Applications of Nanotechnology in the Immunogenic Detection of Allele Risk Gene MBL2 in Teeth Plaque Patients

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Nanotechnology has been explored in the development of dental bioactive materials to reduce or modulate the activity of caries-related bacteria. Nanostructured platforms based on calcium phosphate and metal particles have been developed to provide anti-caries potential to restorative materials. The bioactivity of these platforms prevents the loss of minerals of the hard tooth structure and antibacterial activity against caries-related pathogens. Immunogenetic study in teeth plaques patients was revealed that the primer pair flanking of promoter of MBL2 gene was successfully amplified the target under amplification. The PCR products of target of MBL2 shown 614bp. And sequence analysis sown present SNP rs7096206 G>C. The polymorphism of MBL2 shown The Odd Ratio was higher in genotype GC with OR= 1.56(0.4-5.7) with P value 0.5 and CC with OR=1 (0.2-4.7) with P-value 1, and the allele frequency was higher in with C allele 16(40%) in patient group with high value of OR=1.24(0.5-3.1). Fifty samples were collected from patients suffering from severe teeth decay from different outpatient clinics of dentistry, periodontitis patients attended to specific dental health center and outpatient clinics of dentistry during the duration from (September 2022 to February 2023). Patient age ranges from 10 to 50 years. The blood sample was taken by syringe save in EDTA tube for DNA extraction. The PCR products of target of MBL2 shown 614bp. And sequence analysis shown present SNP rs7096206 G>C, The polymorphism of MBL2 shown the Odd Ratio was higher in genotype GC with OR= 1.56(0.4-5.7) with P value 0.5 and CC with OR=1(0.20-4.7) with P value 1 , and the allele frequency was higher in with C allele 16(40%) in patient group with high value of OR=1.24(0.5-3.1). P.value = 0.6, while the allele frequency low 14(35%) in control group. The polymorphism of MBL2 based on sequence assay shown role of allele risk correlated with patient group more than in healthy group.

Keywords: Dental caries, Nanotechnology, applications of nanomaterials, MBL2, rs7096206 , Allele frequency.

1. Introduction

Dental caries, additionally called cavities and teeth decay, are each not unusual place and preventable, Dental caries was considering one of the popular human health problems that need health service [1]. Different bioagents associated with teeth decay; Bacteria, fungi and protozoa., a significant pathogenic specialist of dental caries, [2]. Candida have essential role in the occurrence and progressionnof infection, due to virulence factors. Where One group of these factors leads to the colonization to take place, or the initiation of an infection, whils the other group helps to spread of the infection [3]. Mannose-binding lectin (MBL) is a protein molecule inherent to the immune system, in which the activation of lectin domains are found in relation to collagenous structures [4]. It has an important role in the autoimmune system, as studies have shown that MBL2 gene polymorphism is susceptible to autoimmune diseases, infections and diabetes [5].

2. Materials and Methods

Sample collection : Fifty blood sample were collected from patients suffering from severe teeth decay from different hospitals and were diagnosed by special physician, the control group was composed of 50 randomly healthy persons without teeth decay. All do not up take any antibiotic before three days ago, both with age ranged (10-50) years during the period from the beginning of September 2022 to February 2023. Also, 3ml of blood samples were collected in EDTA tubes from same patient for molecular study, specimen be saved under -20 in frozen stat.The genotype distributions of SNPs in genes by used the PCR-sequences and sequence analysis polymorphisms for MBL2 after using PCR.

