC4R	Satiety regulation, fats	+0.3	Increased risk of compulsive eating behaviors, potentially associated with cavities.

In addition, genetic preferences for sweet taste influence not only eating behaviour but also essential biological functions such as the composition of saliva and the oral immune response. Frequent consumption of sugars, favoured by certain polymorphisms such as those in TAS1R2, TAS1R3, or GLUT2, tends to lower the oral pH, creating an acidic environment conducive to the development of cariogenic bacteria. This chronic acidification can alter the composition of the salivary microbiota, reducing beneficial bacterial diversity in favour of pathogenic species, as shown by the study conducted by Cruz de Jesus in 2022[41]. This imbalance, or dysbiosis, compromises not only microbial

stability but also the activity of solitary chemosensory cells (SCCs) located in gingival tissue. These cells, which have taste receptors sensitive to bacterial signals, are usually involved in innate defence by stimulating the release of antimicrobial substances. However, an unbalanced microbiota disrupts this detection, reducing the effectiveness of local immune responses and weakening oral protection mechanisms[41]. This process is exacerbated by interference with other innate immunity pathways, notably via TLR4 and CXCR1 receptors, which are involved in pathogen recognition and immune cell activation. Thus, a diet rich in sugar, influenced by genetic predisposition, is not limited to a direct cariogenic risk but more broadly affects the oral ecosystem and its natural defences[37,41].

After detailing the genes linked to tooth formation, immunity, saliva and taste preference (Fig. 3), it should be noted that other approaches have identified additional, sometimes unexpected, loci associated with susceptibility to dental caries. There are two main methodological approaches to exploring the genetic component of this disease: the candidate gene approach and genome-wide association studies (GWAS genome-wide association study). The first is based on targeted biological hypotheses, testing genes whose function is known, such as ENAM and AQP5, which are involved in amelogenesis and salivary metabolism, respectively. These studies, which are often limited in size, have identified genes relevant to specific biological processes. In contrast, GWAS explore the entire genome without any preconceptions, allowing the construction of polygenic scores that integrate several hundred thousand common genetic variants. A recent study by Fries et al. (2024)[6], based on a polygenic score applied to a Swedish cohort of 15,460 adults, identified ten loci with the strongest predictive contributions. These loci include genes involved in cell signaling (ADCY9 Adenylate Cyclase 9, CHRNA3 Cholinergic Receptor Nicotinic Alpha 3 Subunit), oral microbiota regulation (FUT2 Fucosyltransferase 2), salivary metabolism (CA12 Carbonic Anhydrase 12), and chromatin structure (HIST1H2BE Histone cluster 1 H2B family member E). Other regions involving non-coding or poorly characterised genes also emerged, such as PITX1-AS1 (PITX1 Antisense RNA 1), BAHCC1 (BAH domain and Coiled-coil Containing 1), FAM118A (Family with sequence similarity 118 member A), and C10orf11 (Chromosome 10 open reading frame 11). Although the exact function of some of these genes in the pathogenesis of dental caries remains to be elucidated, these results highlight the complex polygenic architecture of the disease and the value of considering new mechanisms. Still, the type

of method used influences the kind of genes that are detected, how we interpret their role, and what they might mean for future treatments. While GWAS are useful for discovering new genetic signals, they also have important limits. They require large numbers of participants to obtain reliable results and are less effective at detecting rare genetic variants. Their findings can also be affected by differences in ancestry between study groups, which may lead to misleading results. In addition, GWAS usually explain only a small part of the genetic influence on disease, leaving much of the heritability unexplained. Ultimately, these studies reveal statistical correlations, but they do not establish which genes are responsible for the disease. Because of these limitations, GWAS are most helpful when combined with other approaches, such as lab experiments and long-term studies, to better understand the genetic factors behind dental caries[6].

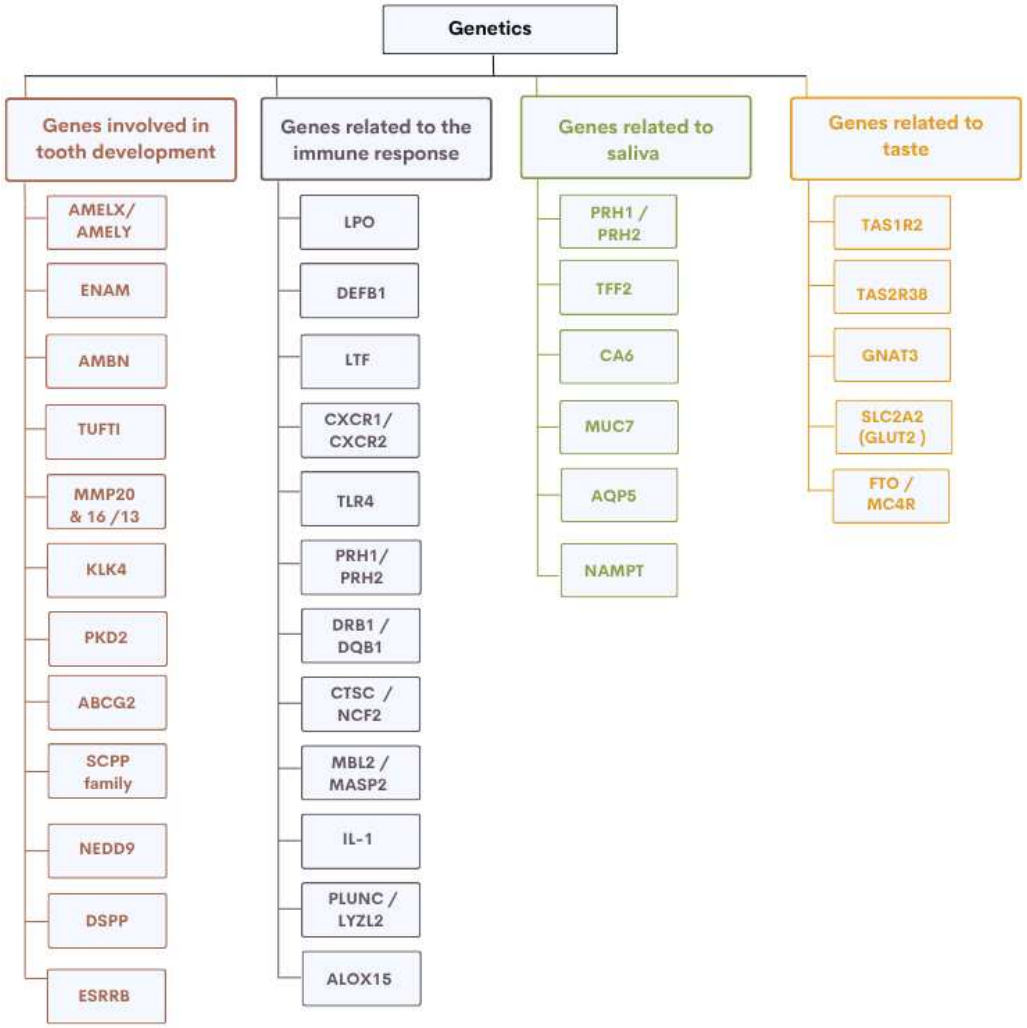

Figure 3: Classification of genes implicated in dental caries according to biological function[4,5]. Figure developed by Ines Bouaita using Canva software.

2. Epigenetics: A Dynamic Modulation

2.1 Definition And Mechanisms

Epigenetics refers to all modifications in gene expression that are not caused by alterations in the DNA sequence. These mechanisms can be influenced by various environmental, nutritional, physiological, or pathological factors. These changes are often reversible but can be mitotically stable and, in some cases, heritable across generations. These modifications enable the dynamic adaptation of cellular responses to the environment by modulating gene activation or repression in response to received signals[7].

DNA methylation is one of the most extensively studied epigenetic mechanisms. It involves the addition of a methyl group (-CH_3) to the cytosine base, mainly in regions rich in CpG dinucleotide (Fig.4). In the human genome, there are regions with a high density of CpG dinucleotides called CpG (Cytosine-phosphate-Guanine) regions, which usually are unmethylated and located near gene promoter regions: approximately 70% of promoters reside in these CpG regions[7]. This modification is catalysed by enzymes known as DNA methyltransferases, particularly DNMT1, DNMT3A, and DNMT3B. When it occurs in gene promoter regions, methylation prevents transcription factors from accessing the DNA, resulting in the inhibition of gene transcription (Fig. 5). Since methylation patterns are dynamic and responsive to stimuli, DNA demethylation can occur passively in dividing cells or actively in non-dividing cells, through the oxidation of 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC) by TET enzymes (Ten-Eleven Translocation enzymes) (Fig.4)[42]. This mechanism plays a crucial role in dental development by regulating the expression of genes involved in enamel formation, cell differentiation, salivary secretion, and local immune response. Excessive or inappropriate methylation can disrupt these processes, increasing susceptibility to dental caries[7,42,43].

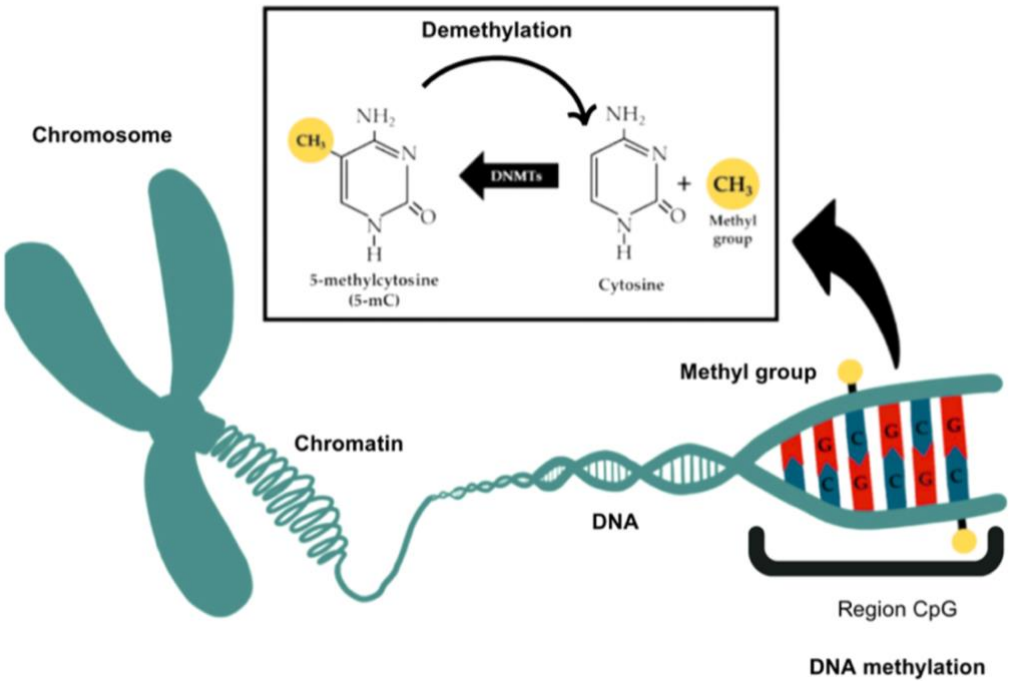

Figure 4: Simplified schematic representation of DNA methylation and demethylation molecular mechanisms, adapted from Valente et al. (2023)[42]. Developed using Canva software.

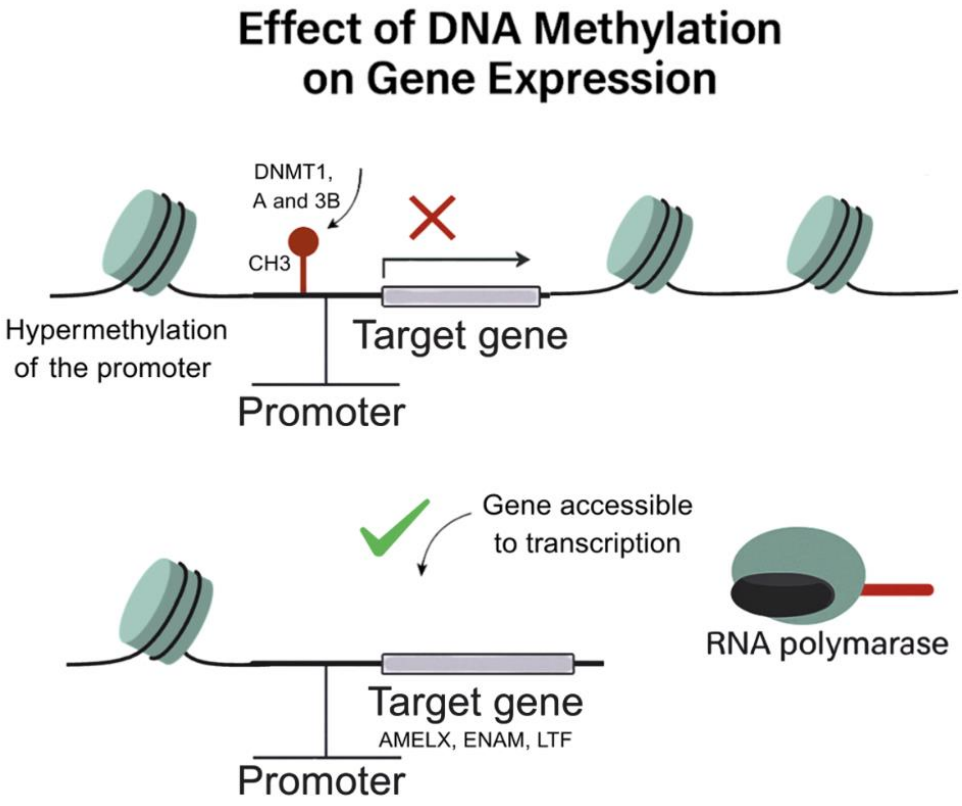

Figure 5: Illustration representing the effect of DNA methylation on gene expression[43]. Figure developed by Ines Bouaita using Canva software.

A study conducted by Silva et al. as part of the Peri/postnatal Epigenetic Twin Study (PETS) cohort[44], which follows pairs of twins from birth, has provided a better understanding of the link between the environment, epigenetics and the risk of dental caries. By comparing genetically identical twins, this approach helps isolate the influence of environmental factors on the differences observed (since genetic bias is taken out of the equation), particularly in terms of DNA methylation. The study revealed that some children with advanced dental caries or hypomineralisation of the second primary molars (HSPM) share specific epigenetic modifications in regions known as differential methylation regions (DMRs). Several genes involved in tooth development and structure show these modifications. The PBX1b (Pre-B-cell leukaemia homeobox 1b) gene, which is expressed very early in tooth formation, shows a different level of methylation in affected children, which could disrupt its role in normal tooth development. The LTBP3 (Latent Transforming Growth Factor Beta Binding Protein 3) gene, which regulates the Transforming Growth Factor Beta (TGF-β) pathway and is already known for its involvement in dental abnormalities such as amelogenesis imperfecta and oligodontia, also shows altered methylation, suggesting a direct influence on enamel quality. Other genes such as ACAT2 (Acetyl-CoA acetyltransferase 2 is linked to tooth wear), DDR1 (Discoidin Domain Receptor Tyrosine Kinase 1 is a collagen receptor expressed in the dental epithelium), AGPAT1 (1-Acylglycerol-3-Phosphate O-Acyltransferase 1 is involved in lipid metabolism and possibly in cellular processes related to tooth development), as well as VGLL4 **(**Vestigial Like Family Member 4) and DCLRE1C **(**DNA Cross-Link Repair 1C), have also been identified as carriers of DMRs in cases of advanced dental caries. The study also highlights that these epigenetic alterations are not random but result from early environmental factors such as maternal diet during pregnancy, exposure to tobacco, stress, or an imbalance in the oral microbiome[44].

