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# Association between MMP 13 Gene Polymorphism [rs2252070] and Irreversible Pulpitis- A Case Control Study

**Running title**: Association between MMP 13 Gene Polymorphism and Irreversible Pulpitis

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**Abstract**

**Introduction**

Microarray is a recently developed simultaneous analysis of expression patterns of thousands of genes. Expression profiling using the microarray technology represents a powerful approach in the study of gene function in human

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cells and tissues. The aim of this study is to find the association between MMP 13 gene polymorphism [rs2252070] and irreversible pulpitis.

**Materials and methods**

The present study was carried out at the Department of Endodontics , Molecular study lab at Saveetha Dental College, Chennai. This pilot study included a total of 100 participants in the 20-40 year age group. The study participants were divided into two groups, Group A (n=50)- Case group ( Patients with irreversible pulpitis) Group B (n=50)- Control group. Genotyping was performed with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique.

**Results**

Data entry and analysis were performed using SPSS version 17.0. The Hardy–Weinberg equilibrium (HWE) was tested in each group using v2-test. MMP13- A>G gene polymorphisms can be considered a significant genetic marker, emphasizing the pivotal role of genetic components in the pathogenesis of irreversible pulpitis .

**Conclusion**

This study showed the association between MMP-13 gene polymorphism with irreversible pulpitis in the South Indian population. From genotyping, predictions can be made for patients' genetic predispositions to disease, allowing strategies for care to become more individualized.

**Keywords:** Gene expression, irreversible pulpitis, Microarray, Matrix metalloproteinase, DNA genotyping.

**Introduction**

Microarray is a recently developed simultaneous analysis of expression patterns of thousands of genes. Expression profiling using the microarray technology represents a powerful approach in the study of gene function in human cells and tissues.(Russell, Meadows and Russell, 2008; Hardiman, 2009) Due to its unique ability to analyze simultaneously the expression levels of thousand genes in a single experiment, this approach represents a good tool in the identification of genes involved in different physiological and pathological conditions and has been largely used in the molecular characterization of different diseases. Different reports have demonstrated the usefulness of the microarray technology in the study of the gene expression profiles of healthy and carious pulp tooth as well as of other dental disorders.(Zhao, 2003; McLachlan, Do and Ambroise, 2005; Müller and Nicolau, 2006)

The identification of genes involved in the molecular response of pulp tissue under carious lesions would be very important for improvement of diagnosis and treatment of pulpal diseases. However, to generate sufficient amounts of RNA to allow microarray analysis, these experiments are generally carried out by pooling samples from different subjects.(Torun *et al.*, 2017; Chen *et al.*, 2020) As a consequence, data reported in the literature provides results representing the average gene expression profile in different patients. Since each individual shows a different genetic background, the use of pooled samples from several subjects increases the inter-patient variability in case-control studies.(Zhang *et al.*, 2018, 2019)

Moreover, this genetic heterogeneity makes it difficult to discriminate between transcripts that specifically play a role in the disease from those that may be altered due to allelic variation. To increase the specificity of the results provided by this approach, the use of samples obtained from single individuals would be of great usefulness, allowing for an inter-patient variability to be ruled out affecting the quality of the results.(Zhang *et al.*, 2018; Chen *et al.*, 2020)

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Previous studies have reported the possibility of obtaining a sufficient amount of RNA to be used in microarrays experiments by means of the total amplification of the RNA obtained from as little as a single cell.(Fujii *et al.*, 2018; Kim and Choung, 2020)This approach allows microarray experiments to be performed when a limited tissue amount is available, avoiding the necessity of pooling RNA from different individuals.Previously our team has a rich experience in working on various research projects across multiple disciplines (Soh and Narayanan, 2013; Campeau *et al.*, 2014; Christabel, 2015; Thamaraiselvan *et al.*, 2015; Christabel *et al.*, 2016; Kumar and S, 2016; Ramesh *et al.*, 2016; Thangaraj *et al.*, 2016; Govindaraju and Gurunathan, 2017; Kumar and Rahman, 2017; Sridharan, Ramani and Patankar, 2017; 'Fluoride, fluoridated toothpaste efficacy and its safety in children - review', 2018; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Mehta *et al.*, 2019; Ponnulakshmi *et al.*, 2019) Now the growing trend in this area motivated us to pursue this project. The aim of this study is to find the association between MMP 13 gene polymorphism [rs2252070] and irreversible pulpitis.

