Original Article

# Effect of Breastfeeding on the HMGA2 Single Nucleotide Polymorphisms in Relation to First Primary Tooth Emergence among Iraqi Infants

**Shaimaa Thabit Salih, Ban Sahib Diab**

Department of Pediatric and Preventive Dentistry, College of Dentistry, University of Baghdad, Baghdad, Iraq



## Abstract

**Backgrounds:** Breastfeeding provides a large group of functional components that improve the infant's health and development. ADditionally, the impact of human milk and genetic variants on complicated outcomes has also been the subject of several gene–nutrition interaction studies. This could also play a key part in the kinship that exists between offspring and their parents. **Objectives:** The purpose of this study was to compare breastfeeding to infants who were fed formula in order to determine if human milk affected the timing of teething in relation to genetic polymorphisms. **Materials and Methods:** This study is a cross-sectional comparative study concerning genetic analysis with a prospective view concerning the timing of eruption. The chosen sample included 40 breastfed infants and 40 infants who consumed formula milk. It was compiled from Baghdad's health centers. For the genetic analysis, DNA samples were obtained from saliva throughout the buccal swab. Standard DNA extraction and real-time PCR techniques have been used according to the manufacturer's instructions for the Quick-DNA™ MiniPrep kit (catalog nos. D3024 and D3025). For the whole sample, clinical examination and monitoring for emerging teeth were performed using a particular criterion. **Results:** findings showed that there were highly significant differences between breastfeeding and early eruption time for the first primary tooth. Also, there was a significant association between single nucleotide polymorphisms in the heterozygous AG genotype and eruption time. **Conclusion:** The results of the current study may support the genetic influence of breast milk on early primary teeth eruption when juxtaposed with infants fed formula.

**Keywords:** Breastfeeding, Eruption, single nucleotide polymorphisms

## Introduction

The cornerstone for developing an infant's physique and preserving his health status throughout the first 6 months of life is breastfeeding. An infant's feeding throughout this crucial stage of development has a number of short- and long-term consequences. Therefore, after delivery and for the first 6 months, it is advised to breastfeed exclusively; after that, supplemental foods must be introduced to suit the baby's growing nutritional needs.[1-3] Several kinds of research have verified the possible gene–environment association; breastfeeding has been recognized as a key environmental influence for infants.[4] This could be due to the fact that it contains more free nucleotides than formula milk. Furthermore, various associated substances, including nucleosides, purine and pyrimidine bases, nucleic acids, and items made from them (such uridine diphosphate galactose), have been discovered in human milk,[5] which are crucial for a number of metabolic activities, including the transmission of energy, the production of nucleic acids (DNA and RNA), and the synthesis of carbohydrates, lipids, and proteins. Human milk advantages, such as those related to infant health, may be achieved via modifying genotypes since genetic

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**Address for correspondence:** Dr. Shaimaa Thabit Salih, Department of Pediatric and Preventive Dentistry, College of Dentistry, University of Baghdad, Health Directorate, Baghdad 10047, Iraq.
E-mail: shaima.hamoudi1901@codental.uobaghdad.edu.iq

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studies have demonstrated an association between nursing and genetic polymorphisms. When it comes to complex phenotypes like cognitive outcomes and asthma risk in children, gene–nutrition interaction studies suggest that these polymorphisms modulate the effect of dietary fatty acid intake. This may discuss the implications for child health and provide an outlook on how this association might translate into adulthood.[6,7]

Breastfeeding and genetic interactions generally take place via a variety of mechanisms, including genetic and epigenetic impacts.[8,9] High mobility group AT-hook 1 and 2 (HMGA1 and HMGA2) are small, non-histone proteins that have the ability to bind to DNA and change the chromatin structure, which affects how readily regulatory factors can bind to DNA. They act as tightly linked "nodes" of protein-DNA and binding proteins within the cell, with impacts on many common biochemical tasks, including growth, multiplication, specialization, and apoptosis. As a result, evidence was mounting for HMGA2's involvement in both the development and growth of human bones.[10,11] Single nucleotide polymorphism (SNP) rs17101923 of the HMGA2 gene was linked to tooth erupting and craniofacial growth according to genome-wide association studies.[12,13]

Vitamins A, C, and D, calcium, and phosphate belong to the elements found in breast milk that are essential for the formation of healthy teeth. Calcium and phosphorus are required for the proper crystallization of hydroxyapatite. Therefore, tooth growth may be impacted by a lack of the aforementioned nutrients.[14,15] As a result, human milk has the potential to influence how teeth develop.[16] Evolutionary teeth develop across jaws and the underlying mucosa into the mouth cavity in a continuous biological process known as tooth eruption.[17] The eruption of teeth also reflects the overall development of the body. Thus, it may be argued that when tooth eruption is postponed, overall body growth is restricted. It is a difficult procedure that is, heavily controlled. Therefore, a variety of variables, including genetics, ethnic, racial, regional, hormonal, cytokine, gender, and dietary variances, might affect tooth eruption.[16,18] Furthermore, several human and animal research lends credence to the notion that hereditary variables may be connected to early or delayed tooth eruptions.[12,19]

Despite the plethora of research that has shown the many benefits of breastfeeding for mothers and infants; yet, there have been no preceding studies that have addressed the information gap about the genetic effects of human milk. That's a key component of the kinship bond between offspring and their parents. Consequently, in order to shed new insight into the genetic consequences of nursing on the emergence of an infant's first primary tooth, this study was conducted.