Post-translational modifications of histones represent another fundamental epigenetic mechanism. Histones, around which DNA is wrapped to form chromatin, can suffer various modifications such as acetylation, methylation, phosphorylation, or ubiquitination. Acetylation refers to the transfer of an acetyl group from acetyl-CoA, catalysed by histone acetyltransferases (HATs) (Fig. 6), which neutralises the positive charges of histones, reduces the histone-DNA interaction force, and facilitates chromatin decompaction, thereby promoting gene expression. Conversely**,** histone deacetylases (HDACs) tighten the chromatin structure and repress transcription. Histone methylation

consists of the addition of a methyl group catalysed by histone methyltransferases (HMTs) that use S-adenosylmethionine (SAM), which can either activate or repress transcription depending on the site and degree of modification (Fig. 6). Phosphorylation consists of the addition of a phosphate group derived from ATP (adenosine triphosphate) to a hydroxyl group located on the side chain of serine, threonine, and tyrosine residues in histone proteins. This phosphorylation, catalysed by kinases, results, like acetylation, in a reduction of the histone's positive charge, thus decreasing its electrostatic interaction with DNA, leading to chromatin decompaction and making it more accessible to transcription factors, thereby activating gene expression. Ubiquitination refers to the addition of multiple ubiquitin molecules. This modification may play a role in spermatogenesis, the stress response, heterochromatin formation, and finally, transcriptional regulation[43].

Histone modifications play a critical regulatory role during odontogenesis. For example, acetylation of histones H3 and H4 permits the expression of key genes such as DSPP and DMP1 (Dentin Matrix Acidic Phosphoprotein 1), which are necessary for dentin formation. Conversely, the PRC2 (Polycomb Repressive Complex 2) repression complex, through trimethylation of histone H3 at lysine 27 (H3K27me3) via EZH2 (Enhancer of Zeste Homolog 2), inhibits proliferation-related genes during odontoblast differentiation. In caries models, an increase in H3K27me3 levels has been associated with the downregulation of AMELX and ENAM, two critical genes involved in enamel matrix formation, leading to enamel hypomineralisation and structural defects. In contrast, enrichment of acetylation marks, such as H3K9ac (Histone 3 Lysine 9 acetylation), has been linked to the upregulation of inflammatory mediators, including IL-6 (Interleukin 6) and TNF-α (Tumour Necrosis Factor-alpha), which contributes to the pro-inflammatory microenvironment characteristic of advanced carious lesions. This constant interplay between histone acetylation and methylation allows fine regulation of gene expression throughout dental development[43].

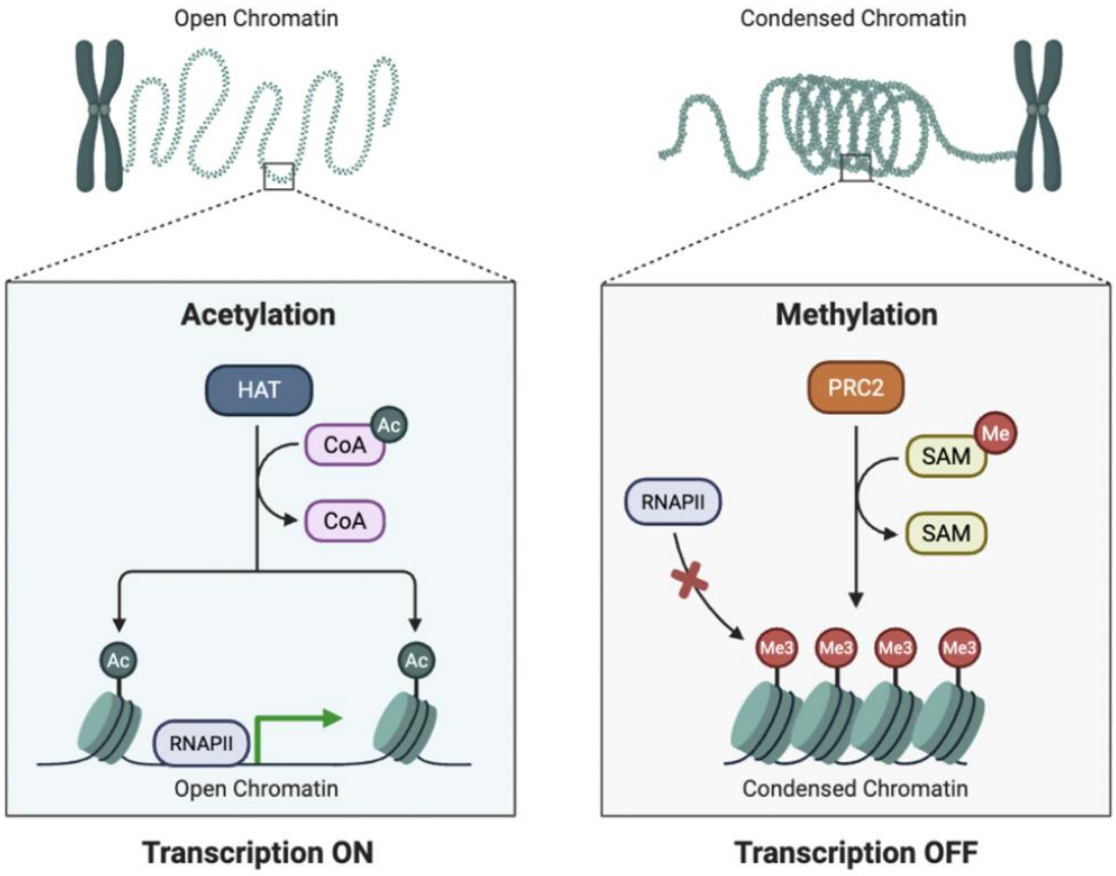

Figure 6: Illustration representing the mechanisms of post-translational histone modifications[43], including acetylation and methylation. Developed using BioRender software.

In addition to DNA methylation and histone modifications, a third fundamental pillar of epigenetic regulation is based on non-coding RNAs (ncRNAs). Contrary to messenger RNAs (mRNAs), which serve as templates for protein synthesis, non-coding RNAs are not translated into proteins but are involved in post-transcriptional and epigenetic regulation of gene expression. These include long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) (Fig.7)[43].

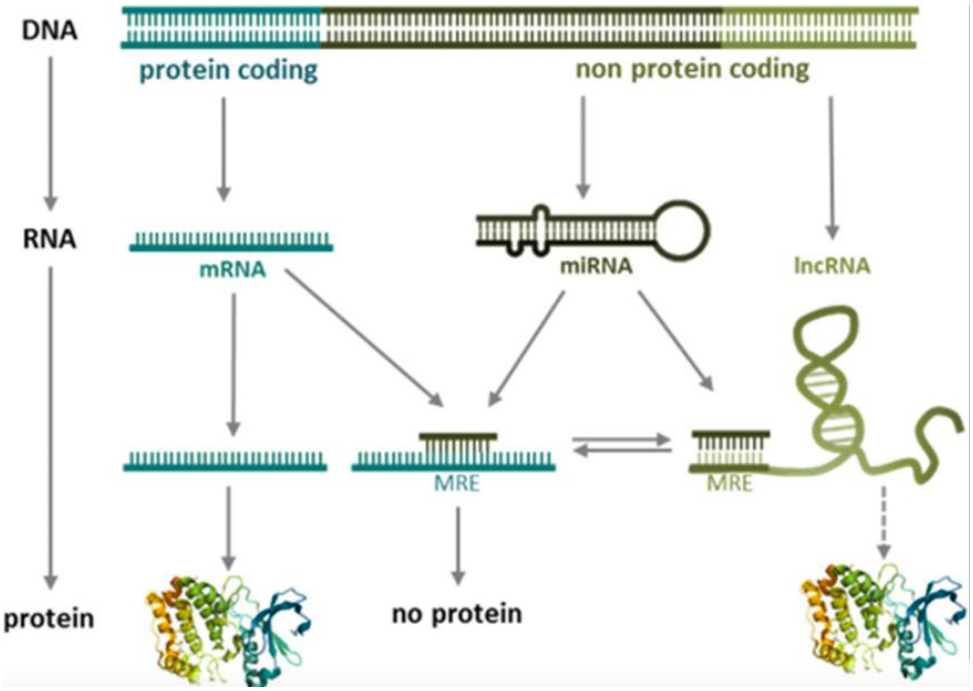

Figure 7: Illustration of the origin of non-coding RNAs from the genome: miRNAs and lncRNAs, adapted from Ramón et al.(2019)[45].

lncRNAs are long RNA molecules transcribed from DNA but not translated into proteins. They play a central role in epigenetic regulation by serving as recruitment platforms for chromatin-modifying protein complexes[43]. A well-known example is the long non-coding RNA Xist, which is essential for the inactivation of the X chromosome in females. Transcribed from the X chromosome destined for inactivation, Xist attaches to it and attracts repressive complexes, including PRC1 PRC2 (Polycomb Repressive Complex 1/2), and sometimes chromatin remodelling complexes such as SWI/SNF, which use ATP to reposition nucleosomes and compact DNA. These complexes are particularly sensitive to environmental signals such as oxidative stress, diet, or the oral microbiota, which allows them to influence gene activity depending on the external context[46].

This same environment-epigenetic interaction principle applies to the development of oral structures. Specific lncRNAs play a key role in tooth formation by regulating the expression of genes involved in cell differentiation and dental tissue development, such as PITX2, FGF3 and DSPP. The lncRNA DLX6-AS1, for example, promotes the transformation of mesenchymal cells into odontoblasts, responsible for dentin production. Its inhibition leads to a marked reduction in the expression of several markers of this differentiation, including SP7, ALPL and DSPP. If such regulatory mechanisms are disrupted, tooth development may be compromised, resulting in weaker dentin or enamel

structure. This structural fragility increases susceptibility to acid and bacterial attacks, thereby raising the risk of dental caries. Thus, lncRNAs not only regulate developmental programs but also mediate the impact of early environmental exposures on oral health, reinforcing their role in both tissue homeostasis and disease onset[47].

Other lncRNAs can also interact with miRNAs (by "sponging" them or by being targeted themselves), participating in a finely regulated network of post-transcriptional control. miRNAs, which are shorter (approximately 22 nucleotides), act after transcription of genes. They bind specifically to mRNAs at sites called MREs (miRNA Response Elements), often located in the 3'UTR region (3' untranslated region) (Fig. 7). This binding prevents translation into protein or induces degradation of the target mRNA[48, 49].

In the oral context, several miRNAs have been identified as key regulators of development and immunity. For example, microRNA-31 (miR-31) is significantly overexpressed in carious dental tissues and targets SATB2 (Special AT-rich Sequence-Binding Protein 2), a transcription factor involved in enamel mineralisation and ameloblast differentiation[50]. Its dysregulation may impair proper enamel formation and contribute to structural enamel defects observed in caries-prone individuals. MicroRNA-200c (MiR-200c)**,** which plays a role in ameloblast polarisation through the regulation of epithelial-mesenchymal transition (EMT), has also been implicated in enamel development; alterations in its expression could interfere with the cellular architecture required for optimal enamel matrix secretion[51]. In addition, the microRNA-202 (miRNA202) has been identified as a potential regulator of the expression of the DEFB1 gene, which encodes β-defensin 1, an essential peptide of oral innate immunity. As previously discussed, this peptide plays a key role in protection against cariogenic bacteria. A polymorphism in miRNA202 (rs12355840) has been associated with reduced DEFB1 expression and increased susceptibility to caries in specific populations of children[52]. Moreover, microRNA-146a (miR-146a), known for its anti-inflammatory properties, is found to be downregulated in active carious lesions, potentially leading to the overexpression of pro-inflammatory cytokines such as IL-1β (Interleukin-1 beta) and IL-6[53]. These findings reinforce the idea that miRNAs are key molecular links between early developmental processes and the immune response to cariogenic challenges[43,49].

2.2 Influence Of Environmental Factors

The environment plays a crucial role in modulating epigenetic mechanisms and, consequently, gene expression. Exposure to various environmental factors can induce epigenetic modifications that influence overall health and the body's response to various diseases, including those affecting oral health.

Postnatal environmental factors

One of the most important environmental factors is diet. A diet rich in sugars and carbohydrates can induce epigenetic modifications in genes regulating inflammation, immunity, and cellular differentiation. For example, high carbohydrate consumption can increase the methylation of genes responsible for cellular protection, thereby decreasing their effectiveness and impacting overall health[54,55].

Chronic stress is another environmental factor that influences epigenetic mechanisms. Excessive production of cortisol, a stress hormone, can disrupt the expression of defence genes, thus affecting immune responses and various biological functions[54,55].

Exposure to environmental toxins can significantly affect epigenetic mechanisms. Heavy metals such as lead, mercury, arsenic, cadmium, chromium, nickel**,** and aluminium are known to disrupt gene expression. Tobacco smoke contains harmful chemicals like benzene, acrolein, formaldehyde, and polycyclic aromatic hydrocarbons, which also interfere with epigenetic regulation. In addition, pollutants such as bisphenol A (BPA)**,** phthalates**,** pesticides**,** and particulate matter < 2.5µm in diameter (PM2.5, measured in µg/m^3) have been shown to compromise the body's defence mechanisms through epigenetic alterations[54,55].

An imbalanced microbiome can mediate the effects of environmental factors by altering host gene expression through epigenetic mechanisms. Some pathogenic bacteria, such as those present in the oral cavity, can secrete metabolites that influence the expression of host genes through epigenetic mechanisms. For example, poor dietary habits and microbiological imbalances can impact the expression of genes involved in immune regulation, thereby increasing the risk of various diseases[54,55].

These diverse environmental factors, whether chemical (e.g., heavy metals, BPA, PM2.5), nutritional (e.g., folate, choline), psychological (e.g., chronic stress), or microbial (e.g., oral pathogens), influence gene regulation mainly through three main mechanisms involving DNA methylation, one of the key epigenetic marks. Other pathways, such as histone modifications and microRNAs, also play a role in the response of the body to these exposures but are not discussed here[56].

First, certain exposures directly alter the activity of the enzymes responsible for DNA methylation. For example, cadmium acts on the enzyme DNMT1. At the same time, air pollution affects the enzyme TET1, which is involved in active demethylation, meaning the direct and enzymatic removal of methyl groups from DNA. This process, carried out by the TET family of enzymes, differs from passive demethylation, which results from a simple maintenance defect during cell division. These disturbances can lead to global or targeted changes in methylation. These disturbances may result in widespread or gene-specific changes in DNA methylation, particularly in genes linked to inflammation (TLR2, Toll-Like Receptor 2, IL6) or salivary function (MUC5B, Mucin 5B), thereby enhancing susceptibility to dental caries[56].