## Materials and Methods

The present study was carried out at the Department of Conservative dentistry and Endodontics , Molecular study lab at Saveetha Dental College, Chennai, India. This pilot study included a total of 100 participants in the 20-40 year age group (mean age:25.5 + 11.2). The study participants were divided into two groups,

Groups (N=100)

Group A (n=50)- Case group ( Patients with irreversible pulpitis)

Group B (n=50)- Control group

Inclusion criteria were as follows: Patients of irreversible pulpitis, Patients of age group between 20-40 years. Exclusion criteria were as follows: known systemic diseases diabetes, having deleterious oral habits (e.g. smoking, tobacco chewing, pan chewing) and associated lesions (e.g. leukoplakia, erythroplakia, oral submu- cous fibrosis) and pregnant or lactating.

### Collection of Samples

Buccal smears were collected from the patients in the posterior region of back and forth motion without touching in labial mucosa. Smears were stored in the refrigerator. Samples from 50 patients with irreversible pulpitis were taken followed by 50 control samples. Both case and control groups were labelled accordingly.

### DNA Extraction and Genotyping

Genotyping was performed with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique : forward primer: 50 –TTGTCAAGACATG, CCAAAGTG-30 reverse primer: 50 - TCAGACATCTCCAG ; TCCTATA-30. Cycling was carried out as follows: initial denaturation at 94C for 3 min, 32 cycles each at 94°C for 30 s, 55 °C for 30 s, 72°C for 45 s, and one cycle at 72°C for 5 min. Digestion of PCR products with NIaIII (Neisseria lactamica) restriction endonuclease enzyme yielded 13 + 54 + 233 bp fragments in the (GG) homozygous state and 13 + 122 + 111 + 233 bp fragments in the (GC) heterozygous genotype.

### Statistical Analysis

Data entry and analysis were performed using Statistical Package for Social Sciences for Windows, version 17.0 (SPSS; IBM, USA). The Hardy–Weinberg equilibrium (HWE) was tested in each group using v2-test. The allele ratio and genotype distribution of irreversible pulpitis patients and healthy controls were analyzed with v2-test. $P \leq 0.05$ was considered to be statistically significant.

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**Results**

Table 1: Genotype frequencies of G>A MMP-13 (rs2252070) gene polymorphism among the cases and controls

| Groups | AA | AG | GG | A | G | HWE value |
|---|---|---|---|---|---|---|
| Case ( N=50) | 22 | 17 | 11 | 0.61 | 0.39 | 0.043 |
| Control(N=50) | 25 | 15 | 10 | 0.65 | 0.35 | 0.016 |

For departure from Hardy-Weinberg equilibrium (HWE), chi-square with one degree of freedom. The genotype frequency of cases and controls do not differ significantly $\chi$ 2df (P = 0.835).

Table 2: Overall genotype distribution of the MMP-13 (rs2252070) gene polymorphism in cases and controls

**Dominant**

| Genotypes | Case | Control | Unadjusted OR (95% CI) | p value |
|---|---|---|---|---|
| AA | 22 | 25 | 0.7857 (0.3577-1.7258) | 0.5480 |
| AG+GG | 28 | 25 | | |

**Recessive**

| Genotypes | Case | Control | Unadjusted OR (95% CI) | p value |
|---|---|---|---|---|
| AG+AA | 39 | 40 | 0.8864 (0.3383-2.3226) | 0.8061 |
| GG | 11 | 10 | | |

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**Allele**

| Genotypes | Case | Control | Unadjusted OR (95% CI) | p value |
|---|---|---|---|---|
| A | 61 | 65 | 0.8422 (0.4740-1.4963) | 0.5581 |
| G | 39 | 35 | | |

