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# Association of COL1A2 gene polymorphism with dental fluorosis in children of Jaipur district of Rajasthan, India

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## ABSTRACT

In India major districts are affected through dental fluorosis. In the Rajasthan, almost all the districts are affected from the fluorosis due to excess ingestion of fluoride through drinking water. Fluoride content calculated in the drinking water and blood through fluoride ion specific electrode method. A case-control study was conducted in two blocks (Jamwaramgarh, Amber) of Jaipur district of Rajasthan, India to investigate the COL1A2 gene polymorphisms with relation to dental fluorosis in Indian children (6-14 years). COL1A2 gene polymorphisms were genotyped using polymerase chain reaction-restriction fragment length polymorphism analysis. Two sites (rs2621208, rs414408) were analyzed for the polymorphism. PCR performed for respective amplicon size for RsaI (865bp) and PvuII (401bp). For the rs414408, PCR product (401bp) was digested with Pvu II enzyme and band pattern was C/C for 240 and 161 bp band, A/C/T for 401,240 and 161 bp band, A/A, T/T 401 bp band. In the control sample pattern type is A/C/T. In the fluorosis affected patients sample band pattern is C/C, A/C/T and C/C. For the rs2621208 site, PCR product (865 bp) was digested with Rsa-I enzyme and expected band pattern was C/C for 412,301,128 and 24 bp band, G/C for 412, 301,152, 128 and 24 bp band, G/G 412, 301 and 152 bp band. In the control sample pattern type is A/C/T. In the fluorosis affected patients sample band pattern is C/C, G/C and G/G. An association between dental fluorosis and the COL1A2 gene polymorphism was observed in fluoride-exposed populations of Jaipur district of Rajasthan, India.

**Key words:** Dental Fluorosis, COL1A2, gene polymorphism, Indian Children.

## Introduction

In India, Fluorosis (due to consumption of excess quantity of fluoride elements) is the most prevalent endemic disease which coexists in certain regions in the country. Fluorine is the most abundant element in nature, and about 96% of fluoride in the human body is found in bones and teeth [1]. Fluororsis is mainly three types i.e., dental, skeletal and non skeletal Fluororsis. Dental fluorosis is a major disease due to the shortage of good quality potable water and consumption of fluoride enriched water by people in the urban as well as in the rural areas. Fluorides are mainly found in ground water and rocks [2]. Higher fluoride concentration exerts a negative effect on the course of metabolic processes and an individual may suffer from skeletal fluorosis, dental fluorosis, non-skeletal manifestation or a combination of the above [3]. Fluoride plays a key role in the prevention and control of dental caries. Dental fluorosis is one of the important performances of skeletal lesion of fluorosis. Excessive fluoride intake has also been shown to affect dentin and cementum mineralization throughout life, diminishing bone density and adversely impacting bone health. Previous study showed that not all the children with high fluoride exposure suffer from dental fluorosis. Therefore; we subsequently hypothesized that the genetic susceptibility would be associated with dental fluorosis status [4, 5]

Collagen is a protein that strengthens and supports bones as well as many other tissues including cartilage,

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tendons, and skin. Two genes, COL1A1and COL1A2 (collagen type I alpha 1 and collagen type 1 alpha 2) provide structions for making components of collagen [6]. Polymorphisms in COL1A1 and COL1A2 may influence the occurrence of dental fluorosis [7]. To date, mutations in the COL1A2 gene in particular have been linked to a wide spectrum of diseases of bone, cartilage, and blood vessels [8]. Although previous research has reported COL1A2 allele frequencies in different populations [9–12], A genetic study done on Chinese population living in fluoride endemic village concluded that children with homozygous P allele of COL1A2 gene had about five times the risk of dental fluorosis compared to the children with homozygous p allele. This association between the COL1A2 gene and dental fluorosis was only observed for children who came from endemic fluoride villages. It is possible that this polymorphism may be in linkage disequilibrium with a causal mutation in the same gene [13]. A case-control study was conducted in two blocks of Jaipur district of Rajasthan, India to investigate the COL1A2 gene polymorphisms with relation to dental fluorosis in Indian children.