Other environmental factors act by disrupting the availability of cofactors necessary for methylation, such as S-adenosylmethionine (SAM). This cofactor is synthesised from nutrients in the diet, such as folate, choline and vitamin B12. Dietary deficiency reduces the body's ability to methylate DNA properly, while supplementation can increase it. For example, hexavalent chromium may interfere with folate metabolism, thereby reducing SAM levels. Studies have shown that adequate prenatal folate intake is associated with changes in methylation on genes such as IGF2 (Insulin-Like Growth Factor 2) and RXR-α (Retinoid X Receptor Alpha), which are involved in development, including oral development[56].

Finally, a more targeted mechanism is based on the transcription factor occupancy theory. According to this model, transcription factors, which are regulatory proteins that bind to DNA to activate or inhibit gene expression, temporarily protect certain regions of DNA from methylation. In the absence of these factors, methylating enzymes (DNMTs) can access the DNA and add methyl groups, often leading to gene repression. Thus, what determines whether a gene is affected or not is mainly whether or not it is active at the time of exposure. When a gene is actively transcribed, its promoter regions are occupied

by transcription factors and are therefore protected. Conversely, an inactive, unoccupied gene is more vulnerable to methylation. This degree of activation depends on the cell type, the physiological context, the stage of development and the local presence of environmental or microbial signals. This mechanism provides a mechanistic framework to understand how specific exposures such as stress, pollution, or microbiota disturbances induce targeted epigenetic modifications, particularly in genes involved in the immune response (IL6, TLR2), enamel formation (AMELX, ENAM) or salivary secretion (MUC7), all of which are essential in the development of dental caries[56].

These observations remind us that epigenetic changes are not always deleterious: they can reflect the body's adaptive responses to its environment. However, when these alterations are persistent, poorly targeted or occur at sensitive periods of development, they can contribute to the onset of diseases, including oral health conditions.

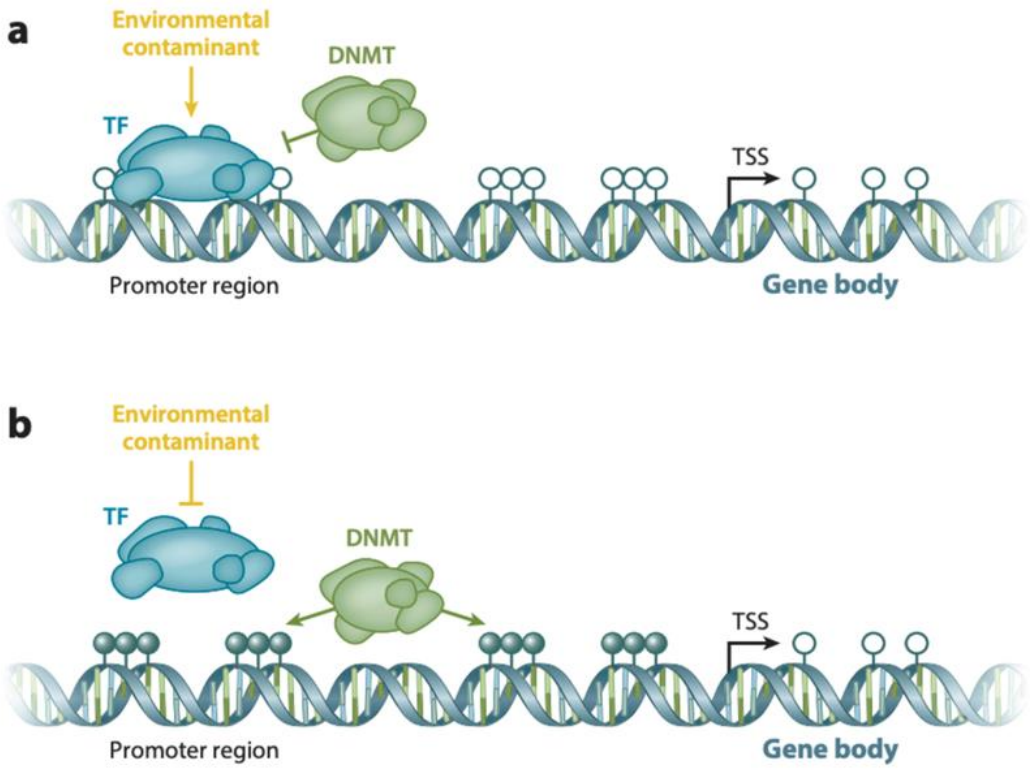

Figure 8: Illustration of the transcription factor occupancy theory. Adapted from Martin and Fry, 2018[56]. This model suggests that the presence or absence of transcription factors on DNA influences access to DNA methylation enzymes. In response to environmental exposures, activated transcription factors may block DNMTs from binding, leading to hypomethylation (a), while repression of these factors can allow DNMT access and result in hypermethylation (b).

Maternal influence: a critical period of epigenetic programming

It is imperative to direct particular attention to the prenatal and perinatal period, during which the maternal environment exerts a profound influence on the epigenetic programming of the foetus. Maternal obesity has been identified as a significant contributing factor to the oral health of their offspring. The intrauterine environment, modified by the diet of the mother and metabolism, has been shown to lead to epigenetic changes that affect not only general development, but also tooth formation and the immune response in the oral cavity of the future child. The issue of maternal obesity has been demonstrated to induce hormonal and metabolic dysregulation. In combination with suboptimal dietary patterns, this may lead to epigenetic alterations that affect odontogenesis and immune function. Furthermore, research has shown that this can render children more vulnerable to oral conditions, such as dental caries and periodontal disease[57,58,59].

Birthweight also plays an important role in this process. Studies show that low birth weight may dysregulate the expression of developmental genes, thereby increasing susceptibility to systemic and oral health disorders, including dental caries, including dental health (Fig. 9). Epigenetics may explain how environmental factors such as nutrition or stress during pregnancy influence the expression of genes related to long-term dental health[57].

Maternal smoking is another critical factor. Substances in cigarette smoke, such as nicotine, carbon monoxide and heavy metals, cross the placenta and expose the foetus to toxins that may induce persistent epigenetic modifications, particularly in genes involved in immune defence and craniofacial development (Fig. 9). These alterations can affect genes linked to the development of oral structures and immune defence mechanisms, thereby increasing susceptibility to oral diseases[57,58,59].

It is important to note that not only maternal smoking but also paternal and environmental exposure to tobacco can exert similar epigenetic influences. Passive smoking in the home, including paternal smoking, has been shown to influence epigenetic programming by altering DNA methylation profiles in offspring, underlining the need for a comprehensive approach to the prevention of tobacco exposure in the early years of life[60].

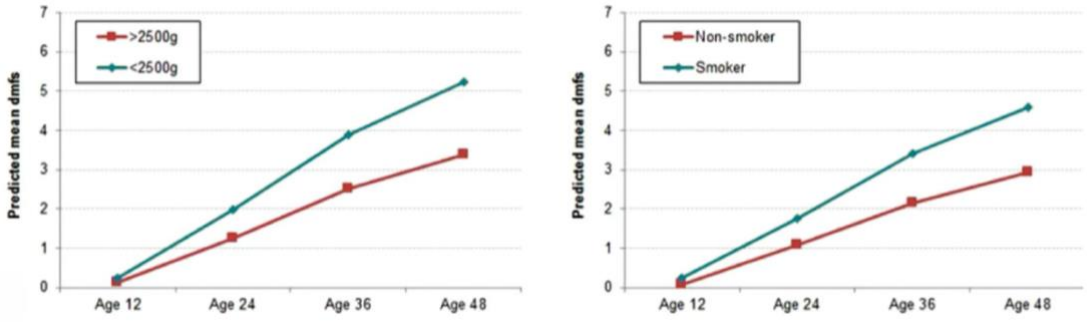

Figure 9: Graphs illustrating the impact of birth weight and maternal smoking on predicted DMFS scores. Adapted from Bernabé et al. (2016)[57]. These graphs show the predicted mean number of decayed, missing, and filled tooth surfaces (DMFS) at different ages, based on birth weight (left) and maternal smoking status during pregnancy (right). Children with low birth weight (<2500g) or those exposed to maternal smoking exhibit higher DMFS scores, highlighting increased caries susceptibility.

Conversely, breastfeeding appears to have a protective effect. Breast milk contains bioactive components such as microRNAs, cytokines and immunomodulatory factors, which may beneficially regulate gene expression through epigenetic pathways, supporting immune development and microbial homeostasis. This promotes the development of a balanced oral microbiome and supports the maturation of the child's immune system, potentially reducing the risk of dental caries[57].

Therefore, environmental factors not only influence biological processes through epigenetic modulation but can also directly affect oral health, promoting the development of dental diseases, such as cavities. In addition to inducing epigenetic modifications that affect gene expression, the environment can directly influence the oral cavity, for example, through dietary habits, oral hygiene, exposure to toxins, or imbalances in the oral microbiome. This complex interplay between genetics, epigenetics, and environmental factors highlights the importance of an integrative approach in the prevention of dental diseases.

3. Application To Dental Caries: The Intersection Of Genetics, Epigenetics, And Environment

Dental caries is one of the most widespread chronic diseases in the world, affecting individuals of all ages and ethnic groups. Its development results from multifactorial interactions involving biological, behavioural, and environmental determinants between various biological, behavioural and environmental factors (Fig. 10)[2].

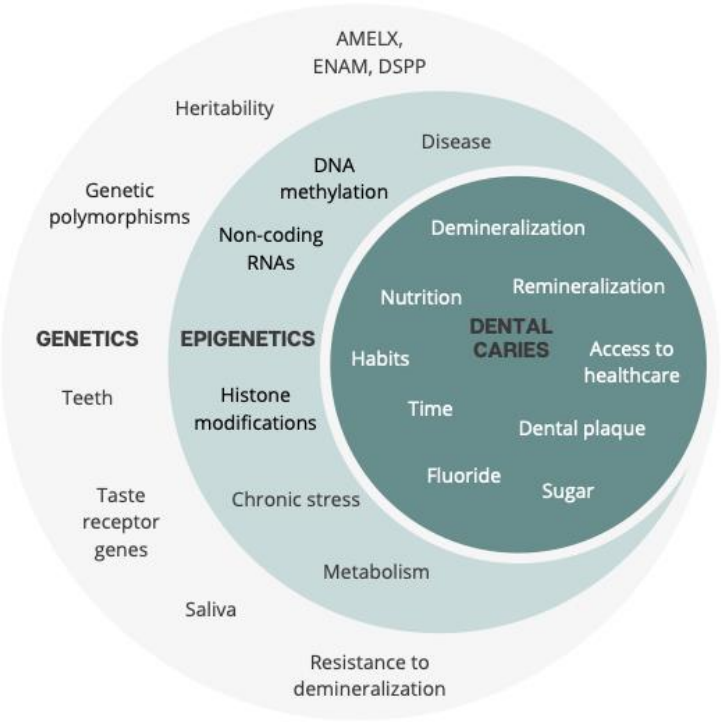

Figure 10: Multifactorial aetiology of dental caries: interaction of genetic, epigenetic and environmental factors[2]. Figure developed by Ines Bouaita using Canva software.

Even though environmental and host factors, like diet and oral hygiene, have been known for a long time, differences in the onset and severity of dental caries among individuals exposed to similar risk factors suggest a genetic contribution, although there is no general agreement on this yet[5]. A key factor in disease progression is the duration of exposure to cariogenic conditions. A diet rich in sugars and refined carbohydrates promotes the growth of acidogenic bacteria, which lower the pH within the biofilm and initiate enamel demineralisation[2]. In parallel, genetic traits can modulate individual susceptibility by influencing saliva flow, enamel resilience, immune function and taste preferences factors that also shape eating habits (Fig. 11)[2,4].

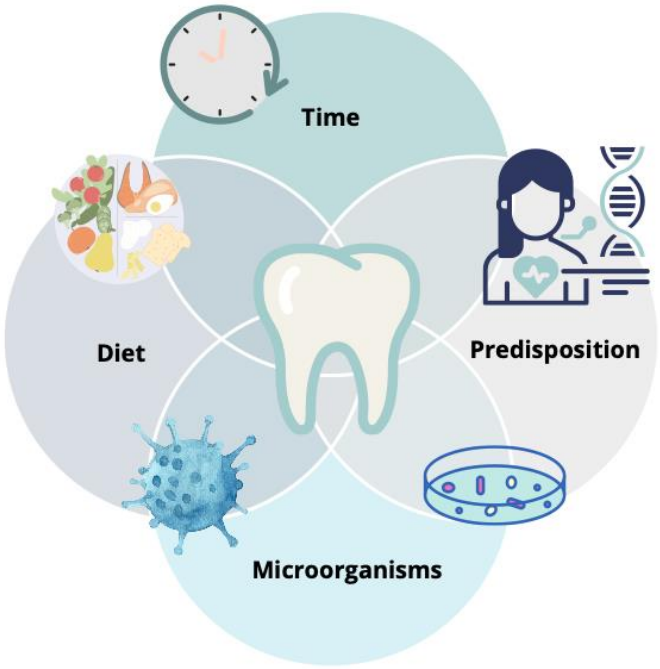

Figure 11: Venn diagram illustrating the key factors involved in dental caries development: time, microorganisms, susceptibility and diet[2]. Figure developed by Ines Bouaita using Canva software.

In this context, several studies, including those based on twin registries in Sweden, have demonstrated that genetics plays a significant role in dental caries, accounting for between 50% and 60% of the variation observed in dental caries indices. However, this research also highlights the impact of non-shared environmental factors, such as dietary habits and oral hygiene, which significantly influence the progression of the disease, representing between 33.1% and 46.4% of the variation in dental caries indices. This means that, despite a strong genetic component, individual behaviours and living conditions continue to significantly influence the occurrence and progression of the disease[61].

Also, the study by Bretz et al. in 2015[5] estimated that the heritability of dental caries was particularly high, with a genetic contribution of 64.6% for the prevalence of surface dental caries. These results reinforce the conclusion of twin registries in Sweden, further emphasising the importance of the genetic component in susceptibility to dental caries[5].

Moreover, some research has shown that the interaction between genetics and environment begins in the prenatal period. The results, which primarily come from studies of twins raised separately, suggest that environmental factors, such as maternal diet or exposure to toxins, can influence genetic susceptibility to dental caries even before birth. These influences occur through epigenetic mechanisms, which modify gene expression

without altering the DNA sequence. For example, DNA methylation, influenced by maternal diet or exposure to toxins, can modulate the expression of genes involved in tooth enamel formation and immune response, thereby impacting susceptibility to dental caries as early as prenatal development[5].

