A SCOPING REVIEW OF THE GENOTYPIC DIVERSITY OF STREPROCOCCUS MUTANS

ABSTRACT

Aim: This paper reviews the literature on the pathogenic genotypes of S. mutans that may be more virulent colonizers and the phenotypic variability of its main virulence factors.
Methods: A thorough literature search on S.mutans was performed and the relevant datas supporting its association to dental caries were extracted.
Results: Dental caries is a microbial disease caused by frequent intake of dietary sugars. Fermentation of sugars by biofilm microbiota produces acids that disrupt microbial homeostasis and cause dissolution of tooth minerals. Identifying S. mutans as the most important cariogenic microorganism has led to the design of target specific preventive measures that intend to reduce its presence in oral cavity.
Conclusion: Due to environmental changes and selective pressure in the oral cavity, Streptococcus mutans endure extensive genotypic diversity thereby exhibiting new physiological and metabolic properties. However, the role of the variants are poorly understood.

Keywords:*Dental caries, Genotypic Diversity, Streptococcus mutans*

Introduction

Dental caries is a ubiquitous chronic diseases affecting people all through their lives. It is a complex multifactorial microbial disease affecting billions of people globally, thereby posing a major public health concern. Although diverse factors aid in the development of dental caries, the disease is mainly driven by the resident cariogenic microbiota which produces acids by metabolizing the dietary carbohydrates. The production of such acids and maintenance of its low pH over prolonged duration in the biofilm of the microbiota, present in proximity to the teeth, predisposes to formation of dental caries by dissolution of the tooth minerals, in an opportunistic manner. Currently the models of dental caries etiology focuses on relating this disease to a microbial ecological shift, which is based on a physiologic imbalance between tooth mineral and biofilm.[1, 2] While dental caries is not a typical infectious disease it is important to know about the infectious and transmissible characteristics of the endogenous oral microflora.

Amongst the plethora of microorganisms present in the oral cavity, the Mutans streptococci have been implicated as the primary agent related to dental caries initiation in humans.[3-5] By virtue of its contribution to the formation of the dental biofilm matrix, its capacity to produce large quantities of organic acids, and its propensity to outcompete non-cariogenic commensal species at low pH conditions.[6] Decades of research on Mutans Streptococci association to dental caries has conclusively narrowed down on to S.mutans. However S.mutans can be

isolated from individuals either with or without a history of caries.[7,8] Thus existence of variations among the colonizingS.mutans is the reason for the subsequent development and progression of caries among different individuals. To understand the infectious nature of dental caries it may be useful to study the genotypes of S.mutans to appreciate its diversity. According to the official Guidelines for Human Gene Nomenclature, a gene is defined as a DNA segment that contributes to phenotype/function.[9] Genotype is the genetic constitution of a cell, an individual or an organism. Also gene regulation is found to play a vital role in the control of the expression of virulence factors such as synthesis of polymeric substances, adhesion, acidogenicity, aciduricity and variability. Diversity is the number of different S.mutans genotypes found within an individual. Genetic diversity of S.mutans remains as a topic of interest, as this provides an explanation for the contrasting caries status in people harbouring it.[10] Thorough understanding of these microbial species and their colonisation stratagems may help in the diagnosis, risk assessment, development of new treatment strategies for caries, so as to prevent disease and promote health in addition to standard prevention treatments.