## Materials and methods

**Study areas**: The study was conducted in the two blocks (a) Palera, Heerawala, Nayabas, Saipur and Birasana of Jamwaramgarh block, (b) Chitanukalan, Jugalpura, Sunder ka bas ,Peelwa and Sirsali of Amber block of Jaipur district of Rajasthan, India with drinking water F levels of more than 1.5 ppm, respectively ( Ministry of Drinking water and sanitation, Government of India and Public Health and Engineering Department, Government of Rajasthan, Jaipur ).Except for the drinking water, there were no other sources of F exposure in the villages.

A sample group of 150 male and female children 6 to 14 years old exhibiting dental, skeletal and non-skeletal fluorosis consuming fluoride-contaminated water in endemic fluorosis areas of Jaipur district of Rajasthan, India were selected through a village level survey was conducted.

A detailed questionnaire regarding their demographic details, written consent was taken and duration of F exposure.

All participants were examined for dental fluorosis using the Dean's Method [14]. According to specific clinical diagnostic criteria of the development of fluorotic enamel opacities, dental fluorosis was categorized as 0 (normal), 1 (questionable), 2 (very mild), 3 (mild), 4 (moderate), and 5 (severe). Children who were diagnosed as grade 0 or 1 were classified as controls, whereas those who were diagnosed as grade 2, 3, 4, or 5 dental fluorosis cases.

**Sample collection and analysis***:* A 4 ml venous blood sample was collected from each selected participants after overnight fasting in a plain plastic Vacutainer (BD) tubes without any anticoagulant and stored at –20 ° C. A 24 hr urine sample and Drinking water were collected in plastic falcon tubes (Tarsons) and investigated for fluoride levels. F concentration in each of the prepared solutions was estimated with the help of a F ion specific electrode (Thermo Scientific Orion Star A329).Fluoride determination in the drinking water was carried out potentiometrically with a fluoride ion specific electrode (Thermo Scientific Orion Star A329). Urinary fluoride was estimated by the method of Hall et al [15].

Blood samples were left to clot at room temperature, and serum was separated by centrifugation. Serum fluoride was also estimated by the method of Hall et al [15]. Using the Thermo Scientific Orion Star A329.

**DNA Extraction**

Genomic DNA was extracted from the blood samples of participants with a DNA extraction kit (centrifugal column type; ***Aristogene*** Biosciences (P) Ltd,. Bangalore, India) and subject to PCR-RFLP.

**PCR RFLP**

DNA genotype was done by using following markers: the PvuII RFLP (rs414408) site and RsaI RFLP (rs2621208) site inside the COL1A2 gene. For the PvuII RFLP, the forward primer (5'CATACGAGATTGAATTGGCT'3), and the reverse primer (5' TCCAGTCTCAGGGAGTTTCC'3) were used in polymerase chain reaction (PCR) to produce a 401-base pair (bp) DNA fragment. For the RsaI RFLP, the forward primer (5'CTGCCTCTACAGCCCATC'3), and the reverse primer (5' AGGACCTCCTGGGCTGAGAGTAG'3) were used in polymerase chain reaction (PCR) to produce a 865-base pair (bp) DNA fragment. For The PCR amplification was conducted in reaction mixtures each containing 21 µl of ddH2O, 25 µl 2X PCR Master Mix ,1X components (***Aristogene*** Biosciences (P) Ltd,. Bangalore.,India), 1µl each of the two Primers for each marker, and 2 µl of genomic DNA Template. Mixing, instantaneous centrifugation and PCR amplification were performed using a programmable thermal cycler. The cycling conditions were as follows: 1 cycle at 94 ° C for 5 min, 30 cycles at 94 ° C for 30 Second, 55 ° C for 30 s, 72 ° C for 1 min, and a final extension 1 cycle at 72 ° C for 7 min. The PCR products were subjected to gel electrophoresis. The PCR was performed on ABI 9600 Thermocycler (***Applied Biosystems***, Foster ***City***, CA, ***USA)***. After PCR amplification, 20 µl of the respective PCR products was removed and digested

with 10 U of PvuII or 10 U of RsaI Incubated at 37˚C for 2hr.