Despite the findings of Swedish twin registries and other studies suggesting that genetics plays a significant role in dental caries, other research has provided different perspectives, highlighting the absence of consensus on this issue. For example, a study conducted by Gao et al. in 2016[5] found that the genetic contribution to dental caries was relatively low, with a heritability index of only 8.7%. In this study, the authors emphasised that environmental factors, such as dietary habits and oral hygiene practices, have a significantly more significant impact than genetics, contributing substantially to the occurrence and progression of dental caries[5].

Similar findings were reported by Kuppan et al. in 2016[62], who studied monozygotic and dizygotic twins aged 6 months to 3 years in India and estimated the heritability of early childhood caries (ECC) at only 15%. This relatively low genetic influence, observed in very young children, reinforces the idea that environmental exposures during the early years of life, such as diet, oral hygiene, parenting practices and socioeconomic status, play a central role in the development of the disease[62].

Faced with this heterogeneity of findings, it becomes clear that focusing on only one dimension (genetic or environmental) is insufficient. A more comprehensive model is needed, one that articulates genetic inheritance**,** epigenetic regulation**,** and environmental exposure across the life course. The following section will therefore focus specifically on environmental factors to complete this tripartite synthesis.

3.1 Environmental And Behavioural Factors: Diet And Oral Hygiene

One of the most studied factors in the pathogenesis of dental caries is the consumption of fermentable sugars. These sugars, such as sucrose, glucose, and fructose, serve as substrates for cariogenic bacteria, particularly *S. mutans,* which metabolise them into acids responsible for enamel demineralisation. The frequency of consumption is as

critical as the quantity: frequent snacking and regular intake of sugary drinks maintain an acidic environment in the oral cavity, reducing the enamel's natural remineralisation ability and thus increasing the risk of dental caries[2,63].

In addition to sugars, the nature of the foods consumed also influences oral health. Sticky or acidic foods prolong the exposure of teeth to adverse conditions, while fibre-rich foods stimulate saliva production, aiding in the removal of food residues and neutralisation of acids[2,63].

Along with diet, oral hygiene plays a crucial role in preventing dental caries. Regular and effective tooth brushing removes plaque and limits the proliferation of microorganisms. The use of fluoride toothpaste strengthens enamel and promotes remineralisation, thereby reducing the risk of tooth demineralisation. However, poor hygiene combined with an unbalanced diet favours bacterial biofilm accumulation and accelerates the progression of carious lesions (Fig. 12)[2,63].

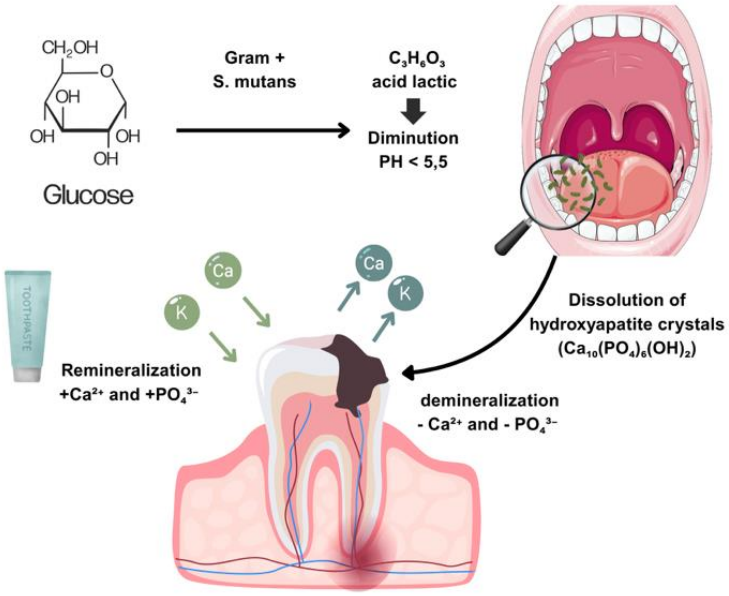

Figure 12: Simplified illustration of the biological process of demineralisation and remineralisation in dental[2]. Figure developed by Ines Bouaita using Canva software.

3.2 Socioeconomic And Environmental Factors In Dental Caries

In addition to biological and behavioural aspects, living conditions and socioeconomic context significantly influence the prevalence of dental caries (Fig.13)[2].

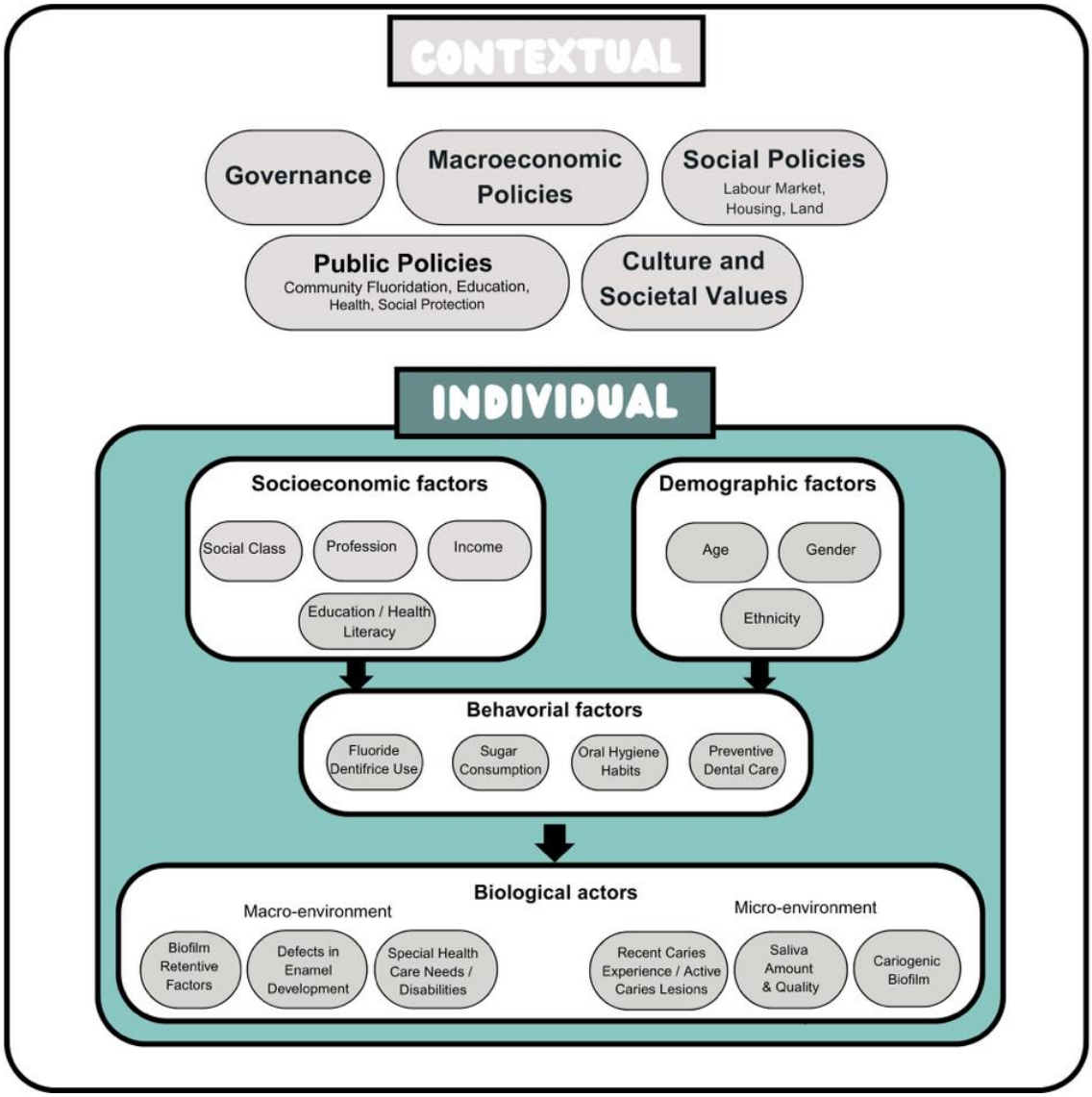

Figure 13: Illustration of contextual and individual determinants of oral health[64]. Developed by Ines Bouaita using Canva software. This diagram highlights the interaction between macrosocial factors (governance, economic policies, culture) and individual determinants (socioeconomic status, behaviours, biological factors) in the aetiology of oral diseases, particularly dental caries[2].

The social determinants model by Dahlgren and Whitehead[64] highlights the impact of education level, income, and access to healthcare on oral health. Disadvantaged populations often face financial and structural barriers that limit access to preventive and curative care[64]. The lack of regular check-ups and preventive treatments, such as fluoride application or scaling and polishing, promotes the onset and worsening of dental caries[2].

Education level also plays a crucial role: lack of information about good hygiene and nutrition practices can increase the prevalence of risky behaviours. In some parts of the world, particularly in developing countries, access to dental care remains a significant issue, often overlooked in public health policies[64].

Environmental factors, such as water fluoridation, also influence the prevalence of dental caries. Fluoridated drinking water is an effective measure for reducing dental caries incidence, particularly among vulnerable populations. Fluoride strengthens enamel resistance and promotes its remineralization. However, its effectiveness depends on several factors, including age, timing of exposure, and the association with other preventive practices. In regions where drinking water is not fluoridated, dental caries incidence tends to be higher[2].

Urbanization and changes in lifestyle have also modified the food environment, promoting higher consumption of processed foods rich in sugars. These changes, combined with limited access to dental care in some rural or socioeconomically disadvantaged areas, exacerbate oral health inequalities[64].

3.3 The Oral Microbiota: Mediator Between Genetics and Environment

3.3.1 Technological Advances And Microbial Balance: Towards A New Understanding Of The Oral Microbiota

For a long time, the study of the oral microbiota was limited by traditional culture methods, which could only identify a small proportion of the microorganisms present in the mouth. As most bacteria cannot be cultured in laboratories, our understanding of the microbiota was very incomplete. The emergence of molecular techniques, such as PCR (Polymerase Chain Reaction) targeting 16S ribosomal RNA, was a major first step forward, making it possible to detect species that were previously invisible[3,65].

However, it was the arrival of next-generation sequencing (NGS) technologies that truly revolutionised this field. These high-throughput techniques now make it possible to analyse not only bacteria but also other microorganisms, such as fungi (e.g., *Candida albicans*), archaea, and viruses (including bacteriophages). Above all, they no longer just identify the species present but also enable us to study their activity using complementary approaches, such as metagenomics (gene analysis), transcriptomics (analysis of expressed RNA), and metabolomics (analysis of metabolic products). This provides a better

understanding of how the microbiota responds to its environment and participates in pathological processes, such as enamel demineralisation[3,65].

The oral microbiota is now seen as a living, structured ecosystem composed of microbial communities organised into adherent layers on the surfaces of the mouth. When in good health, this ecosystem remains generally stable, helping to regulate pH levels, maintain good tooth mineralisation, and prevent the proliferation of pathogenic bacteria. There are six main bacterial phyla: *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, *Bacteroidetes* and *Spirochaetes*, each playing a specific role in the balance of the oral flora[3,65].

However, this balance is fragile and can be disrupted by environmental factors. A sugar-rich diet, poor hygiene or reduced salivary flow promote dysbiosis within the dental biofilm, favouring aciduric bacteria such as *Streptococcus mutans (S. mutans)* or *Lactobacillus fermentum (L. fermentum)*. These bacteria ferment sugars into organic acids mainly lactic, but also acetic, formic and propionic acids which accumulate in the biofilm and lower the local pH to values around 4.0–4.5. This acidic microenvironment initiates enamel demineralisation, as it falls below the critical threshold of pH 5.5[2,3,65].

These discoveries have led to a profound change in our understanding of the causes of dental caries. The traditional hypothesis, known as the “Specific Plaque Hypothesis”, attributed the disease to a few specific bacteria, principally *S. mutans*. This theory is now being questioned by the “Ecological Plaque Hypothesis” developed by Marsh and Takahashi. According to this model, dental caries is not simply the result of the presence of certain bacteria but of an overall imbalance in the microbiota caused by environmental factors, such as excessive sugar consumption. These conditions favour acidogenic and acid- tolerant species and lead to a loss of beneficial bacterial diversity[2,3,65].

Thus, the development of a carious lesion results from a dynamic interaction between protective and pathogenic forces. When defence mechanisms such as saliva, natural remineralisation and microbial diversity are sufficient, the lesion can stabilise or even heal. However, if these mechanisms are overwhelmed by conditions favourable to dysbiosis, demineralisation progresses and dental caries worsens (Fig. 14)[2,3,65].

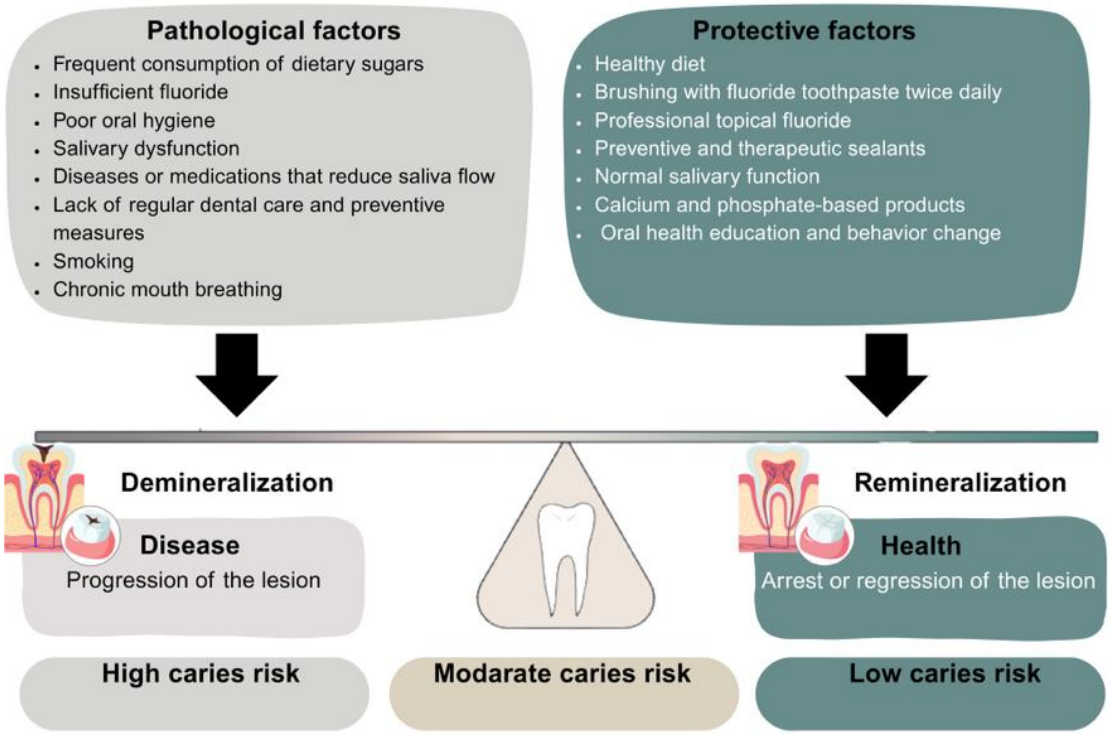

Figure 14: Balancing pathological and protective factors in dental caries: "a visual model of risk dynamics". Adapted from Pitts et al. (2017)[2]. Developed by Ines Bouaita using Canva software.