## Materials And Methods

Cross-sectional comparative study concerning salivary genetic analysis with longitudinal prospective concerning the examination of first primary tooth eruption. The selected subject included 40 breastfed infants and 40 infants who were fed formula milk. Both groups were inspected when they attended to the Baghdad Health Clinics for primary medical care. The research only included infants between the ages of 4–9 months. For genetic analysis, a salivary swab (Saliva Bio Infant's Swab SIS) was utilized. Using buccal swabs throughout, individually rubbing along the inner surface of the cheek for a maximum of 1 min.[20] Finally, insert the saturated swab into the swab storage tube to be recovered by centrifugation after cutting it to remove the free saturated area before inserting it into the centrifuge tube. According to the kit's manufacturer's instructions, saliva was stored and examined. Real-time PCR and standard DNA extraction techniques were both employed using the Quick-DNA™ MiniPrep kit (catalog nos. D3024 and D3025).

The time of primary tooth emergence was examined through intraoral examinations. The mother and child were positioned knee to knee for the intraoral examination. Infants whose first tooth had not yet erupted were observed monthly until the exact eruption time had been established.[21] Mothers were asked to record the date of their infant's first tooth eruption, which they were to consider a tooth erupted as soon as any part of the crown emerged through the gingival surface. Infants with no tooth emergence were followed at monthly intervals after the monitoring for the first time. The exact eruption time was determined through several call interviews. Mothers were questioned once more for each sample following the recording of the first primary tooth's eruption time to ensure the accuracy of the answers.[22]

### Exclusion criteria

Infants with malnutrition, chronic illness, or systemic diseases were excluded. Infants with mixed feeding also were excluded.

### Inclusion criteria

Apparently, healthy infants are absent of systemic congenital abnormalities. Only Iraqi Arab infants aged between 4 and 9 months were included in this study; at this age, the infants depend mainly on breastfeeding as essential nutrition, taking into consideration the matching in age, gender, and complementary food intake.

### Ethical considerations

The ethical committee of the College of Dentistry, University of Baghdad, approved this study on the date April 4, 2021, and number: 325. Legal permission was obtained from the Ministry of Health to perform clinical examinations at health centers. Also, a special informed consent in accordance with the College of Dentistry at

the University of Baghdad was prepared and distributed to mothers to obtain permission to participate in this study. The Declaration of Helsinki, the World Medical Association's code of ethics for studies involving humans, guided the conduct of this work.

### Statistical analysis

Statistical analysis using Statistical Package for Social Science developed by IBM (SPSS version 22, Chicago, Illinois), Statistical analyses can be classified into three categories:

1. Descriptive analysis: frequency and percentage for qualitative variable while mean and standard error (SE) for quantitative variable.
2. Inferential analysis: Student *t* test, one-way analysis of variance, level of significance is at 0.05.
3. Hardy–Weinberg equilibrium law was used for calculating allele and genotype frequencies.

## Results

Genetic analysis in the current study revealed that there was a significant association between the HMGA2 gene/SNP (rs17101923) and feeding pattern in both heterozygous AG and homozygous GG genotypes. However, AG is found to be more among the breastfeeding group, while the opposite result was found concerning genotype GG as it was found to be more among the bottle-feeding group, as shown in Table 1.

Infants' eruption time for primary teeth in relation to feeding pattern is shown in Table 2. Data revealed that the mean eruption time of primary teeth among the breastfeeding group was significantly higher than bottle-fed infants. ADditionally, the distribution of infants according to the teething eruption time by feeding pattern, the timing of teeth eruption ranged from 5 to 11 months and more. The data showed that the percentage of infants with an eruption time of 5–6 months was higher among the breastfeeding group, while infants with delayed eruption time of 11 months and more were found to be higher among bottle-feeding, as demonstrated in Table 3].

The data also revealed a significant association between SNP (rs17101923) of HMGA2 gene and eruption time by feeding pattern concerning heterozygous AG genotype, which is higher in frequency among bottle feeding group; on the other hand, data showed no significant differences in the mean values of eruption time for both homozygous AA and GG genotypes by feeding pattern, as revealed in Table 4.