Then the samples were electrophoresed in 1.5% agarose gel in 1x TAE buffer using 100bp ladder. Gels was visualized on a transilluminator under UV light and photographed under UVI doc gel doc system (UVItec Limited Cambridge, UK).

**Ethical approval:** The protocol for this study was approved from the Ethical Committee of Desert Medicine Research Centre (ICMR), Jodhpur. All work was performed according to the ICMR guidelines, New Delhi, India for human experimentation in biomedical research. Before the sample collection a written consent was obtained from each participants or their parents or legal guardians.

**Results and discussion**

High concentration of fluoride has been detected in the underground water in many countries, e.g. Kenya, Nigeria, South Africa, Tanzania, USA, China, India, Japan, Korea and Australia [16]. Fluorosis is also major problem in India, particularly in Rajasthan and Gujarat in North India and Andhra in South India are worst affected. Punjab, Haryana, Madhya Pradesh and Maharashtra are moderately affected states in India, while the states Tamil Nadu, West Bengal, Uttar Pradesh, Bihar and Assam are mildly affected [17]**.** Endemic fluorosis is a major public health concern due to the excessive consumption of fluoride in drinking water. Gene polymorphism also plays an important role in the fluorosis problem. A genetic study done on estrogen receptor (ESR) gene polymorphism in Chinese children concluded that since estrogen polymorphism were associated with bone metabolism-related diseases. Therefore it is possible that the variation in ESR gene will be associated with dental fluorosis status [18]. This study provides the first evidence of an association between COL1A2 gene polymorphism with DF in high-fluoride-exposed populations. [19]. To date, the role of genetic susceptibility in relation to fluorosis, particularly dental fluorosis, has been unclear. Rajasthan is severely affected by fluororsis problem. Huang et al., 2008 had reported that children with homozygous P allele of COL1A2 PvuII had about five times the risk of dental flouorosis compared to children with homozygous p allele after adjusting for age and gender [13]. COL1A2 gene polymorphism study is undertaken and study area is Jaipur District of Rajasthan, India. Age of the children is 6-14 years old school going children. For the purpose of COL1A2 gene polymorphism, DNA isolation was carried out for the PCR-RFLP purpose from the collected blood samples (Fig.1).

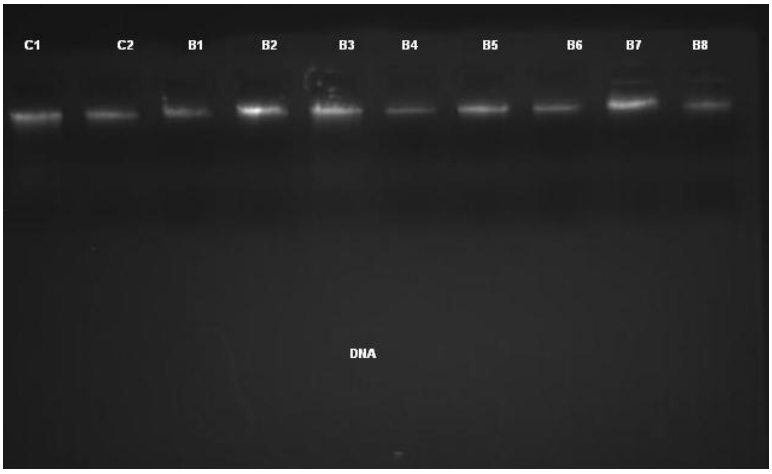


**Fig 1: Isolated DNA from blood sample.**

PCR RFLP study performed to check the polymorphism of COL1A2 gene association with dental fluorosis. Two sites (rs2621208, rs414408) were analyzed for the polymorphism. PCR performed for respective amplicon size for RsaI (865bp) and PvuII (401bp) fig.2 & Fig.3.