Human oral microflora is composed of an abundant mix of microorganisms that are part of a multispecies biofilm complex. The oral cavity being a dynamic environment allows for the selection of certain organisms to not only adapt metabolically but also to develop its potential and genomic content in the biofilm.[11]. Although microorganisms belonging to the same genus and species present a common gene set denoted core-genome, which is essential for cellular functioning and the survival of the species, they can differ in their physiological and virulent properties because of their strain specific genes.[12-15] The rest of the genome, referred to the accessory functions, is generally known as the not essential or dispensable genome and is not shared by all strains. Comparative genomic analysis between multiple genomes of individual species has revealed an extensive intra-species genomic diversity. The dispensable genome contributes to the diversity of the species and probably provides functions that are not essential for survival. It also confers selective advantages such as survival and adaptation to different ecological niches, antibiotic resistance, and the capacity to colonize new hosts.[14] This diversity is generated by a variety of processes which include genome rearrangements, horizontal gene transfer by natural genetic transformation, and exposition to genetic mobile elements such as bacteriophages, plasmids, insertion elements, transposons, and genomic islands.[12,16] With the advent of new molecular tools over the past few decades there has been a substantial change in the understanding of dental caries microbial pathogenicity relative to bacterial detection and genotyping.

Rationale: The dental biofilm is composed of complex bacterial community, specific strains of S.mutans have to compete with other strains to establish colonization.[17] Gene regulation resulting in differences in genetic content of each S.mutansstrain will also influence exhibition of phenotypic variability between the strains.[18-20] Knowledge of the various genotype of S.mutans with varied virulence factors and their correlation with other species is fundamental to understand their colonization and establishment in the same individual.[21] It has been suggested that not all strains have the same virulence capacity to promote the

formation of dental caries.[20,22] Therefore, it seems reasonable to expect that at least some of the genes related to virulence are not distributed in the same way in the strains according to their caries status.[23]

S.mutansserotypes

S.mutansbelong to the Mutans streptococci group along with nineteen other species.[24] It can be further classified into specific serotypes based on the cell wall antigenic polysaccharides known as rhamnose-glucose polymers.[25,26]. S.mutans is found to have the highest genetic variation thanother species present in humans and is classified into fourserotypes (c, e, f, and k).[18] Useof polymerase chain reaction (PCR) with primers designed based on the sequence differences of the rgpgene[18,27], has facilitated in determining the frequencies and distribution of these serotype in clinical isolates. Studies have revealed that serotype c is the frequent one corresponding to approximately 70-80% of the isolates in the oral cavity, followed by serotype e (20%), and serotypes f and k corresponding to less than 5% and 2% respectively.[18,28-30] This distribution also highlights different pathogenic patterns in S.mutans.

Environmental changes, selective pressures, and the presence of a variable genome in S.mutans cues its adaptation as well as strongly influence the acquisition of new physiological and metabolic properties that alter the dental biofilm homeostasis, leading to development of dental caries. Natural genetic transformation is a genetically programmed process which offers the recipient microorganism with the ability to acquire new phenotypic traits by facilitating the integration of dispensable genes.[21,31] Resulting in generation of a wide range of genome heterogeneity thereby promoting the emergence of resistance, genetic variation, and the rapid evolution of virulence factors.[14,21,31,32] This process is carried out as long as the cells have the ability to enter a physiological state known as competence which allows them to take up exogenous DNA from their environment and incorporate it into their genome.[33] It a mechanism of ecological importance as S.mutans manages to adapt to the changing environments, originating from different selective pressures.[21,34]

Although the existence of heterogeneity of S.mutansis widely known, very little is known about the mechanisms for the expression of pathogenic properties in specific genotypes. The employment of the state of the art molecular biology techniques in the various studies on S.mutanshas provided enormous information on the evolution, pathogenesis, diversity, metabolic activities, and virulence properties of this species.[35] The three main natural strategies that generate genetic variations are:
(1) Small local changes in the nucleotide sequence of the genome, which explains the presence of four serotypes
(2) Intragenomicreorganization of segments of genomic sequences, and
(3) Acquisition of DNA sequences from another organism

Intragenomic rearrangements

Genomic rearrangements are mutations that change the gene content of a genome or the arrangement of the genes in a genome.They can be categorised as deletions, duplications, insertions, inversions, and translocations. They occur due to break in long stretches of DNA involving at least two different locations, followed by a re-ligation of the broken ends to produce a new chromosomal arrangement.[36] Sometimes this rearrangement could encompass a cluster of genes depending on the size of the rearranged fragment.[37] Rearrangements therein influence the structure of the chromosome through disruption of an existing gene or by creation of a new gene, thereby affecting its expression instigating phenotypic variability.[36]