3.3.2 Origin, Formation and Modulation Of The Oral Microbiota: Between Heredity And Environmental Influence

The oral microbiota does not develop randomly; its formation begins at birth and follows a trajectory shaped by multiple factors. Initially, microbial colonisation is mainly of maternal origin. During childbirth, particularly vaginal birth, the child meets its mother's oral flora. Breastfeeding, early physical interactions, and even the lifestyle of the home (including eating habits, hygiene, and environment) contribute to enriching this initial reservoir of microorganisms. However, this initial biological imprint alone is not sufficient to determine the final composition of the microbiota. It constantly evolves throughout a person's life under the influence of numerous environmental factors. Diet plays a central role here. Regular consumption of fermentable sugars, for example, promotes the development of acidogenic bacteria such as *S. mutans* or *L. fermentum*. Conversely, a varied diet low in simple sugars can maintain a more stable microbial balance. Other factors, such as oral hygiene habits, antibiotic use, stress, passive smoking, and exposure to environmental toxins, influence the diversity and dominance of certain

species. This close link between the environment and microbial composition can be observed at several levels. In one way, the child's general environment, such as their socioeconomic status, the location where they live (rural or urban), or access to healthcare, indirectly influences the development of their microbiota. On the other hand, individual behaviours play a direct role. The frequency of toothbrushing, dietary habits (particularly sugar consumption), and exposure to fluoride (through water or toothpaste) all modulate bacterial composition daily[2].

This influence of the environment on the microbiota has also been demonstrated in longitudinal studies, i.e. studies that follow the same individuals over several years. These studies indicate that, over time, certain species become more abundant, particularly among individuals whose lifestyle habits promote the development of dental caries. For example, bacteria from *Tannerella* spp are more commonly found in adulthood and appear to be associated with the progression of carious lesions, illustrating the cumulative role of environmental exposures.

3.3.3 Genetic Predisposition, Environmental Plasticity And Lessons From Discordant Twins

If the environment modulates the diversity of the oral microbiota daily, it does not act alone. Host-specific characteristics, such as enamel structure, salivary flow, immune response, and taste receptors, influence the development of this microbial ecosystem. This genetic background provides a basis for susceptibility, but it cannot explain the development of dental caries on its own. It is the complex interactions between genetic factors, epigenetic regulation, individual behaviour, and the microbial environment that determine the phenotypic expression of dental caries risk. Studying these relationships, particularly through discordant twin models, provides a better understanding of this multifactorial dynamic[67,68].

However, it is not so much the presence of hereditary factors that raises concerns but rather how the environment modulates their expression over time. This is where epigenetic mechanisms come into play: without altering the DNA sequence, they regulate gene activity in response to external signals. Diet, stress, toxins and early disruptions to

the microbiota are all stimuli that can activate or inactivate certain genes at key moments in life[54].

This plasticity of gene expression, modulated by the environment, is reflected in food preferences. Studies conducted on monozygotic twins, who share the same genetic material, have revealed marked differences in their eating habits. This finding suggests that family traditions, cultural norms and early exposure to certain foods can have a lasting impact on behaviours, regardless of genetic makeup[34].

This malleability is even more pronounced in children. As Birch (1999)[34] showed, an initial aversion to bitterness can be overcome through repeated exposure, illustrating how tastes are shaped by experience. Drewnowski (2000)[34] confirmed that this ability to adapt persists into adult life, with preferences changing in response to motivation or information about nutritional benefits. This link between behaviour, information and environment highlights how genetics provides a template, but experience constantly reshapes it[34].

These observations are supported by longitudinal studies such as the Perinatal and Postnatal Epigenetic Study (PETS), which analysed the combined impact of genetic, environmental and epigenetic factors on oral health. By following a cohort of 250 mothers and their twins, this study showed that factors such as maternal diet, prenatal stress, breastfeeding and fluoride exposure were associated with the risk of dental caries at the age of 6. The fact that the concordance rate for dental caries is similar in monozygotic (70%), and dizygotic (63%) twins suggests that the environment and epigenetics are just as important as genetics[69].

In summary, the environment acts both as a modulator of genetic potential and as a driver of changes in the microbiota through progressive influences related to behaviour, lifestyle, and developmental conditions. These data reinforce the idea that prevention must be rooted in an integrated approach that considers the dynamic interactions between heredity, environment, and microbiota from an evolutionary perspective.

The modulating influence of the environment on gene expression, particularly via epigenetic mechanisms, has been confirmed in studies conducted on twins with discordant dental caries status (i.e. when one twin has dental caries and the other does not). These natural models offer a unique opportunity to observe the differential impact

of the environment on two genetically identical individuals who often share the same living conditions.

Although the studies by Zheng and al. in 2018[66] and Zhang and al. in 2015[68], which looked at pairs of twins aged 3 to 6 years (Zheng) and 46 to 71 months (Zhang), did not specifically quantify the heritability of oral microbiota composition, their results converge on one key point: the variation in species associated with dental caries is mainly modulated by the environment[66,68].

Zheng's study[66], using Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) technology on pairs of discordant twins for dental caries (i.e., only one of the two children had one or more dental caries), revealed that the relative abundance of bacteria such as *Streptococcus mutans, Lactobacillus fermentum, Neisseria sicca, Actinomyces israelii* and *Veillonella dispar* was significantly higher in the group with dental caries. Although monozygotic twins displayed a more similar overall microbiota composition than dizygotic twins ($P = 0.011$), this similarity did not necessarily imply identical susceptibility to dental caries. Indeed, the species identified as cariogenic were strongly correlated with dietary factors, particularly the frequency of sweet consumption, rather than hereditary parameters. This suggests that the immediate environment, particularly individual dietary habits, plays a significant role in the development of dental caries, even among genetically identical individuals. Thus, it is not the global similarity of the microbiota that determines the risk of dental caries but rather the increased presence, even in low abundance, of specific acidogenic bacteria, which is modulated by the environment[66].

This result highlights that ecological imbalances leading to dental caries do not necessarily rely on massive changes in the dominant microbiota but rather on a targeted increase in particular low-abundance species. Although bacteria such as *Lactobacillus* spp, *Scardovia* spp and *Propionibacterium* spp represent only a small fraction of the overall oral microbiota, their relative abundance is significantly higher in children with dental caries. This localised increase, sufficient to disrupt microbial homeostasis, supports the Ecological Plaque Hypothesis, according to which gradual changes in the oral environment (particularly frequent exposure to sugars) promote the development of acidogenic and aciduric microflora, even in the absence of major changes in the dominant species[3].

In addition, Zhang's study[68] analysed the supragingival microbiota of 10 pairs of twins who were also discordant in terms of dental caries, using pyrosequencing of bacterial DNA. This technique enables the rapid and sensitive identification of bacterial species by reading DNA sequences in real-time using light emission, which occurs when nucleotides are added to a DNA strand during synthesis. More specifically, the researchers targeted regions of the 16S ribosomal RNA gene, commonly used for the taxonomic identification of bacteria. The variable regions V3 to V5 were selected because of their high diversity among bacterial species, enabling precise differentiation of microbial communities[68,70]. Although global alpha diversity did not differ significantly between healthy and dental caries groups, certain species such as *Veillonella dispar*, *Corynebacterium matruchotii*, *Selenomonas noxia* (in children with dental caries) or *Fusobacterium canifelinum* and *Kingella denitrificans* (in healthy children) showed differential abundance patterns. However, it was species with low overall abundance, such as *Lactobacillus* spp, *Scardovia* spp, *Propionibacterium* spp, *Moraxella* spp and *Alysiella* spp, that showed the most significant differences between the groups. Although present in small quantities, these bacteria are known for their acidogenic and aciduric properties: their overrepresentation in children with dental caries, even at low concentrations, indicates a subtle but decisive ecological imbalance in favour of a cariogenic biofilm. This observation perfectly illustrates the ecological plaque shift hypothesis. Analysis of behavioural surveys reinforced these observations. In several pairs of genetically identical twins living in a shared environment, differences in eating behaviour, particularly more frequent consumption of sweets or sugary drinks in one of the two children, coincided with specific alterations in the microbiota. In concrete terms, despite genetic similarity and similar living conditions, these differences in diet were sufficient to alter the balance of the bacterial biofilm, leading to the dominance of acidogenic species in the affected child. This result confirms that the oral microbiota is highly sensitive to the individual environment. Thus, even though the twins shared a broadly similar microbiota, slight variations in the proportion of key species induced by behaviour may be sufficient to trigger disease in one of the two children[68].

These results confirm the observation by Gomez et al. (2017)[67] that host genetics influence the overall similarity of the microbiota in twins (MZ twins being more similar than DZ twins), but that the species involved in dental caries pathogenesis in particular *S. mutans, Tannerella* spp and *Rothia* spp are highly dependent on non-shared

environmental factors, such as diet and hygiene. Therefore, just because twins share a similar microbiota does not mean that they have the same susceptibility to dental caries: the species that "make the difference" are modulated by the environment[67].

Zheng[66] and Zhang's[68] studies also highlight that hereditary component (such as enamel structure, immune response and salivary metabolism) influence individual susceptibility to dental caries. In contrast, progression to dental caries largely depends on external factors.

In summary, the common conclusion of these studies is that genetic factors primarily influence susceptibility to dental caries. In contrast, environmental factors determine the composition of biofilm and the progression of dental caries. Such a distinction between susceptibility and phenotypic expression has significant implications for targeted prevention of dental caries[66,68].

Although the question of whether genetic or environmental factors cause dental caries has long divided researchers, recent data from epigenetics, microbiota studies and twin studies now make it possible to move beyond this opposition by linking these dimensions in a complementary way. It is recognised that there is a genetic predisposition to dental caries linked to hereditary characteristics such as enamel structure, saliva composition, immune response and taste preferences. Although these factors are real, they are not sufficient on their own to explain the onset or severity of the disease.

The environment plays a decisive role in modulating genetic susceptibility to dental caries. Whether these are global factors, such as socio-economic context, access to care or diet quality, or individual factors, such as oral hygiene, exposure to fluoride or eating habits, the environment has a decisive influence on the development of the dental caries process. This influence is exerted through epigenetic mechanisms, which are capable of modifying gene expression without altering the gene sequence in response to stimuli such as stress, toxins or nutritional habits[66,67,68].

It also manifests itself in the composition of the oral microbiota, which constitutes a dynamic interface between the genome and living conditions. The oral microbial structure is constantly evolving in response to exposures, behaviours and contexts, making it a particularly sensitive marker of interactions between biology and the environment. In this

regard, the work of Gomez[67], Zheng[66] and Zhang[68] provides valuable insights. Based on twin studies, it has been shown that while monozygotic twins have a more similar microbiota than dizygotic twins, this similarity primarily concerns common dominant species, which are influenced mainly by genetic factors. On the other hand, bacterial species closely associated with dental caries pathogenesis, particularly acidogenic and acid-tolerant strains, are much more sensitive to environmental exposures specific to each individual, such as diet or oral hygiene practices[66,67,68].

In this context, time appears to be a cross-cutting, cumulative and decisive factor. Biologically, dental caries is a slow and chronic process, the progression of which results from the accumulation of imbalances. Each repeated exposure to an acidic environment, linked to a sugary diet or hyposialia, triggers a new phase of demineralisation. Without intervention, the initial lesions become profound and then irreversible. Time thus amplifies the effects of local disturbances until a pathological threshold is reached[2].

At the same time, time acts as a revealer of lifestyle patterns. Dietary habits, oral hygiene, exposure to toxins, and hormonal or medical changes gradually alter the composition of the microbiota, promoting a state of dysbiosis in the long term. Epigenetic mechanisms follow the same logic: their influence accumulates and permanently alters the expression of genes involved in mineralisation, immune defence and taste perception processes[2].

Time should, therefore, not be considered a simple monitoring variable but an essential factor in the development, progression, and prevention of dental caries. It requires a continuous, evolving and personalised preventive approach tailored to different stages of life. This integrated dynamic between genetics, environment and time highlights the importance of strategies that act upstream on modifiable determinants[2].

Ultimately, while genetics shapes individual susceptibility, it is the environment, over time, that triggers and directs the course of the disease. This understanding must guide prevention policies towards a comprehensive, educational and behavioural approach that is firmly rooted in the long term[2].

Finally, recent work has added an extra layer of complexity. in genetic predisposition to dental caries. Some studies suggest that this predisposition may vary depending on dental topography, with specific areas such as occlusal fissures being more influenced by

hereditary influences[61]. On the other hand, a pioneering study has highlighted interactions between genes and gender, revealing that certain polymorphisms only act in women or men[71,72]. This finding could help explain certain epidemiological paradoxes, such as the higher prevalence of caries in women despite better hygiene on average. The same study recommends that future research systematically include gender stratification[71].

4. Biological And Economic Challenges In The Quest For A Dental Caries Vaccine: A Scientific Revolution In Progress

4.1 Dental caries: A Multifactorial Disease With A Significant Impact On Health

Dental caries, also known as cavitated caries or common dental caries, is one of the most prevalent infectious diseases worldwide, affecting billions of people to varying degrees. The World Health Organization (WHO) estimates that around 2.3 billion individuals are affected, with a particularly high incidence in children and young adults[1]. Its public health relevance lies not only in its high frequency but also in the wide-ranging consequences it entails. Without appropriate treatment, dental caries can cause chronic pain serious infections, and lead to premature tooth loss, compromising essential functions such as eating and speech. In children, it is a well-documented cause of school absenteeism and impaired academic performance, while in adults, it contributes to loss of productivity and increased absenteeism in the workplace. In advanced cases, the progression of untreated lesions may lead to systemic complications, including cellulitis or sepsis, requiring emergency intervention and, in some instances, hospitalisation. These functional, educational, and economic repercussions are particularly marked in socioeconomically disadvantaged groups, making dental caries not only a clinical issue but a significant public health challenge[73].

Dental caries is a multifactorial disease that cannot be reduced to a simple infectious process. It results from a dynamic interaction between various factors, which have been thoroughly detailed in this thesis but are worth briefly revisiting to clarify the main mechanisms involved[2].

One of the primary aspects to consider is the presence of a cariogenic oral microbiome. The oral cavity hosts an impressive bacterial diversity, with over 700 identified species, some of which play a central role in the development of carious lesions. Among them *S. mutans* holds a prominent position, which is why it has been the focus of several investigations in the field of prevention and vaccination. This bacterium, identified in 1978 by Russell and Lehner as an important agent in the aetiology of dental caries, has a high capacity to adhere to tooth surfaces, thanks to the expression of specific proteins such as antigen I/II (PAc) and glucosyltransferases (GTF). However, *S. mutans* does not act alone. Other bacteria, such as *Streptococcus sobrinus (S. sobrinus), Lactobacillus* spp, and *Scardovia wiggsiae*, also participate in the cariogenic process, particularly in the later stages of the disease, promoting acidification of the environment and lesion progression[3].