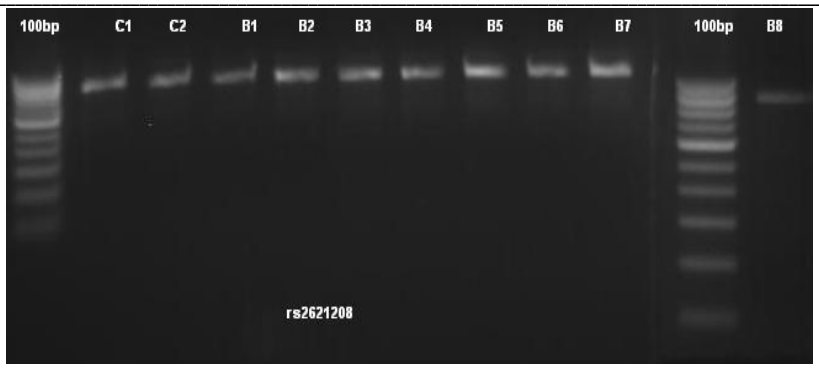


**Fig 2: PCR amplification for rs2621208 site using 100 bp DNA marker**

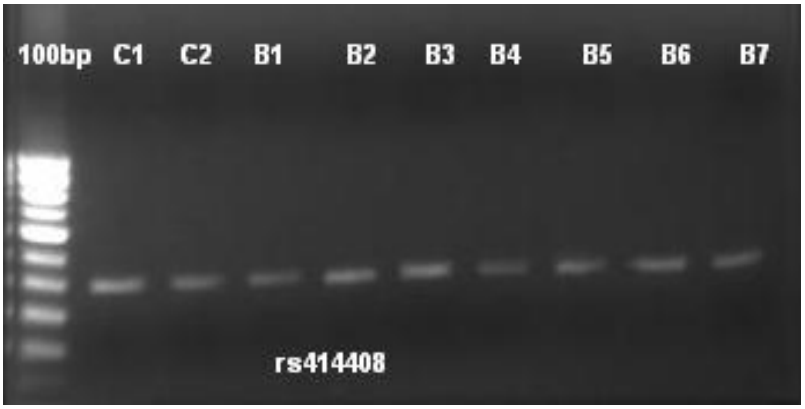


**Fig 3: PCR amplification for rs414408 site using 100 bp DNA marker**

Amplified PCR product digested with restriction enzyme PvuII and RsaI Fig.4 &Fig.5.