In 2002 the complete genome sequencing of the S.mutans strain UA159 has helped unriddle the complexity and genetic specificity of S.mutans.[21] Since then UA159 is frequently used as a reference strain for variability studies.[13] Till date, a large number of S.mutans genomes have been sequenced and 188 genomic sequences of S.mutans are available in the NCBI database for the researchers. In comparative genomics studies the genomic sequences are often used to identify the virulence factors. It involves comparing genomes from different strains, then detecting the differences and similarities, therebyrevealing common molecular and pathogenic mechanisms which can be related to specific phenotypiccharacteristics.[38]

A pioneering study by Maruyama et al on S.mutans using comparative genomic analysis provided extensive information on the species-specific genetic content of S.mutans, while comparing strain UA159 with strain NN2025. The findings of the study were, althoughthese strains belonged to the same serological group(serotype c) with expression of the same biochemical, adhesive, and cariogenicproperties as well as similarity in 90% of their genes the strains presented eight variable regions with more than 30 genome rearrangements. The NN2025 strain genome contained eight strain-specific regions while the UA159 strain genome contained nine strain-specific regions.[34]

Shields et al. utilized transposon sequencing technology and discovered that only 11% of the S.mutans UA159 genome is essential, with the presence of genes encoding products required for replication, translation, cell wall biogenesis, and the lipid metabolism, as well as genes necessary for survival, growth, and persistence colonization in both experimental and clinical conditions. It was suggested that S.mutans strains evolve through a process of chromosomal shuffling, which plays an important role in the genomic diversity of this species.[32]

Song et al. sequenced the genomes of six S.mutans clinicalisolates and compared them to the reference strains UA159 andNN2025, focusing on characteristics related to pathogenicity. Genome alignment study revealed existence of chromosomal rearrangements amongst the strains. High variations in virulence-related genes between the strains was apparent. In addition to these findings the genomic regions required for S.mutans survival in differentenvironments were also discovered.[31]

Horizontal gene transfer(HGT):

It is the movement of genetic information within a wide range of the bacteria which inhabit the human oral cavity.[11] As a result of it strain variants can be spawned due to loss, duplication, or modification of existing genes.[12]Often through this mechanism new phenotypic traits are acquired to provide a selective advantage to the microorganism. Therefore, due to the genomic diversity presentamong different isolates, the genome content of a single strain does not necessarily represent the genomicpotential of certain species.[34]

Hoshino et al. and Argimón et al. determined the origin of glycosyltransferases (GTFs) in the Streptococcus genus. This enzymecatalyses the synthesis of glucans from sucrose and are is encoded by gtf genes. The authors proposedthat the Streptococcus acquired the genes gtf by HGT and then were capable of forming cariogenic biofilms.[39,40]

Cornejo et al. sequenced 57 S.mutans isolates to determine the general structure and the potentialadaptive characteristics of both the core and dispensable genomes. The genomes of the studied strains were highly variable and their global genetic compositions were found to differ markedly from one isolate to anotherowing to the high HGT rate.[15]

Meng et al. performed a pan-genome analysis of 183 S.mutansstrains and determined that this species has an open pan-genome, thereby indicating that new genes can befound as more genomes are sequenced. An open pan-genome has been found to beassociated with the adaptation of the bacterial species.[38]In addition, this is a distinctive characteristic of species whichcolonize diverse habitats and coexist with other microorganisms in large communities. It is typical ofspecies that have the ability to exchange genetic material and have a high rate of HGT.[41]Hence, these findings suggest that the genome of S.mutans may be expanding gradually over time.