Another key factor in the development of dental caries is diet. Various studies have shown that the frequency of sugar exposure has a more significant impact than the quantity consumed. Sucrose, fructose, and glucose are metabolised by cariogenic bacteria into organic acids, primarily lactic acid, which cause a reduction in local pH and progressive enamel demineralisation. In the early stages, this phenomenon can be reversed through remineralisation mechanisms, but it becomes irreversible if the balance is compromised by prolonged acidification[2].

Oral hygiene and access to dental care also significantly influence the course of the disease. The absence of proper brushing, not using dental floss or antiseptic solutions, as well as irregular dental check-ups, promotes the accumulation of dental plaque and the formation of calcified deposits (tartar), creating an environment conducive to the development of dental caries. Access to preventive care, such as scaling and fluoride application, plays a key role in limiting the disease's progression but remains unequal across different populations and healthcare systems[2].

In addition to these environmental factors, individual susceptibility also plays a crucial role in the progression of the disease. Some individuals develop dental caries more easily due to genetic factors that influence the structure of their enamel, the composition of their saliva, and their oral immune response. It has been demonstrated that variations in genes such as AMELX and ENAM, which are involved in enamel formation and maturation, can influence tooth resistance to acid attacks. Likewise, saliva, which plays a protective role by neutralising acids and promoting remineralisation, is influenced by specific

proteins such as PRH1 and TFF2, whose expression varies among individuals. Furthermore, the local immune response, largely regulated by molecules such as SLPI and LPO, determines the body's ability to control bacterial proliferation and the inflammation associated with carious lesions[4].

From the prenatal period, certain factors also contribute to dental susceptibility. Studies have shown that events occurring in utero, such as maternal nutritional deficiencies, tobacco exposure[58], or even maternal stress, can affect the formation and maturation of a child's dental enamel, increasing the risk of early carious lesions[57]. These early-life influences are primarily mediated by epigenetic mechanisms that modulate the expression of genes involved in dental structure and oral immune response[8]. Considering the evidence presented above, dental caries should not be seen merely as a bacterial infection but as a multifactorial condition that encompasses biological, environmental, and behavioural dimensions. Its development results from a complex interaction between the oral microbiome, diet, oral hygiene, genetic susceptibility, and prenatal influences. This comprehensive view is essential for developing effective prevention and treatment strategies, whether through hygienic and dietary measures, targeted therapeutic interventions, or innovative approaches such as vaccination.

4.2 Why Target *Streptococcus Mutans* In Dental Caries Prevention?

Streptococcus mutans is frequently considered the primary pathogenic agent in the development of dental caries, making it an ideal target in combating this condition. Firstly, *S. mutans* has a strong ability to adhere to dental enamel due to the expression of PAc, which allows it to firmly attach to the teeth and initiate the formation of a bacterial biofilm. This biofilm is further consolidated by the production of extracellular glucans through GTF, which promotes bacterial aggregation and makes the biofilm difficult to remove through brushing or rinsing. This dual capacity for adhesion and biofilm formation enhances the resistance of *S. mutans*[3,11].

Furthermore, *S. mutans* is capable of metabolising fermentable sugars to produce lactic acid, which lowers the pH of dental plaque and leads to enamel demineralisation. This phenomenon is central to the progression of dental caries. Moreover, *S. mutans* can resist

highly acidic environments, allowing it to survive even when acidity in the mouth is elevated, making its elimination even more challenging[2,3].

Streptococcus mutans is characterised by three major virulence traits: strong adhesion to dental enamel, high acid production, and the capacity to form a protective biofilm. These features contribute to enamel demineralisation and support its persistence in the oral environment. These characteristics make it an ideal target for the development of vaccines against dental caries. By targeting this bacterium and preventing its adhesion to enamel as well as the formation of pathogenic biofilms, it would be possible to reduce the risk of dental caries and better control the progression of the disease.

4.3 Principles Of Vaccination Applied To Dental Caries

Vaccination against dental caries is based on inducing a specific immune response against the pathogen *S. mutans*, allowing the body to recognize and neutralise it in case of future exposure. This response is primarily aimed at preventing *S. mutans* from adhering to teeth, thereby limiting biofilm formation. Vaccination against dental caries is based on inducing a specific immune response against the pathogen *S. mutans*, allowing the body to recognise and neutralise it in case of future exposure. This response is mainly directed at preventing *S. mutans* from adhering to teeth, thereby limiting biofilm formation and reducing its cariogenic potential[11,74,75,76].

In this context, Secretory Immunoglobulin A (SIgA), found in mucous membranes such as saliva, mother's milk, and respiratory and intestinal secretions, plays an essential role in local defence. It is produced by plasmocytes, cells specialised in antibody production, which themselves originate from B lymphocytes. These B lymphocytes are born in the bone marrow and, in the case of mucosal immunity, are activated in the Gut-Associated Lymphoid Tissue (GALT) before migrating to glands such as those in the oral cavity. In saliva, immunoglobulin A (IgA) prevents *S. mutans* from attaching to tooth surfaces and blocks the formation of bacterial biofilm. This mechanism is crucial for preventing dental caries[74,75,76].

There are different types of antibodies, and two are particularly important in fighting oral infections: IgA, which is involved in local mucosal immunity, and immunoglobulin G

(IgG), which is involved in systemic immunity. IgG is produced by other plasmocytes derived from activated B lymphocytes and circulates in the blood and gingival fluid. It neutralises bacterial antigens present in the body, thus complementing the defence provided by IgA[74,75,76]. The production of these antibodies is subject to rigorous regulation through the interaction with T lymphocytes, particularly T helper cells (CD4+). These cells perform a supportive function by stimulating B lymphocytes to differentiate into IgA or IgG-producing plasma cells. These T lymphocytes originate in the bone marrow but complete their maturation in the thymus, hence their name (Fig. 15). Among these helper T cells, T helper 17 cells (Th17) cells play an essential role in mucosal immunity. Their differentiation occurs when naive T lymphocytes are exposed to a specific combination of cytokines, such as IL-6, IL-1β, and interleukin 23 (IL-23), which directs their development specifically toward the Th17 pathway. This subtype is distinguished by its ability to migrate to mucosal tissues, such as the oral cavity, and to produce two major cytokines: interleukin-17 (IL-17) and interleukin-22 (IL-22). IL-17 acts as a local immune alarm: it attracts neutrophils, stimulates the production of antimicrobial peptides and promotes the switching of B lymphocytes to IgA production, thereby strengthening specific immunity in saliva. IL-22, on the other hand, acts directly on epithelial cells to strengthen the mucosal barrier and accelerate tissue repair in the event of inflammation. Together, these cytokines provide rapid, localised and effective defence against pathogens that colonise the mouth, such as *S. mutans*[74,75,76].

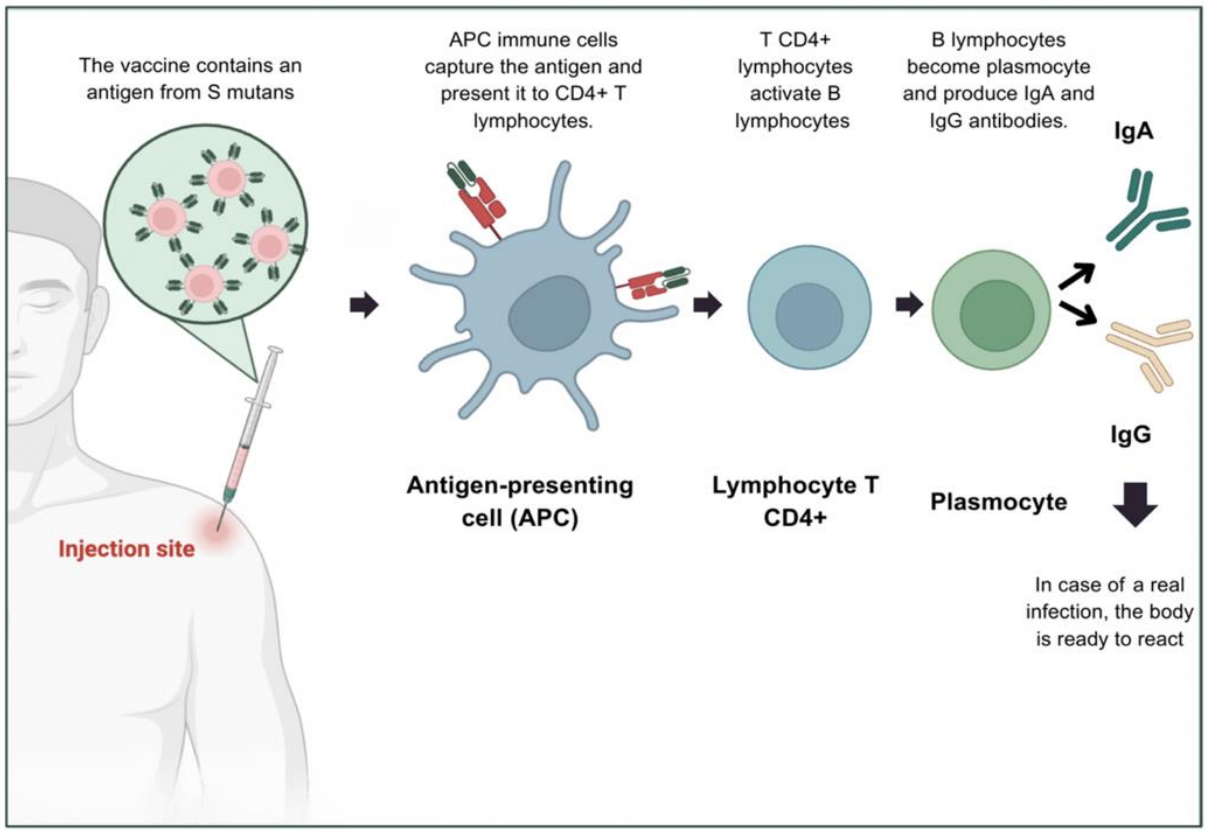

Figure 15: Schematic representation of the immune response initiated by an anti-caries[74]. Developed by Ines Bouaita using Biorender software.

To guarantee optimal protection against dental caries, it is essential to combine these local and systemic immune responses. Vaccines can induce the production of specific IgA and IgG antibodies against key proteins in *S. mutans*, such as PAc, which are responsible for the bacteria's adhesion to teeth, and GTF and Glucan-Binding Protein (GBP), which facilitate glucan production and the bacteria's anchoring in the biofilm. By targeting these proteins, the vaccine can prevent biofilm formation, thereby reducing the cariogenic impact of *S. mutans*[74,76].

4.3.1 Antigenic Targets For Anti-Caries Vaccines

Vaccination against dental caries primarily targets *S. mutans,* a key pathogen in the development of dental caries. Several specific antigens have been identified as promising vaccine candidates, each playing a pivotal role in colonisation or biofilm formation[75].

4.3.1.1 Antigen I/II (PAc)

It is a long surface glycoprotein, measuring 190 kDa, anchored to the bacterial membrane via a C-terminal LPXTG motif recognised by sortase, a transpeptidase enzyme specific to Gram-positive bacteria. Its N-terminal domain interacts with salivary agglutinin, facilitating the initial adhesion of *S. mutans* to enamel surfaces. Mutant strains lacking PAc show a marked inability to aggregate in the presence of saliva, highlighting its central role in early oral colonisation and biofilm formation.

In addition to its functional importance, the PAc protein contains multiple B- and T-cell immunogenic epitopes, enhancing its potential as a vaccine target. However, structural homologies between certain regions of PAc and human immunoglobulin domains have raised concerns about potential autoimmune cross-reactivity, which could limit its clinical applicability[75].

In this context, the study conducted by Liu in 2024[77] provides convincing evidence of the relevance of PAc as a mucosal vaccine target. By designing a recombinant fusion protein (KFD2-rPAc) to enhance immune recognition, they showed that intranasal immunisation of adult rats induced robust PAc-specific salivary IgA responses. It should be noted that the vaccine was administered before exposure to *S. mutans*, unlike many previous studies using simultaneous infection and immunisation protocols. To overcome the low sensitivity of adult rats to *S. mutans* infection, the bacteria were grown under anaerobic conditions (like those found in the air), which increased their virulence and colonisation capacity. In this optimised challenge model, vaccinated rats showed a significant reduction in bacterial colonisation on teeth and an 83% reduction in caries development compared to controls. These results validate the prophylactic potential of PAc-based vaccines and highlight the importance of appropriate antigen preparation and infection models for evaluating vaccine efficacy[77,80].

4.3.1.2 Glucosyltransferases (GTFs)

Glucosyltransferases are key factors in the virulence of *S. mutans*, as they catalyse the synthesis of extracellular glucans from dietary sucrose. These glucans play two essential roles: they promote bacterial cell aggregation and reinforce the three-dimensional

structure of cariogenic biofilms. This matrix not only facilitates the adhesion of *S. mutans* to the enamel surface but also protects against environmental stress factors, including mechanical removal and antimicrobial agents.

By interfering with GTF activity, vaccines can disrupt both the initial colonisation phase and the long-term stability of the biofilm, thereby limiting the cariogenic potential of the pathogen. GTFs, particularly Glucosyltransferase B (GtfB) and Glucosyltransferase C (GtfC), are highly immunogenic and accessible on the bacterial surface, making them promising antigenic targets for the development of anti-caries vaccines[75].

4.3.1.3 Glucan-binding Protein B (GbpB)

Glucan-binding protein B (GbpB) is another important structural component of the biofilm formation mechanism of *S. mutans*. Rather than synthesising glucans itself, GbpB acts by cross-linking glucans produced by GTFs. This “adhesive” role contributes significantly to the architectural integrity and cohesion of the dental biofilm, enabling bacteria to adhere firmly to the tooth surface and resist physical or chemical disturbances.

Vaccines targeting GbpB aim to destabilise this matrix, making the biofilm more vulnerable to elimination by the host's immune system and to common mechanical action[75].

4.3.1.4 Glutamine-binding Periplasmic Protein (GlnH Protein)

The GlnH protein from *S. mutans* has recently emerged as a new antigenic target in the development of mucosal vaccines against dental caries. GlnH is involved in nutrient uptake and bacterial colonisation, and its exposure at the surface makes it accessible to immune surveillance. In a recent preclinical study, researchers genetically modified *Bacillus subtilis (B. subtilis)* to express the GlnH protein, which was then formulated into a sublingual vaccine and tested in mice[13].

After administration, the mice developed a robust immune response characterised by the production of specific antibodies capable of inhibiting the adhesion of *S. mutans* to oral

surfaces. This blocking of initial colonisation is essential to prevent the formation of cariogenic biofilms. In addition, vaccinated mice showed partial protection against infection with *S. mutans* NG8, a well-characterised and highly cariogenic strain.