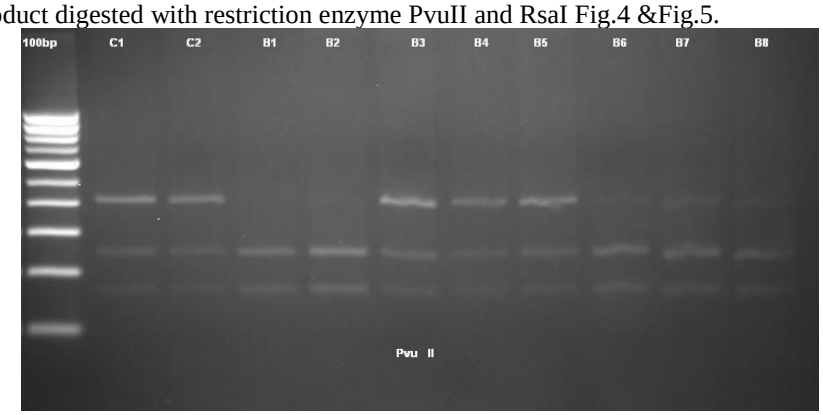


**Fig 4: Restriction digested PCR amplicon for rs414408 site using Pvu-II restriction enzyme and 100 bp DNA markers**

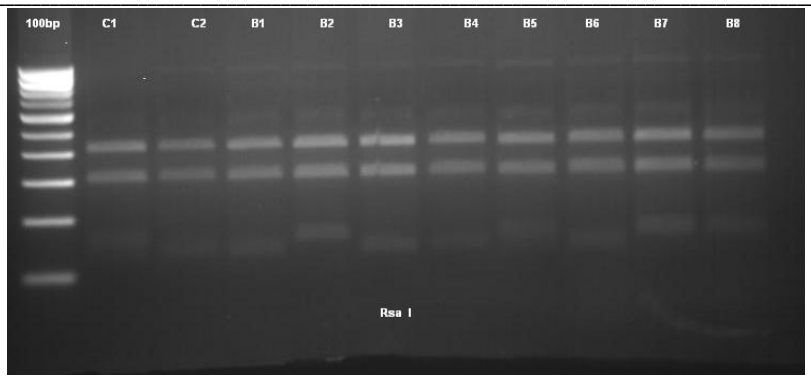


**Fig 5:Restriction digested PCR amplicon for rs2621208 site using Rsa-I restriction enzyme and 100 bp DNA markers**

For the rs414408, PCR product (401bp) was digested with Pvu II enzyme and expected band pattern was shown in table.1. Band pattern after digestion with the Pvu-II restriction enzyme i.e. C/C for 240 and 161 bp band, A/C/T for 401,240 and 161 bp band, A/A , T/T 401 bp band.

**Table 1: Expected band pattern for the rs414408 site using Pvu II restriction enzyme.**

| C/C | A/C/T | A/A T/T |
|---|---|---|
| **240** | 401 | 401 |
| **161** | 240 | |
| | 161 | |

In our experiment, we obtained band pattern according to table.2 for rs414408 site using Pvu II restriction enzyme. In the control sample pattern type is A/C/T. In the fluorosis affected patients sample band pattern is C/C, A/C/T and C/C.

**Table 2: Experimental band pattern for the rs414408 site using Pvu II restriction enzyme**

| Sample | Genotype |
|---|---|
| **C1** | A/C/T |
| **C2** | A/C/T |
| **B1** | C/C |
| **B2** | C/C |
| **B3** | A/C/T |
| **B4** | A/C/T |
| **B5** | A/C/T |
| **B6** | C/C |
| **B7** | C/C |
| **B8** | C/C |

For the rs2621208 site, PCR product (865 bp) was digested with Rsa-I enzyme and expected band pattern was shown in table.3.Band pattern after digestion with the Pvu-II restriction enzyme i.e. C/C for 412,301,128 and 24 bp band, **G**/C for 412, 301,152, 128 and 24 bp band, G/G 412, 301 and 152 bp band.

**Table 3:Expected band pattern for the rs2621208 site using Rsa-I restriction enzyme**

| C/C | G/C | G/G |
|---|---|---|
| **412** | 412 | 412 |
| **301** | 301 | 301 |
| **128** | 152 | 152 |
| **24** | 128 | |
| | 24 | |

In our experiment, we obtained band pattern according to table.4 rs2621208 site using Rsa-I restriction enzyme. In the control sample pattern type is A/C/T. In the fluorosis affected patients sample band pattern is C/C, G/C and G/G.

**Table 4: Experimental band pattern for the rs2621208 site using Rsa-I restriction enzyme**

| Sample | Genotype |
|---|---|
| **C1** | G/C |
| **C2** | C/C |
| **B1** | C/C |
| **B2** | G/G |
| **B3** | C/C |
| **B4** | C/C |
| **B5** | G/C |
| **B6** | C/C |
| **B7** | G/G |
| **B8** | G/G |

Thus, our data indicated that the COL1A2 gene polymorphism is associated with an increased risk of fluorosis. Gene-gene or gene-environment interactions could lead to the varying genetic effects observed in different populations. Our results further confirmed that there were significant differences in the distribution of alleles among fluorosis patients and control subjects.

## Conclusion

Our data supported the hypothesis that the COL1A2 gene polymorphism may be one of the genetic components associated with fluorosis. Due to the modest sample size, it is essential to replicate these findings in different populations with larger sample sizes, and investigation of other related genes will be needed to clarify the relationship between this polymorphism and fluorosis.