Gene regulation:

S.mutanshave been studied in some detail at genetic level, however many of the discovered genes have not been studied in the context of genetic competenceuntil the past few years.[42]This can actually help us in understanding the cell-cell communication in S.mutans.[43]The various mechanisms of gene regulation in S.mutans include competence, signal transduction, quorum sensing, and small RNA (sRNA) regulation. For survival in a dynamic environment such as oral cavity, microorganisms were found to use regulatory systems to detect and respond rapidly to the stimuli generated.[44] Presentation of these regulatory genes involves complex coordinated processes that depends on the internal and external signals.[45]

Shields et al. detected and characterized 20 novel genes that have a substantial impact on competition regulation and competence-related phenotypes.It has been studied that acquisition of natural competence is mostly transient and the genes encodingare not expressed as well.[44] The activation of the transcription of these genes take place in reaction to specific signals, and only when the environmental conditions are conducive. It isfound out

that in S.mutans, a networkof genes and at least two peptide signaling molecules are accountable for the development of its genetic competence and its expression varies among the clinical isolates.[31,40,46]This was verified by Palmer et al., when they revealed that 15 isolates from geographically diversepatients with caries presenteddifference in the genetic content and phenotypiccharacteristics associated with virulence.[47]

Strain UA159 were found to be naturally competent, transformable, and have a systemcommitted to competence and quorum sensing, which is regulated byspecial signal peptides. Additionally, this systems also coordinates the development of antagonistic interactions and the synthesis of antimicrobial bacteriocins as a means to acquire the transformingDNA by competing with others. This is achieved either by eliminating closely related streptococcal species or through an altruistic suicide mechanismamong a subpopulation of competent cells within thecommunity of S.mutans.[47-49]

S.mutanshave also been found to be able to trigger a competence cascade. Besides the existing association between natural genetic competence and stress response pathways,multiple environmental factors of the oral cavity canprovoke a direct impact on the competence cascade of S.mutans.[50] This phenomenon was evidenced in the results of the experiments carried out by exposing the cells to low pH conditions. Similarly, oxygen is also considereda key factor which significantly alters S.mutans transcriptional regulation. It is found to strongly alter the competition by decreasing bacteriocins expression.[51]Carbohydrate source was as well found to have a considerable effect on theprogression of the competent state of cells.[50] Hence these studies approve that the S.mutanshas evolved from a network of regulators to integrate its cellular response to environmental change.[52]

Phenotypic diversity:

As a consequence of the differences in genetic content and gene regulation, S.mutans strains exhibit phenotypic variability.[18-20] Around 52 different S.mutans genotypes have been reported in saliva and oral biofilms, with the existence of 1–5 different genotypes of S.mutans species in the same individual.[53,54] It has beensuggested that not all strains have the same virulence capability to encourage the development of dental caries.[20,22]So, practically some of the genes related to virulence are notdistributed in the same way in the strains according to their caries status.[23]Acidogenicity, aciduricity, and adhesion properties of S.mutans are considered as the key virulence factors.[3,55,56]The study of these virulence factors and their correlations with other species present in the biofilm is crucial in understanding the role of colonization of multiple genotypes in the same individual.[53]

Acidogenecity:

The ability to produce organic acids from the carbohydrate metabolism under anaerobic conditions, with a drop in the pH value below 4.0.[3,57] Expression of the genes related to carbohydrate absorption and metabolism seem to be responsible for the effective adaptation of S.mutans in the oral cavity. Carbohydrate fermentation is the key strategy for survival of

S.mutans. They have been found to have all the genes necessary for a complete glycolytic pathway and they can produce acids even when the pH of theoral cavity is relatively low. There is sufficient evidence that S.mutans is one of the most acidogenic species inhabiting the dental biofilm.[58]They can also lower the dental biofilm pH due to the intracellular polysaccharide (IPC) metabolism in the absence of fermentable carbohydrates from the diet. This was confirmed in a study by Harris et al. where they generated an IPC-deficient strain (SMS203) from the cariogenic strain UA130 and assessed itsacidogenecity. It was found to belower than the progenitor strain, thereby resulting in reduced cariogenic potential.[59]