To enhance the local immune response at the mucosal level, the vaccine formulation included LTK63, a detoxified derivative of a thermolabile enterotoxin from *Escherichia coli* that acts as a mucosal adjuvant. LTK63 potentiated the production of secretory IgA in the oral cavity, thereby strengthening the mucosal barrier against microbial invasion. These results suggest that GlnH, particularly when combined with an effective adjuvant and administered via the mucosal route, represents a promising target for future anti-caries vaccination strategies[13].

4.3.2 Vaccination Strategies And Routes Of Administration

A wide range of immunological approaches has been studied to prevent colonisation by *S. mutans* and the subsequent development of dental caries. These strategies are generally classified as active immunisation and passive immunisation, depending on whether the host produces the protective antibodies or receives them directly. At the same time, the route of administration (systemic, mucosal, plant or topical) plays a crucial role in the quality and location of immune responses, particularly IgA secreted in the oral cavity. This section presents the most promising active and passive vaccination strategies against *S. mutans*, with a particular focus on their mechanism, route of administration and transfer potential[11].

4.3.2.1 Active Immunisation Against *Streptococcus Mutans*

Active immunisation strategies aim to induce a specific and long-lasting immune response to the antigen by stimulating the host's immune system. In the context of dental caries, the goal is to promote the production of antibodies, particularly secretory IgA in the mucosa, which can prevent the initial adhesion of *S. mutans* to the enamel, neutralising its virulence factors and inhibiting biofilm formation. To this end, various platforms have been explored, ranging from protein subunit vaccines and DNA-based constructs to new delivery methods such as nasal sprays and plant-derived antigenic vectors. These approaches share the common goal of mimicking natural exposure to *S. mutans* antigens to prime the immune memory before colonisation occurs, particularly in young children[11].

Recombinant Protein-Based Vaccines

Injectable protein subunit vaccines, which contain both purified native antigens and recombinant proteins, have been extensively studied as an active immunisation strategy against *S. mutans*. Early formulations relied on partially purified surface components such as PAc, GTF, and GbpB extracted from bacterial cultures. However, concerns about cross-reactivity with human tissues, particularly heart muscle, led to a shift towards recombinant subunit vaccines, which offer several advantages, including precise antigen design, scalable production, and reduced risk of contamination by host-derived proteins[11].

These characteristics make recombinant subunit vaccines desirable for injectable and mucosal delivery platforms. In this context, nanoparticle-based delivery systems have recently emerged as innovative strategies to improve vaccine efficacy. By encapsulating antigens in biodegradable nanoparticles, these systems protect the antigens from enzymatic degradation and enhance their uptake by antigen-presenting cells, resulting in stronger and more durable immune responses. This strategy stimulates both systemic IgG and local IgA responses, resulting in more durable and adequate immune protection. Preclinical studies in mouse models have shown that these approaches significantly reduce *S. mutans* colonisation and the incidence of dental caries. A notable example is the ZIF-8 NPs PAc formulation, which combines recombinant PAc, a key adhesin of *S. mutans*, with zeolite-8 imidazolate structure nanoparticles (ZIF-8 NPs) acting as an injectable adjuvant and delivery vehicle. In the study by Yu et al.[78], subcutaneous immunisation of mice and rats with this formulation significantly improved antigen internalisation by dendritic cells, elicited a robust Th2-dominant IgG response, and

increased the generation of central memory T cells. More importantly, it significantly reduced *S. mutans* colonisation and carious lesions in immunised animals, confirming its potential as an effective injectable recombinant nanoparticle-based vaccine for caries prevention[78,79].

Despite these promising results, no injectable candidate has yet progressed beyond preliminary clinical trials. Nevertheless, these platforms remain a fundamental model in caries vaccine research and continue to guide the development of safer and more immunogenic antigens and adjuvant systems. However, one of their main limitations is their inability to elicit strong immune responses at the mucosal level, which is essential for effectively preventing a disease that originates on the oral surface[78,79].

This limitation has sparked growing interest in mucosal immunisation strategies, which aim to induce immune responses specific to the site of entry of *S. mutans*.

Stimulating the production of secretory IgA (sIgA) in saliva is considered a key objective, as these antibodies are capable of blocking bacterial adhesion to enamel, neutralising virulence factors and inhibiting early biofilm formation. Mucosal routes of administration, including intranasal, oral and sublingual, are therefore being investigated to achieve more effective local immune protection than systemic injections alone[11].

To stimulate this local immunity, several mucosal routes of administration have been explored, including intranasal, oral and sublingual, each offering specific immunological and logistical advantages. The activation of Th17 responses is a key mechanism contributing to the success of mucosal vaccines. These responses promote the switch to the IgA class in B lymphocytes and strengthen the epithelial barrier function, making them particularly relevant in paediatric populations, where early immunisation could prevent colonisation and provide long-lasting protection.

This reasoning guided the development of the recombinant KFD2-rPAc vaccine, evaluated in the study by Liu et al. (2024)[77]. The vaccine combines the bacterial surface adhesin PAc, a key factor in colonisation by *S. mutans*, with the adjuvant properties of bacterial flagellin. Administered intranasally to adult rats, this mucosal immunisation protocol allowed for the full development of protective immune responses prior to bacterial exposure. The results were striking: vaccinated rats produced high levels of IgA

in saliva, which significantly reduced initial and long-term colonisation by *S. mutans*. It should be noted that infection was induced using bacteria grown under air-restricted conditions (similar to anaerobic conditions) to simulate increased virulence. Even under these conditions, the vaccine reduced caries development by 83%. This study not only validates the antigenic potential of PAc but also demonstrates the feasibility and efficacy of a mucosal prophylactic vaccination strategy, which is particularly relevant for paediatric application, where early immunisation before colonisation is essential[77,80].

Among the mucosal routes explored for combating *S.mutans*, the sublingual route has garnered attention for its ability to trigger both systemic and local immune responses while avoiding antigen degradation in the gastrointestinal tract. Based on this reasoning, Ferreira et al. (2015)[81] conducted a key preclinical study evaluating a sublingual vaccine based on recombinant PstS, a phosphate-binding lipoprotein and component of an ATP-binding cassette (ABC) transporter involved in bacterial metabolism and adhesion. The antigen was produced in Escherichia coli using recombinant DNA technology. BALB/c mice (a widely used inbred strain known for its strongly Th2-biased immune responses and suitability for mucosal immunisation studies) received three sublingual doses of rPstS (recombinant Phosphate-specific transport system substrate-binding protein), with the antigen applied directly under the tongue under mild sedation. The vaccine induced robust systemic IgG responses, particularly when the antigen was combined with the mucosal adjuvant LTK4R, and promoted a Th2-dominant profile, as indicated by high IL-6 levels and IgG1 predominance. Significantly, both rPstS alone and the adjuvanted formulation-induced IgA-secreting cells in MALT and serum from vaccinated mice inhibited the adhesion of *S. mutans* to the oral cavity in naïve animals. This confirmed the in vivo expression of PstS and its functional targeting by vaccine-induced antibodies[81].

A more recent study by Pereira in 2022[13] extended this approach by using another component of the ABC transporter, GlnH, a glutamate-binding protein involved in acid tolerance and essential metabolism in *S. mutans*. The recombinant antigen was designed and expressed in *B. subtilis*, then administered sublingually with or without the non-toxic adjuvant LTK63. Like PstS, GlnH triggered strong systemic IgG responses and significantly reduced *S. mutans* colonisation in the oral cavity of immunised mice, even though mucosal IgA responses were not significant. These results highlight the broader

relevance of ABC transporter proteins as promising targets for recombinant vaccines capable of eliciting protective immunity through mucosal administration[13].

Plant-Derived Vaccines

Among emerging platforms in mucosal immunotherapy, transgenic plants are becoming increasingly strategic. Although based on genetic engineering, this strategy is unique in its use of plant hosts as safe, scalable and economical bioreactors capable of producing recombinant antigenic proteins directly in their tissues. The principle is ingenious: plants such as tobacco or lettuce are modified to express a specific *S. mutans* protein, such as the PAc protein, by integrating the gene encoding this antigen into their DNA. Once transformed, these plants produce the antigen as if it were part of their metabolism, thus acting as veritable "biological factories". The plant material obtained can then be administered in powder, capsule or nasal spray form without the need for injections or sophisticated medical equipment. This active immunisation route, therefore, offers major advantages for large-scale vaccination campaigns, particularly in regions with poor healthcare infrastructure. In addition to its simplicity, this approach is attractive due to its low cost, ease of storage (no refrigeration required), simplified logistics and greater acceptability among children and people with a fear of injections[82].

Although no specific clinical studies are yet available for caries, this strategy has been explored in other infectious contexts, and its application to *S. mutans* antigens remains a promising avenue for future research. It has been successfully applied in active immunisation in other contexts (e.g., influenza, HBV, COVID-19). For example, the Covifenz® vaccine, developed by Medicago and GlaxoSmithKline, is based on pseudo-viral particles produced in *Nicotiana benthamiana*. Authorised in Canada in 2022, it has demonstrated 71% efficacy against symptomatic COVID-19, validating the feasibility and relevance of this technology. These results reinforce the credibility of the concept for potential applications in the prevention of oral diseases[83].

DNA Vaccines

DNA vaccines represent an innovative advance over traditional strategies based on purified proteins. Rather than administering a prefabricated antigen, this approach involves injecting a DNA plasmid encoding a specific antigen from *S. mutans*, most often

a surface protein involved in adhesion, such as the PAc protein. Once in the body, this vector (usually circular DNA) enters the host cells and uses their machinery to produce the antigen internally. The antigen is then presented by antigen-presenting cells, triggering an adaptive immune response, particularly in the mucosa.

With this in mind, Jiang[84] conducted an experimental study in 2017 aimed at optimising the immunogenicity of this type of vaccine. They developed a DNA vaccine targeting *S. mutans* using a live attenuated strain of *Salmonella typhimurium* as an oral delivery vector. The vaccine gene, encoding two key antigenic fragments (the saliva-binding region SBR of the PAc protein and the glucan-binding region GBR of Glucosyltransferase of type I GTF-I), was placed under the control of a dual promoter system: nirB, active in the vector bacterium, and Cytomegalovirus (CMV), active in host cells. This device allowed the antigen to be produced both in the vector bacterium and in the infected cells, maximising the quantity and duration of vaccine expression and stimulating a more robust immune response. BALB/c mice (inbred laboratory strain widely used in immunological research) were immunised intragastrically, and the results showed that the vaccine with the dual promoter induced significantly higher levels of IgG (serum) and IgA (saliva) antibodies than the vaccine using the bacterial promoter alone. After a booster vaccination, humoral and cellular immune responses were amplified, with an increase in Th1 and Th2 cytokines. In a challenge test, vaccinated mice showed a reduction of more than 99% in *S. mutans* colonisation in dental plaque compared to controls. These results show that the use of a dual promoter system combined with mucosal immunisation with live attenuated *Salmonella* spp is an effective strategy for improving the performance of anti-caries DNA vaccines[84].

In addition, Yan et al. (2016)[85] evaluated the immunogenicity and protective efficacy of a DNA vaccine encoding a conserved region of the *S. mutans* PAc protein, administered intranasally to rats in combination with CCL17 and CCL19 genetic adjuvants. Vaccinated animals developed significantly higher levels of PAc-specific salivary IgA and exhibited a notable reduction in caries lesions compared to controls, highlighting the potential of this approach to induce local mucosal immunity against dental caries[85].

However, despite their preclinical potential, DNA vaccines still face significant translational barriers. Concerns persist about their long-term safety, the risk of genomic integration, the variability of immune responses between individuals, and the durability

of protection. Further investigations are necessary to optimise delivery systems (e.g., electroporation, nanoparticle vectors), improve immunogenicity, and establish efficacy in human populations before this technology can be applied in clinical dental settings.

Live Attenuated Vaccines (CAIV-Based Vectors)

A particularly innovative and promising strategy relies on the use of a live attenuated vaccine based on the cold-adapted influenza virus (CAIV), a viral vector already approved by the Food and Drug Administration (FDA) for human use to prevent influenza[86]. In this context, CAIV would be genetically modified to incorporate genes encoding antigens from *S. mutans*, thereby serving as a delivery system to induce a targeted immune response against caries[86].

The main objective would be to stimulate a robust mucosal immune response, particularly the production of salivary IgA, prior to colonisation of the oral cavity by cariogenic bacteria. This is particularly relevant in paediatrics, where early intervention could delay or even prevent the establishment of *S. mutans*[86].

However, this approach has not yet been tested experimentally. This is a hypothesis put forward by Yang in 2019[87], who describe a theoretical protocol including in vitro expression tests, followed by preclinical trials in rodents to assess immunogenicity (IgA titres), bacterial colonisation and caries scores. The authors propose inserting well-characterised antigenic sequences from *S. mutans* into the CAIV genome, such as epitopes from the PAc protein, GTF or GBP, all of which are known for their ability to induce protective IgA responses in saliva. They also point out that the CAIV platform is stable, not highly transmissible, and has already been produced on a large scale, including in low-resource countries. It has a good ability to induce mucosal immune memory, non-invasive intranasal administration methods, and well-documented safety in other indications. These arguments make it a relevant candidate for early vaccination against dental caries. As such, the lack of development to date reflects not a limitation of the CAIV vector itself, but rather the early, untested nature of the proposal and the absence of foundational experimental work. The article's intention is to propose a promising avenue for future investigation, rather than to report any active development or ongoing research[87].

4.3.2.2 Passive Immunisation

Contrary to active immunisation, which relies on stimulating the immune system to produce a memory response, passive immunisation involves directly administering preformed antibodies capable of neutralising a pathogen. This approach, which has already been used successfully against several viral and bacterial infections, offers immediate and targeted protection without requiring immune maturation in the host. It represents a particularly interesting strategy in the context of dental caries, especially in young children or immunocompromised individuals, in whom it can be difficult to induce a robust vaccine response[11,75].

The emergence of plant platforms for the production of recombinant antibodies opens up new prospects for the development of effective, safe, and accessible passive immunotherapies. This strategy relies on the use of transgenic plants as scalable and economical bioreactors capable of producing either antigenic proteins (active immunisation) or recombinant immunoglobulins (passive immunisation). In the case of dental caries, the plant production of *S. mutans*-specific IgA antibodies represents a promising approach: when administered locally, these antibodies could directly neutralise the oral pathogen without engaging the host's immune system. In their 2015 review, Vasilev[82] details the technical and immunological advances related to this technology, highlighting the benefits in terms of biosafety, cost and stability. They show that plants such as tobacco and lettuce can efficiently produce mucosal IgA through stable transformation or transient expression via viral vectors. These antibodies retain their activity after purification and can be formulated into sprays, oral gels or tablets. Although few studies have directly targeted *S. mutans*, animal models have demonstrated the effectiveness of these plant IgA in neutralising pathogens at the mucosal level[82].

However, it is important to mention the pioneering study by Ma in 1998[88], which remains the only experimental demonstration in humans of the efficacy of secretory IgA antibodies produced in transgenic plants against *S. mutans*. In this pioneering study, IgA specific for the AgI/II antigen was produced in tobacco plants, purified and then administered locally to volunteers. The results showed a temporary inhibition of biofilm formation, although the antibodies persisted in the oral cavity for no more than three days[88].