Genomic DNA extraction kit (Favrogen) from frozen blood

A 180Microleter of frozen blood were transferred to a 1.5ml for each.microcentirfuge tube. 200ul of FBAG buffer was added to the cells and re-suspend the cells by vortex. The cells were resuspend in 40µl of proteinase was added for each. solution were added , mixed well by vortexing, the samples were incubated at 60°C for 30 min. A 200µl of ethanol (96-100%) was added and intermixes well by vortexing for10 seconds.Aspin Column was placed in Collection Tube.The sample mixture(including anyprecipitate) was transferred carefully to spin Column.Centrifuged at11,000rpm for 30 second then the spinColumn was placed to a new Collection Tube. A total 400µl of W1 Buffer was added to the spin Column.Centrifuged at 11,000 rpm for 30 seconds and discarded the flowthrough.A600µl of Wash Buffer was added to the spin Column.Centrifuged at 11,000 rpm for 30 seconds and discarded the flowthrough, and the spin Column was placed back to the Collection Tube,centrifuged at full speed (12000 rpm) for an additional 3 min to dry the column. The spin Column was placed to a Elution Tube.A 50~100µl of Elution Buffer was added to the membrane center of the spin Mini Column. centrifuged at full speed (12000rpm) for1min to elute total DNA. total DNA was stored at 4°C or -20oC. DNA was amplification according to Suchman, E, [14].

Primer Design and PCR

Molecular detection was conducted by PCR with a primer

ML2F:5'- CCTGGTTCCCCCTTTTCTCC-3', ML2R: 5'-AGATGGACCCGAAGAGGACA-

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Statistical Analysis

Chi square test was used to test the deviation from Hardy-Weinberg Equilibrium (HWE) of SNPs by comparing the observed and expected frequencies. The association of different genotypes with the risk of teeth decay was estimated by calculation of odds ratios (OR) with 95% confidence intervals (CI). Statistical significance was set at $p < 0.05$. based on SPSS software.

3. Results

The profile gel-electrophoresis 23 genomic DNA whole blood samples Figure (1) was shown huge bright bands of DNA extracted from whole blood of human patients undergo teeth plaques symptoms.

Figure(1): Gel-electrophoresis illustration quality of DNA extracted from teeth plaques patients group, 1-21 patient samples, M=molecular marker 100bp for first step.

The results shown success the primer pair efficiency to amplification region 52185415-52186396 as target DNA region of MBL2 gene included, the amplification region with flanking primers 614bp Figure (2).

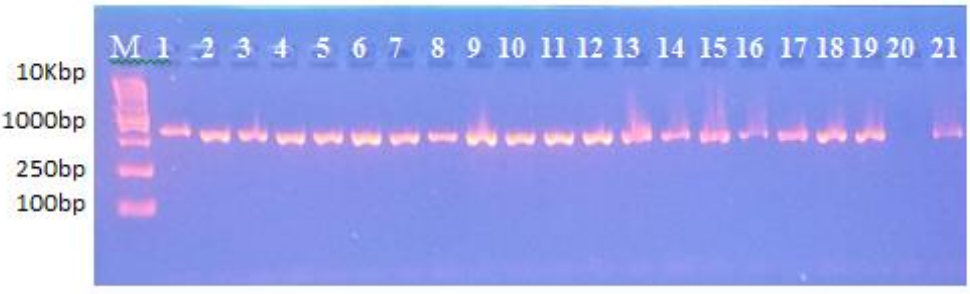


Figure (2): Gel electrophoresis profile of target DNA region of MBL2 gene for patient group amplification region with flanking primers, 1-21 patient PCR products 614bp, M=molecular marker, first step100bp.

Location of Mannose binding lectin 2(MBL2) gene:

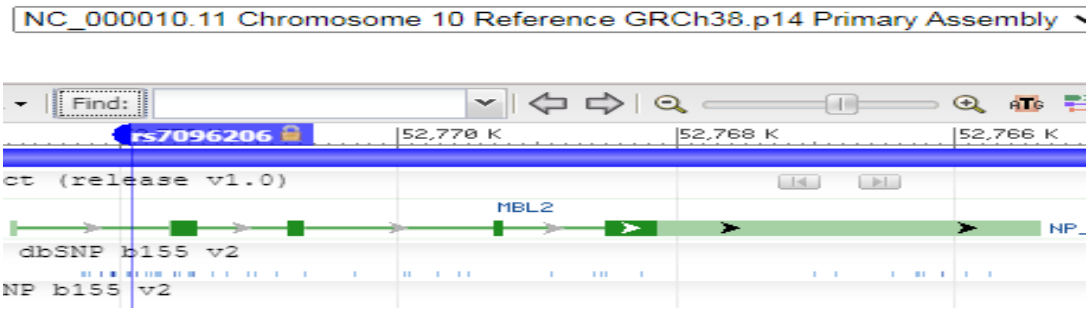
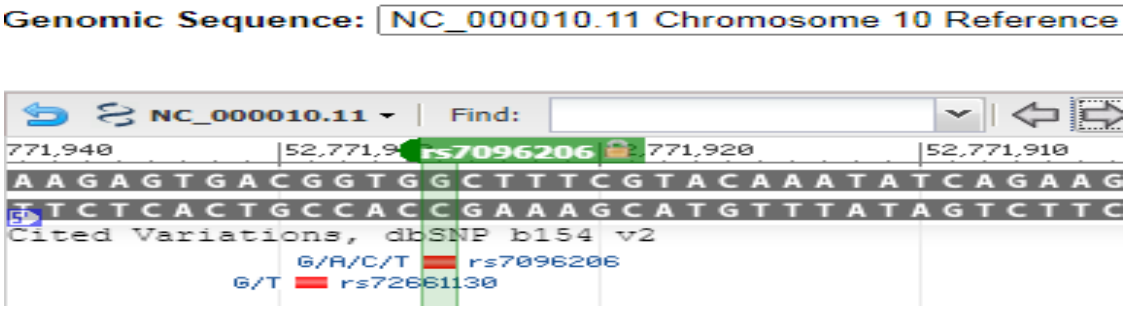


Figure (3): Illustration MBL2 location in Chr:10, and site SNP rs7096206 G>C.

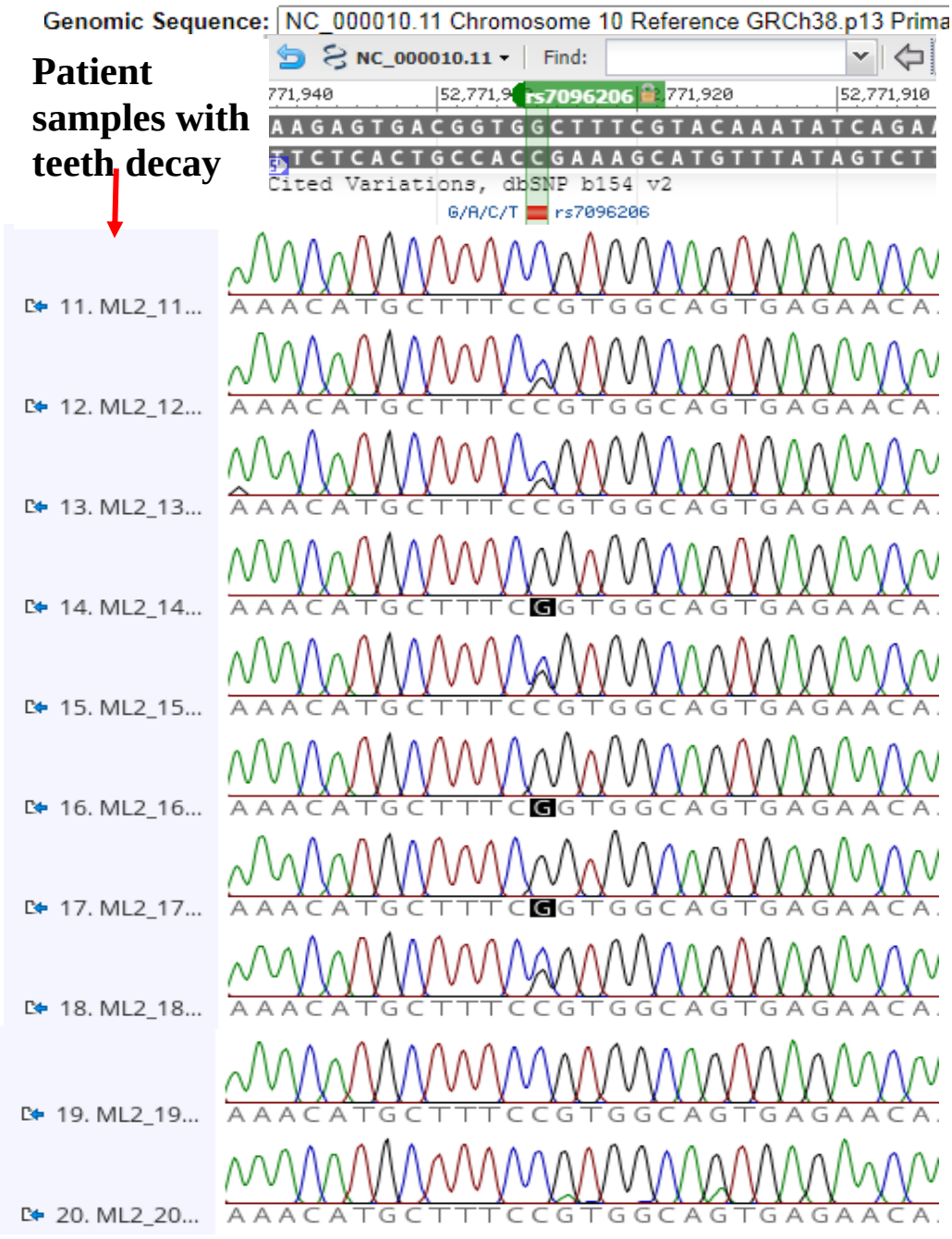
To conformed the validity of SNP: rs7096206 G>C, the site SNP was at 52771925 on Chromosome 10, a combination of SNP site of rs7096206 G>C Homozygous allele C mutant to allele T, Figure (4).