Aciduricity:

It is the ability to survive rapid and extreme changes in pH and is considered to be one of the most important attributes of cariogenic bacteria. S.mutanscan grow and carry out glycolysis at low pH values, thereby establishing a selective advantage over less aciduricspecies.[60,61]In order to do this, S.mutans are able to modify its physiology and survive in these environmentsdue to its adaptive acid tolerance response (ATR) mechanism. This is possible due to at higher levels of expression of theproton translocator F0F1-ATPase pumps.[60,62].This pump works by preserving a more alkaline cytoplasmic pH comparedto the extracellular environment by transporting protons out of the cell and also confers protection to acid-sensitive glycolytic enzymes.[60,63,64]Acidogenicity and aciduricity can be considered as the main factors contributing to S.mutanscariogenicity, as variations in these characteristics could help explain the differences in virulence among clinical isolates.[20,55,56]

Adhesion:

Colonization and survival of S.mutans is facilitated by its adherence to the tooth surface.[60] This adhesion, can be mediated by two mechanisms: sucrose-independent and sucrose-dependentmechanisms.[55,65] The first mechanism is not relevant for S.mutans virulence.[56,60]However, the sucrose-dependent mechanism is considered to be responsible for colonization of oral cavity.[55]The enzyme glycosyltransferase(GTFs), hydrolyses sucrose into glucans and fructans, The extracellular polysaccharide, glucan, not only promotes adhesion of bacteria to tooth surfaces but also promote aggregation and coaggregationof microorganisms favouring biofilm formation.[55,56,65]It has been found that S.mutans possesses three GTFs encoded by the gtfB, gtfC, and gtfD genes.[55,66]The genes encoding gtfB and gtfC are close to eachother, have 95% sequence homology, and are subject to the same regulatory processes. These genes are found to be expressed in response to excess glucose or sucrose.[56] The study by Yamashita et al. demonstrated that the loss of anyof these genes resulted in reduction in the virulence of S.mutans.[67]

Caries and genotypic diversity studies:

Pieralisi FJS et al. and Zhou Q et al. investigated the genotypic diversity of S.mutans in children with and without early childhood caries (ECC) and found a strong genetic diversity among S.mutans strains. They also stated that caries active children carried more genotypes than caries free children.[68,69]

Valdez RMA et al. studied the genotypic diversity and phenotypic traits of S.mutans isolates and concluded that there were no differences in genotypic diversity among CF, ECC, and severe ECC children. However, the phenotypic traits such as, acidogenicity and acidurity were found to be high in children with S-ECC.[70]

Napimoga et al evaluated the relationship between clonal diversity and some virulence traits of S.mutans isolated from caries-free and caries-active subjects. It was found that there was larger number of genotypes of S.mutans with increased ability to synthesize water insoluble glucans in caries-active individuals.[53]

Ravikumar et al. in 2021 reviewed seven studies related to S.mutans genotypic diversity and stated that the number of genotypes of S.mutans varied between caries active and caries-free children. They also insisted on the need for further studies to draw a definitive conclusion.[10]

Conclusion:

This review highlights the complexity and dynamic changes in the genetic and physical components of S. mutan.It is obvious that S.mutans has evolved numerous strategies to become well established in the oral biofilms, toantagonize the growth of commensals, to produce organic acids from dietary carbohydrates, and topropagate and metabolize under acidic conditions thereby favouring the initiation and progression of dental caries. The overall findings indicate that the acquisition of virulence genes is only a first step on the path towardsthe S.mutans pathogenic lifestyle, it additionally requires mild genetic changes mediated by regulatory genes toadapt the expression of phenotypes with pathogenic potential according to the environmental pressures.The association between number, genotypic diversity, and caries status of an individual is still controversial. Future studies should explorethe possibility of simultaneous action of different genotypes, with different phenotypic potentials, resulting in different virulence characteristics,which ultimately alters the risk of developing caries.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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