Figure 1: High molecular weight human genomic DNA isolated from the samples

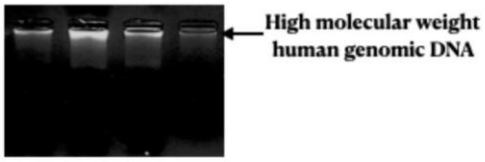


Figure 2: G>A polymorphism of MMP-13 (rs2252070) (A) Agarose gel electrophoretogram of showing 445 bp amplicon in lanes 1-4 (Lanes [M]: 100 bp DNA ladder)

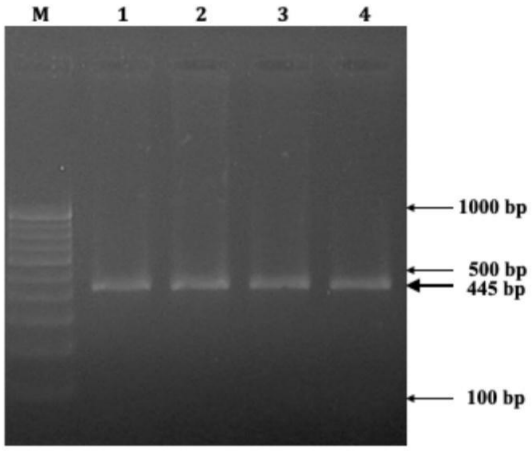

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Figure 3: BfeI digestion of PCR amplified product (Lanes 1-AA Homozygous Wild type, 2-6: GG - Homozygous variant, 7-8: AG- Heterozygous, M-100 bp DNA ladder)

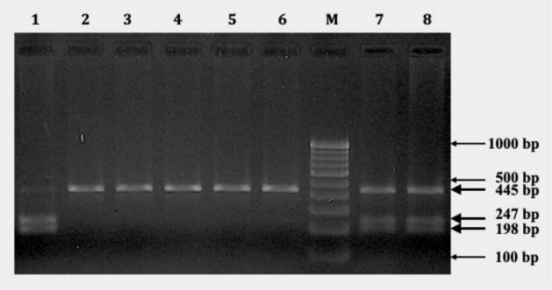
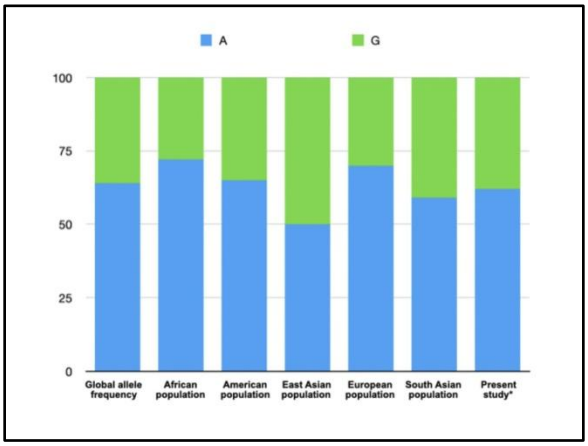



Figure 4: Comparison of allele frequencies of MMP-13 (rs2252070) gene polymorphism among different populations with the present study group*




The graph showed the allele frequencies of MMP-13 (rs2252070) gene polymorphism among different populations and the results of this study correlated with previous studies.

**Discussion**

Many studies have focused on the ultrastructure and molecular aspects of human tissue in physiological and pathological conditions to understand the fine functioning mechanisms. Recently, it has been shown that simultaneous analysis of several genes involved in these processes can be done using the expression profile approach of microarray technique.(Espina, Castellanos and Fereira, 2003; Qi *et al.*, 2021)

Microarray technology has been largely used in recent years for the study of expression profiles of different normal and pathological tissues, providing diagnosis, treatment, and prevention of infectious diseases, identification of genes present in bacteria, diagnosis of allergic diseases, gene expression in disorders.Microarray technology has been used in the study of expression profiles of healthy and carious tooth pulp, to identify genes involved during pathogenesis.(Cai *et al.*, 2020; Guo *et al.*, 2020)(Tóth *et al.*, 2020)