Figure(4): Location validity of SNP rs7096206 0n site 52771925 on Chr:10

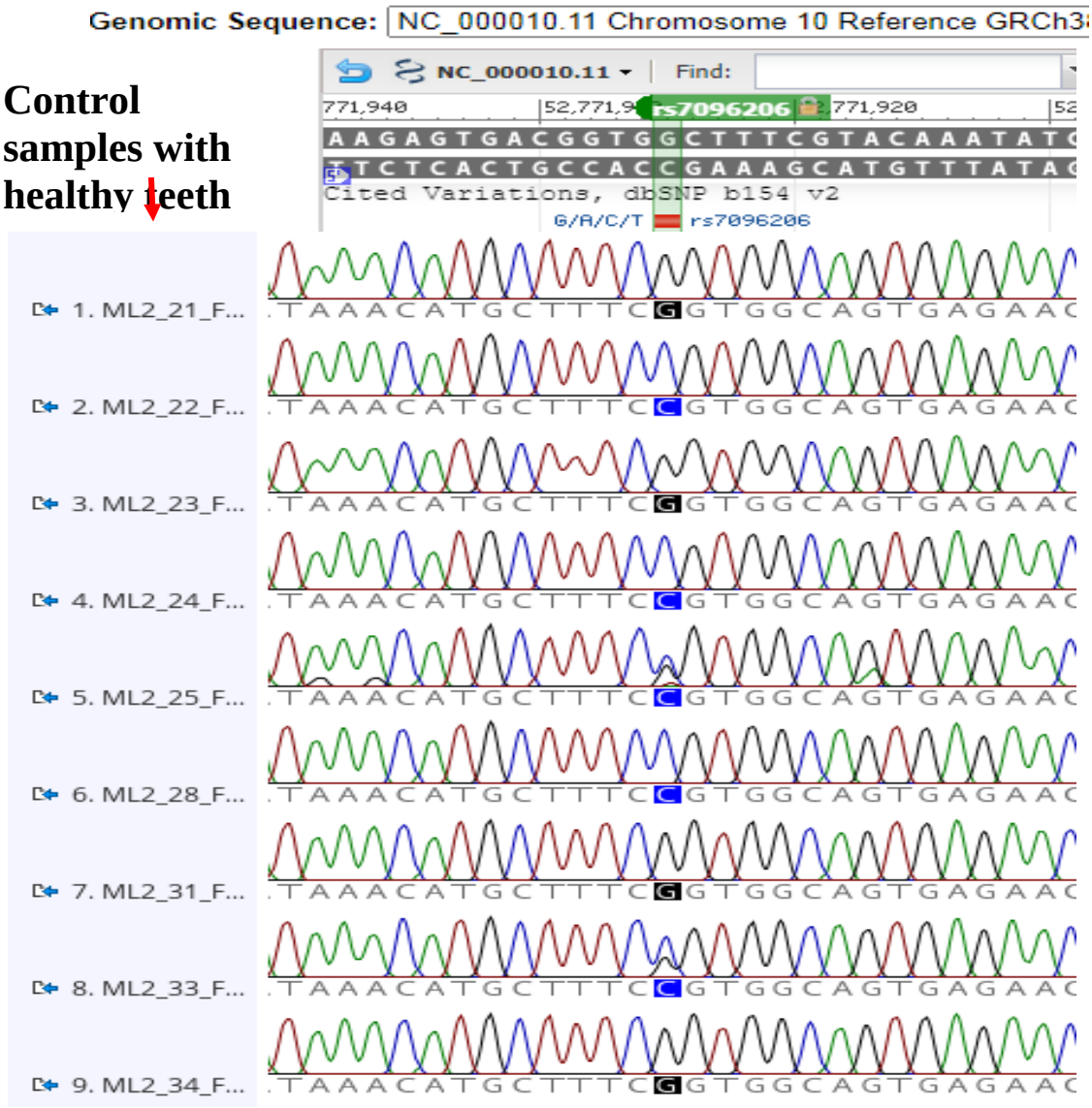
The multiple alignment of chromatograms of targeted region:

Figure(5): The multiple alignment of chromatograms of partial sequence of Chr:10, shown SNP rs7096206 G>C correlated with patient disease(1-10 cases). Alignment performed by Geneious prime softwar.



Continue figure (6) : The multiple alignment of chromatograms of targeted region shown three SNP rs7096206 G>C teeth decay patient disease (continue 11-20 cases). alignment performed by Geneious prime software.

The multiple alignment of chromatograms of Healthy group:The results of targeted region shown SNP rs7096206 G>C correlated with healthy (control group).



Figure(7): The multiple alignment of chromatograms of partial sequence of Chr:10, shown SNP rs7096206 G>C, 1-9 control cases. Alignment performed by Geneious prime software.

Table 1: Genotypes distribution and allele frequency of wild type allele and mutant allele of SNP: rs7096206 G>C, OR values and p values for Candidiasis infection patients.

rs7096206 G>C	Case N=20		Control N=20	OR(95%CI)	P-value
genotypes	GG	8(40%)	10(50%)	Reference group	
	GC	8(40%)	6(10%)	1.56(0.4-5.7)	0.5

	CC	4(20%)	4(20%)	1(0.2-4.7)	1
Allele Frequency	G	24(60%)	26(65%)	0.65(0.26-1.6)	0.6
	C	16(40%)	14(35%)	1.24(0.5-3.1)	0.6