This clinical proof of concept, although dated, highlights the concrete potential of passive plant immunisation in the context of caries. Since then, no other publication has reproduced or extended these results in humans, revealing a significant gap in the contemporary literature.

In parallel with antibodies produced by plants, other biotechnological approaches to passive immunisation have been developed, such as those using human fragment antigen-binding regions (Fabs) produced by phage display.

In a particularly promising study, Alam[12] in 2018 investigated Fabs specifically directed against *S. mutans* and *S. sobrinus*. Fabs correspond to the monovalent variable region of the antibody, responsible for antigen recognition, but lacking the constant region involved in effector functions. Their small size promotes better tissue diffusion, and they are commonly used in passive immunotherapy to specifically block the interaction between a pathogen and its colonisation site[12].

These Fabs were produced using phage display technology, which involves using genetically modified bacteriophages to display antibody fragments on their surface. This process allows the selection of Fabs with high affinity for bacterial antigens in their native conformation, as presented on the surface of living bacteria[12].

The fragments selected in this way showed a high affinity for *S. mutans* and *S. sobrinus* and significantly inhibited biofilm formation in vitro. Furthermore, topical application to the teeth of infected rats fed a cariogenic diet resulted in a marked reduction in the incidence of caries. Evaluation by micro-computed tomography (micro-CT), a high-resolution three-dimensional imaging technique, showed a fivefold reduction in the number of carious lesions in the treated groups. These results highlight the potential of Fabs as an innovative and targeted passive immunisation strategy against dental caries[12].

Despite these encouraging results, passive immunisation still has some limitations, including the short lifespan of antibodies in saliva, which requires repeated applications. In addition, inter-individual variability in dietary behaviour or microbiota may modulate the effectiveness of this strategy. Current research focuses on improving formulations to prolong the oral retention of antibodies and on creating antibody variants with extended

half-lives. Combined approaches, including fluorides, probiotics, or dietary interventions, are also being explored to maximise protection against caries[12].

4.4 Feasibility and Risks

4.4.1 Target Populations And Expected Benefits

Children in preschool, primary school and adolescence

Young children, particularly those between the ages of 2 and 5, are a priority target group for a dental caries vaccination strategy. It is during the preschool years that the initial acquisition of *S. mutans*, the main cariogenic agent, occurs in conjunction with the eruption of primary teeth and vertical or horizontal transmission from the environment. This critical phase of sustained colonisation of the oral microbiota largely determines the risk of subsequent caries, particularly in the form of Early Childhood Caries (ECC)[2,89]. Although this concept has been established for many years, current scientific evidence continues to support the relevance of targeting this age group in preventive strategies.

The idea of administering a preventive vaccine before or during this phase is based on the objective of blocking the establishment of the pathogen through a mucosal immune response, mainly via the production of secretory IgA. However, despite encouraging results in animals, no clinical studies have been conducted in children to date, and there is a complete lack of safety and efficacy data in the paediatric population. This lack of data currently prevents any application in this age group and highlights the need for specific clinical research[90].

In school-age children (6–12 years), a period corresponding to mixed dentition, vaccination could theoretically strengthen or prolong immunity against bacterial recolonisation. Similarly, in adolescents, who are often less involved in oral hygiene and exposed to risk behaviours (sugar consumption, irregular hygiene), a vaccine could be a complementary protective tool. However, the clinical efficacy of such a strategy in these age groups remains entirely speculative at this stage[91].

Vulnerable populations (elderly, disabled, vulnerable)

The elderly are a population that is particularly at risk of oral diseases, especially root caries. This vulnerability is due to several factors, including age-related gum recession, dry mouth caused by the long-term use of certain medications (especially anticholinergics, antidepressants, and antihypertensives), and the gradual loss of independence in oral hygiene. The situation is particularly worrying for people in care homes. A recent international review found that almost all elderly residents in some countries had carious lesions, with average Decayed, Missing, and Filled Teeth (DMFT) scores ranging from 6.9 (Malawi) to 29.7 (South Africa). The prevalence of root caries also reaches up to 74% in Brazil[92].

In this context, vaccination against caries could be a high-impact passive protection tool, reducing the bacterial load of *S. mutans*, limiting chronic infections and delaying irreversible dental caries. This approach would be particularly relevant for dependent older people, especially those with cognitive impairment, who often have limited access to care and irregular oral hygiene. People with physical or mental disabilities also face significant barriers to accessing dental care, compounded by specific oral and facial disorders, a diet that is often high in sugar, and risky behaviours. In these patients, long-lasting protective immunity could reduce the need for invasive treatments under general anaesthesia, which is common in this population.

Finally, vulnerable populations, people without stable social security coverage, living in extreme poverty or experiencing social exclusion, also face limited access to oral care, often poor hygiene and a high prevalence of untreated caries. Free and accessible vaccination could be an equitable response to these structural inequalities.

4.4.2 Reducing Social Inequalities In Oral Health

Dental caries is a condition closely linked to social determinants of health. Numerous studies have shown a significantly higher prevalence of untreated caries among children and adults from disadvantaged socioeconomic backgrounds[2]. Social inequalities in oral health are well established. In the United States, National Health and Nutrition Examination Survey (NHANES) data show that children living below the poverty line

are five times more likely to have dental caries than those from high-income households. These disparities can be explained by a combination of factors: a diet high in sugar, limited access to healthcare, and poor oral hygiene[93].

In this context, anti-caries vaccination accessible to the entire population, particularly through public health channels (such as schools, maternal and child health centres, and medical and social facilities), could be a major lever for reducing inequalities in oral health. By acting independently of socioeconomic status, dental care or family background, it would offer a form of "universal" immune protection against *S. mutans* and associated caries lesions.

If proven safe and effective, this preventive strategy would be particularly beneficial for groups most exposed to structural inequalities, complementing traditional education, hygiene promotion and targeted fluoridation measures. It is entirely in line with the WHO's objectives of universal health coverage and the fight against inequalities in oral health[94].

4.4.3 Health Economics: Direct And Indirect Costs Of Dental Caries

Dental caries is not only a significant global public health issue but also a substantial economic burden on healthcare systems. According to a 2021 study by the World Dental Federation (FDI), the overall costs associated with oral diseases, of which caries is the most common component, exceed $540 billion per year worldwide. Most of this expenditure is on caries-related care, both in children and adults[95].

Direct costs include conservative treatments (such as fillings and endodontics), surgical treatments (such as extractions), prosthetics, and follow-up visits. In France, dental caries is one of the leading reasons for dental consultations, including emergency consultations, and a frequent reason for prescriptions (such as painkillers and antibiotics), generating high costs for the national health insurance system. Added to this are the often underestimated indirect costs associated with school or work absenteeism, chronic pain, loss of productivity and even social stigma in severe cases[96].

In this context, the development of an effective vaccine against *S. mutans* could theoretically lead to a significant reduction in the incidence of dental caries, thereby generating long-term savings Although no economic model has yet been validated for this approach, it is reasonable to assume by analogy with other preventive vaccines that even moderate efficacy, combined with adequate population coverage, could improve quality-adjusted life years (QALYs), a key indicator reflecting both the quality and quantity of life gained through health interventions. Moreover, such a strategy could help reduce the costs associated with curative care, particularly in low- and middle-income countries where access to dental services remains limited[97].

Finally, vaccine prevention could also help relieve pressure on hospital dental services and reduce the need for invasive procedures under general anaesthesia in young children and vulnerable people, which represent a significant source of avoidable expenditure for public health systems.

4.4.4 Oral Microbiota And Mucosal Immunity: Cross-influence With Genes

The development of vaccines against dental caries must consider genetic variability among individuals, particularly differences in immune response, to maximise efficacy and minimise the risk of side effects. Genetic polymorphisms in key genes of the immune system, such as Toll-like receptors (TLRs), cytokines and major histocompatibility complex (MHC) antigens, strongly influence this immune response. For example, variations in the TLR4 gene can modulate how the immune system recognises antigens and produces cytokines, which has a direct impact on vaccine efficacy[98]. Furthermore, recent findings by Shaffer et al.[71] suggest that certain genetic differences may interact with sex-specific immune mechanisms. Their study highlighted that some polymorphisms appear to have a more pronounced effect in women than in men, particularly regarding the immune response to *S. mutans* antigens. This observation raises the hypothesis that the efficacy of anti-caries vaccines could vary according to gender. Such a possibility underscores the importance of designing gender-stratified clinical trials to ensure both equity and optimal effectiveness in future preventive strategies[71].

As we saw in the first part, studies have shown that genetic variations in the HLA-DRB1 and HLA-DQB1 genes, which are part of MHC, are associated with increased susceptibility to dental caries, particularly in young children. These genes play a crucial role in presenting antigens to immune cells. However, certain genetic variations can alter this presentation and disrupt the recognition of bacterial antigens in the oral cavity by immune cells. For example, certain alleles of the HLA-DRB1 and HLA-DQB1 genes can lead to a less effective immune response against *S. mutans*, one of the primary cariogenic bacteria. This makes individuals carrying these variants more vulnerable, as their immune system has more difficulty eliminating them effectively due to incorrect antigen recognition[98,99].

This variability also applies to how an individual responds to a caries vaccine. This is because the response to the vaccine depends on the immune system's ability to recognise, process and remember the injected antigen. This ability is highly dependent on HLA genes. If an individual has an HLA variant that poorly recognises the vaccine antigen, the immune response will be weak, reducing the effectiveness of protection. Conversely, some variants can trigger an excessive response, characterised by increased inflammation or more pronounced side effects. The genetic diversity of HLA genes is therefore a significant obstacle to standardising the vaccine response at the population level[98,100].

In addition to their direct role in antigen presentation, HLA genes also indirectly influence the response to vaccination by modulating the composition of the oral microbiota. HLA proteins help establish immune tolerance towards commensal microorganisms by helping the immune system distinguish between harmless bacteria and potential pathogens.

The presence of specific HLA alleles can thus promote the persistence of certain microbial species in the oral cavity, while others can be more easily eliminated. This selective pressure has a long-term effect on each individual's microbial ecosystem, ultimately shaping their unique microbial profile[98].

This microbe composition, which is specific to each person, plays a key role in regulating mucosal immunity. Some commensals promote the production of secretory IgA, which neutralises pathogens and contributes to mucosal protection. In contrast, others influence T-cell polarisation, notably by stimulating Th17 responses and the production of IL-17, a cytokine involved in antimicrobial defence. Consequently, a balanced and diverse

microbiota creates an immunological environment that promotes a more effective and better-regulated response to mucosal vaccines. Conversely, dysbiosis can disrupt this balance, leading to suboptimal vaccine efficacy or excessive inflammation[98].

Overall, the combined influence of HLA polymorphisms and microbiota composition highlights the rationale for personalised vaccination strategies against dental caries tailored to the host's immunogenetic and microbial profile in order to maximise protection and minimise adverse effects.

However, the implementation of personalised vaccines against dental caries poses several major challenges, both practical and ethical. Logistically, personalisation requires preliminary testing, such as genetic typing (particularly of HLA genes) and analysis of the oral microbiota. These tests require state-of-the-art technological infrastructure, qualified professionals and significant financial resources, which considerably complicates public health programmes. At the individual level, this could lead to longer and more expensive care pathways, potentially making them inaccessible to certain populations.

4.4.5 Experimental Feasibility: Overview Of Data

The narrative review conducted by Contreras et al. identified 11 recent studies evaluating vaccines against *S. mutans*, the majority of which were in vivo animal trials. The reported results converge towards the same conclusion: several vaccine formulations can induce a local or systemic immune response, particularly through the production of specific salivary IgA, and significantly reducing bacterial load and the severity of caries lesions in experimental models[101].

The diversity of approaches explored, including recombinant vaccines, DNA vaccines, attenuated vector vaccines, plant-based platforms, and passive immunisation, as well as the optimisation of adjuvants (such as chitosan, flagellin, and LTK4R), demonstrates significant progress in terms of technology and immunology.

However, the authors emphasise that most of the available data remains preclinical, without validation in humans. Only the pioneering study by Ma in 1998[88], which we

mentioned earlier, reported a humoral immune response in adult volunteers following experimental nasal immunisation. To date, this is the only documented human clinical evaluation, although advanced phase studies have not followed it.

These experimental data, although still largely preclinical, confirm the scientific feasibility of anti-caries vaccination while highlighting the need to validate the durability of the local immune response, the safety of mucosal vectors, efficacy in real-world conditions, and the cost-benefit ratio before any large-scale clinical application.

III) CONCLUSIONS

Our knowledge of dental caries has evolved considerably in recent decades, moving beyond a purely behavioural or microbiological perspective to incorporate a multifactorial approach that combines genetics, the environment and host biology. The work presented in this thesis clearly shows that while some individuals have a genetic predisposition to caries involving polymorphisms that influence enamel quality, saliva composition, mucosal immune response, or even taste preferences for sugar, this predisposition is strongly modulated by epigenetic and environmental factors.

These environmental factors are numerous and act from the earliest stages of life. They include early and regular consumption of fermentable sugars, low exposure to fluoride, poor oral hygiene, and unfavourable socioeconomic conditions that limit access to care and prevention. Perinatal factors, such as mode of delivery, infant feeding (breastfeeding vs. bottle feeding), and exposure to oral microbiota disruptors (antibiotics, passive smoking), can also influence the maturation of the immune system and microbiota, thereby contributing to individual vulnerability to caries. These influences can act via epigenetic mechanisms (DNA methylation, microRNA), interfering with the expression of genes related to immunity, mineralisation and microbiota regulation.

This complex interaction between early environmental exposures, host biology, and epigenetic regulation not only shapes individual susceptibility to caries but also highlights the limitations of conventional preventive strategies. Furthermore, a growing body of evidence suggests that certain genetic determinants, such as dental topographical vulnerability and sex-related differences in immune response, may influence both caries risk and responses to interventions. In this context, innovative approaches such as vaccination appear to be promising avenues for strengthening host defences and disrupting cariogenic mechanisms, while paving the way for gender-differentiated prevention strategies.

Vaccination against *S. mutans* appears to be an innovative avenue with great potential. The preclinical studies summarised in this thesis confirm the biological feasibility of several vaccine platforms (recombinant, DNA, plant-based, attenuated vectors) capable of significantly reducing bacterial colonisation and the severity of carious lesions in

animals. However, their translation into human clinical practice remains subject to several crucial challenges: the durability of the mucosal response, the tolerance of mucosal vectors, efficacy in real-world conditions, and the influence of genetic variability, including gender, on the immune response. In addition, there is a need for rigorous cost-benefit analyses, particularly in low-resource settings.

In summary, this thesis highlights the need to address dental caries from an integrated, personalised and preventive perspective, considering the complex interactions between the genome, environment, immunity, microbiota and social factors. The future of dental caries prevention lies in an integrated approach based on genetic data, immune mechanisms and advances in vaccination.

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