However, studies so far reported have been carried out by pooling samples from different individuals, in order to generate a sufficient pulp amount to be used in microarray experiments. This approach can decrease the specificity of the obtained expression profiles, since at least a portion of the observed variability can be due to the different genetic background of the investigated patients.Variability between hybridizations of different samples was detected suggesting variations among the overall gene expression pattern of individual teeth. In order to reduce this

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variability, likely due to the different genetic background of the investigated subjects, in our study we carried out experiments in order to demonstrate the microarray approach for producing expression profiles from tooth pulp obtained from a single subject. Using an approach able to perform RNA amplification, our research has demonstrated that successful hybridization can be obtained starting from as little as 100g of RNA, corresponding to the amount obtained from a single subject.

Vital pulp therapy aims to treat reversible pulpal injury whilst maintaining cell viability and function. Currently, two therapeutic approaches are frequently used, (i) indirect pulp capping in cases of deep dentinal lesions and (ii) direct pulp capping/pulpotomy in cases of pulp exposure.(Krivanek *et al.*, 2020; Asgary and Parhizkar, 2021a, 2021b; Rodríguez-Lozano *et al.*, 2021) Successful treatment outcomes are dependent on the type and location of the injury, age of the tooth, the pulp capping material used and the integrity of the cavity restoration. In the future, it is hoped that biomimetic approaches will be developed based on a thorough understanding of the molecular and cellular events that occur during dental injury and disease.(Fuks and Peretz, 2016; Charlotte Wells (Clinical reviewer), Dulong and McCormack, 2019; Arora S *et al.*, 2021; Yong and Cathro, 2021) Such therapies aim to maintain the pulp's vitality and stimulate its innate reparative and regenerative mechanisms. Previous molecular and biochemical studies characterizing caries have, however, so far been limited.Notably, the use of microarrays and complex tissues poses challenges as the pulp is composed of heterogeneous and changing cell populations with interactions between immune and non-immune cells being pivotal to disease pathogenesis.(Goldberg, 2014; Chang *et al.*, 2020; Thaw Dar *et al.*, 2020; Demant, Dabelsteen and Bjørndal, 2021)

A better understanding of the roles of the differentially expressed genes identified by our microarray analyses during the caries disease process will require identification of their cellular origin using techniques such as in situ hybridisation and immunohistochemistry in mature and developing dental tissue.(Gutmann, 1994; McLachlan *et al.*, 2005; Sathyanaryanan and Sathyanaryanan, 2014; Galicia *et al.*, 2016; Chinajitphan, Chunhacheevachaloke and Ajcharanukul, 2019) Such analyses will determine whether expression of these molecules is by cells native to the pulp or due to infiltrating immune cells.Our institution is passionate about high quality evidence based research and has excelled in various fields ( (Pc, Marimuthu and Devadoss, 2018; Ramesh *et al.*, 2018; Vijayashree Priyadharsini, Smiline Girija and Paramasivam, 2018; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Ramadurai *et al.*, 2019; Sridharan *et al.*, 2019; Vijayashree Priyadharsini, 2019; Chandrasekar *et al.*, 2020; Mathew *et al.*, 2020; R *et al.*, 2020; Samuel, 2021)

Within the limitations of a relatively-small sample size, the present pilot study enabled us to support the hypothesis that MMP13- A>G gene polymorphisms can be considered a significant genetic marker, emphasizing the pivotal role of genetic components in the pathogenesis of Irreversible pulpitis.

**Clinical Significance**

The present study enabled us to support the hypothesis that MMP13- A>G gene polymorphisms can be considered a significant genetic marker, emphasizing the pivotal role of genetic components in the pathogenesis of irreversible pulpitis .

**Conclusion**

Within the limitations,this study showed the association between MMP-13 gene polymorphism with irreversible pulpitis in the South Indian population.From genotyping, predictions can be made for patients' genetic predispositions to disease, allowing strategies for care to become more individualized. Further genetic studies with larger sample sizes and haplotype analyses, followed by confirmatory clinical studies, will reveal a magnitude of contribution of the susceptibility or resistance allele.

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Nil

**Conflicts of interest**

There are no conflicts of interest.

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