4. Discussion:

The results shown success the primer pair efficiency to amplification region 52185415-52186396 as target DNA region of MBL2 gene included, the amplification region with flanking primers 614bp Figure (1). Many studies than ever before achieving the associated between MBL2 polymorphism and teeth decay disease [6]. Three polymorphic sites have been identified in exon1 of the MBL2 gene, at codon 52 (CGT to TGT), codon54 (GGC to GAC) and codon57 (GGA to GAA) [7].there is study done by [8] that the achievable associated between the MBL2 gene polmorphism and children with early acute dental caries disease three polymorphic sites in the MBL2 gene varying between different ethnic groups. The protein recognizes and binds to mannose and N-acetylglucosamine on many microorganisms. These polymorphisms had been achieving in many diseases, Where in innate immunity plays a crucial role especially in infections of bacteria, tuberculosis, rheumatic fever, sepsis, lupus erythematosus [9].Based on sequencing results of MBL2 gene , one single-nucleotide polymorphisms (SNP) in MBL2 become decided in 30 patients with caries and in 30 age-matched caries-unfastened controls. The SNP rs7096206 G>C examined with inside the on MBL2 gene. The MBL2 gene becomes proven to be related to an excessive occurrence of caries in our cohort. In addition, In conclusion, the facts imply that rs7096206 G>C with inside the MBL2 gene become proven to be related to an excessive occurrence of teeth decay in our cohort, and a pair of haplotypes also are concerned with inside the elevated susceptibility to dental caries[10].The MBL2 gene located on Chr: 10q21.1 with length7405 nt , and it include four exons and three introns thate situated on chromosome10 ,This gene encodes the soluble mannose-binding lectin or mannose-binding protein found in serum. The protein encoded belongs to the collecting family and is an important element in the innate immune system. The protein recognizes and binds to mannose and N-acetylglucosamine on many microorganisms, including bacteria, yeast, and viruses including influenza virus, HIV and SARS-CoV. This binding activates the classical complement pathway. Deficiencies of this gene have been associated with susceptibility to autoimmune and infectious diseases[7].MBL2 is a gene that has ability to infect infectious diseases, the relationship between polymorphisms of this gene and the susceptibility to caries was first reported by [6]. Researchers who did the analysis the two SNPs (codons 54 and 57) using polymerase chain reaction restriction fragment length polymorphism assays they not find any significant associations[11]. A study was about the relationship of MBL2 (rs1100325 and rs1800450) gene polymorphisms and dental caries disease in children where they found that allele G was a risk factor for decay Other studies also Similar results were shown [10,12]. MBL2 genes were determine in the Ensembl database, the gene in the Splice .Genotyping risk allele and allele frequency results of multiple alignments of chromatograms were shown present three genotypes, GG, GC and CC. The allele C was considered mutant allele based on valid SNP rs7096206 G>C pointed on chromosome 1 at site at 52771924

which mutant allele G>C on upstream DNA Figures (5-6).The genotype GC and CC were shown more distribution in patients undergo teeth decay compared with control group, the values of Odd Ratio (OR) were support that C allele in both TC and CC was correlated with disease under interest and considered as risk allele. The Odd Ratio was higher in genotype GC with OR= 1.56(0.4-5.7) with P value 0.5 and CC with OR=1(0.20-4.7) with P value 1 , and the allele frequency was higher in with C allele 16(40%) in patient group with high value of OR=1.24(0.5-3.1) , P.value = 0.6, while the allele frequency low 14(35%) in control group Table(1).while In study done by [10] on gene polymorphisms and dental in Sudia children ,they are clear up that allele G was a risk factor for decay.[12] also found that there were on gene polymorphisms and caries in Iranian adults. In[13] study they found there were no associated with teeth decay were explicating include MBL2 (rs7096206C/G) (odds ratio, 0.721; 95% confidence interval 0.449–1.156) or MBL2 (rs7095891G/A) (odds ratio, 1.076; 95% confidence interval, 0.675–1.177).

5. Conclusion:

Bioactive nanoparticles in restorative polymeric materials were discussed as useful strategies for the prevention and management of caries-related bacteria. This research was carried out to characterize single nucleotide polymorphism at the MBL gene The Immunogenetic study was revealed that the primer pair successfully amplified partial sequence of MBL2 gene. This study attempt to Evaluation MBL2 gene polymorphism by Nano in patients with teeth decay.

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