## References


1. Park K. Park's text book of preventive and social medicine. Ed 21. Banarasis das Bhanot Publishers, Prem nagar, Jabalpur, India. 2011; 1167: 577.
2. Wedepohl KH. Hand book of geochemistry. Springerverlage berlin. (Ed.:Heidelberg). New York. 1974;2: 9 K-1 pp.
3. Susheela AK, Kharb P. Arotic calcification in chronic in chronic fluoride poisoning: Biochemical and electromicroscopic evidence. Expe. Mole. Pathol.1990; 53: 72-80.
4. Aoba T, Fejerskov O. Dental fluorosis: chemistry and biology. Crit Rev Oral Biol Med 2002; 13 (2):155-70.
5. Deng HW, Chen WM, Conway T, Zhou Y, Davies KM, Stegman MR, Deng H, Recker RR. Determination of bone mineral density of the hip and spine in human pedigrees by genetic and life-style factors. Genet Epidemiol. 2000; 19 (2):160-77.
6. Phillips CL, Bradley DA, Schlotzhauer CL, BergfeldM, Libreros-Minotta C, Gawenis LR et al. Oim mice exhibit altered femur and incisor mineral composition and decreased bone mineral density. Bone.2000; 27:219–26.
7. Zhang Y, Yan Q, Li W, Den Besten PK. Fluoride down-regulates the expression of matrix metalloproteinase-20 in human fetal tooth ameloblast-lineage cells in vitro. Eur J Oral Sci 2006; 114 (Suppl. 1):105–10; discussion 127-9, 380.
8. Dalgleish R. The human type I collagen mutation database. Nucleic Acids Res 1997; 25:181–7.
9. Constantinou CD, Spotila LD, Zhuang J, Sereda L,Hanning C, Prockop DJ. PvuII polymorphism at the COL1A2 locus. Nucleic Acids Res 1990; 18:5577.
10. Liu JL, Xia T, Yu YY, Sun XZ, Zhu Q, He W et al. The dose-effect relationship of water fluoride levels andrenal damage in children. Wei Sheng Yan Jiu 2005; 34:287–8.
11. Rickards O, Martinez-Labarga C, Trucchi E, Renzi F,Casalotti R, Babalini C et al. Restriction fragmentlength polymorphisms of type I collagen locus 2(COL1A2) in two communities of African ancestry and other mixed populations of north western Ecuador.Hum Biol 2005;77:115–23.
12. Suuriniemi M, Mahonen A, Kovanen V, Alen M,Cheng S. Relation of Pvu II site polymorphism

in the COL1A2 gene to the risk of fractures in prepubertal Finnish girls. Physiol Genomics 2003; 14:217–24.

**13.** Huang H, Ba Y, Cui L, Cheng X, Zhu J, Zhang Y, Yan P, Zhu C, Kilfoy B, Zhang Y. COL1A2 gene polymorphisms (Pvu II and Rsa I), serum calciotropic hormone levels, and dental fluorosis. Community Dent Oral Epidemiol 2008; 36 (6):517-22.

**14.** Dean HT. Classification of mottled enamel diagnosis. Am Dent Assoc 1934; 21:1421–6.

**15.** Hall LL, Smith F, Hodge HC. Direct determination of ionic fluoride in biological fluids. Clin chem.1972; 18:1455-8.

**16.** Saravanan S, Kalyani C, Vijayarani M, Jayakodi P, Felix A, Nagarajan S, Arunmozhi P, Krishnan V. Prevalence of dental fluorosis among primary school children in rural areas of Chidambaram Taluk, Cuddalore District,Tamil Nadu.India. Indian J Commun Med.2008.33:146-50.

**17.** Mathur SC. Epidemiology of Endemic Fluorosis.http://www.pptuu.com/show_22473_1.html.

**18.** Ba Y, Li HX, Yin GJ, Wu WH, Yu B, Cheng XM, Cui Lx. Study on the relationship between ER Rsa1 gene polymorphism and children's dental fluorosis. Sichuan Da Xue Bao Yi Xue Ban. 2009; 40(5):869-72.

**19.** Dawson DV. Preliminary evidence of an association between COL1A2 polymorphisms and dental fluorosis in a population with high fluoride exposure. J Evid Based Dent Pract. 2010 ; 10(2):96-98.

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