**Table 1: High mobility group AT-hook 2 gene/SNPs (rs17101923) genotype distribution and allele frequency by feeding pattern**

| Genotype | Breastfeeding no. (%) | Bottle-feeding no. (%) | Chi-square ($\chi^2$) | *P* value |
|---|---|---|---|---|
| AA | 5 (12.50%) | 5 (12.50%) | 0.00 | 1.00 |
| AG | 23 (57.50%) | 12 (30.00%) | 3.55* | 0.04 |
| GG | 12 (30.00%) | 23 (57.5%) | 3.55* | 0.04 |
| Total | 40 | 40 | – | – |
| Allele | | Frequency | | |
| A | 0.41 | 0.55 | – | – |
| G | 0.59 | 0.45 | – | – |

* Significant at *P*-value= $P \leq 0.05$

**Table 2: Infants' eruption time (months) (mean ± SE) according to feeding pattern**

| Feeding pattern | Eruption time emergence (months) | | *t*-Test | *P* value |
|---|---|---|---|---|
| | Mean | SE | | |
| Breastfeeding | 8.44 | 0.18 | 2.36* | 0.01 |
| Bottle-feeding | 9.09 | 0.20 | | |

*Significant at $P \leq 0.05$

**Table 3: The distribution of infants according to tooth eruption by feeding pattern**

| Eruption intervals | Feeding pattern | | | |
|---|---|---|---|---|
| | Breastfeeding | | Bottle-feeding | |
| | *N* | % | *N*. | % |
| 5–6 | 19 | 19.00 | 12 | 12.00 |
| 7–8 | 35 | 35.00 | 33 | 33.00 |
| 9–10 | 31 | 31.00 | 27 | 27.00 |
| 11+ | 15 | 15.00 | 28 | 28.00 |

**Table 4: Eruption time (months) (mean ± SE) according to single nucleotide polymorphisms of rs17101923 by feeding pattern**

| Genotypes | | Feeding pattern | | | | *t*-Test | *P* value |
|---|---|---|---|---|---|---|---|
| | | Breastfeeding | | Bottle-feeding | | | |
| | | Mean | ±SE | Mean | ±SE | | |
| rs17101923 | AA | 9.90 | 0.78 | 8.80 | 0.66 | 1.764 | 0.314 |
| | AG | 8.06 | 0.41 | 9.70 | 0.76 | 3.651* | 0.045 |
| | GG | 8.29 | 0.55 | 8.58 | 0.34 | 0.365 | 0.618 |
| *F* | | 0.35 | | 0.57 | | | |
| *P* value | | 0.17 | | 0.28 | | | |

*Significant at $P \leq 0.05$



## Discussion

This study was performed to assess how breastfeeding affects the timing of teething in relation to genetic variants of the HMGA2 gene/SNP (rs17101923). Despite all the diverse proposed benefits of breastfeeding on the factors associated with teeth emergence, there was no previous Iraqi data that studied these relationships concerning the effect of breastfeeding on genetics in relation to teeth emergence. Tooth eruption is a complex and highly regulated process in which multiple factors can affect how quickly the first primary tooth erupts. Strong genetic influence over emergence is shown by assessments of narrow-sense heritability that are greater than 70%.[18,23] External environmental factors, particularly the infant feeding pattern, have a considerable impact on when the primary tooth may erupt.[21,24,25]

The data showed that the breastfed infants had earlier eruption time in comparison with those depending on bottle feeding. This finding is confirmed by many previous studies.[21,26] Additionally, according to the time range of eruption (5–11 months and above), it was found that the percentage of infants with eruption time of 5–6 months was higher among breastfeeding than bottle-feeding infants (19%) and (12%), respectively. However, infants with delayed eruption time of 11 months and above were found more among bottle-feeding (28%) than breastfeeding (15%). This may be related to potential factors associated with the variance of the timing of first tooth emergence as follows:

- Genetics, certain genes are associated with signaling components along with other signaling molecules, which play crucial roles in the morphogenesis of craniofacial growth and tooth development and eruption.[12,13,27]
- Nutritional factors such as vitamins A, D, and C, as well as phosphorus and calcium, are essential nutrients for the growth of healthy teeth. Vitamin D controls the amount of phosphorus and calcium in plasma, which are necessary for the satisfactory formation of hydroxyapatite crystals. Vitamin A is required for the production of keratin, and vitamin C is necessary for the proper conformation of collagen. Therefore, a lack of the aforementioned nutrients may affect both teeth formation and the time of teething.[15,21]

Results regarding genetic analysis reveal that there was a significant difference between HMGA2 gene/ SNP (rs17101923) and feeding pattern according to AG and GG genotype frequencies. This is consistent with many previous studies that approved the genetic effect of milk on infants' health and development.[28-30] According to eruption time and HMGA2 gene/SNP (rs17101923), there was an increase in the mean eruption time, which was significantly higher among bottle feeding concerning AG genotype, in addition to low AG genotype frequency comparable to the breastfeeding group. This may confirm the relation between early eruption time and this genotype (AG) polymorphism, which makes this genotype (AG) variant positively affect on teeth eruption because AG genotype frequency was higher among the breastfeeding group, which have also earlier eruption time infants. This might be attributed to the genetic influence of human milk on the sequencing of this gene (SNPs).[31,32] In addition, this result parallels a wide genome association study, which revealed the positive effects of this gene/SNP and early eruption time of primary teeth.[13] Thus, human milk benefits were not restricted just to the nutritional benefits and growth factors, but the genetic effect of human milk had a crucial role in health and development. Therefore, much high quality, prospective, longitudinal studies need to support this genetic effect of breastfeeding with the underlying mechanisms, and more sample size is recommended.

## Conclusion

This study confirmed the effect of breastfeeding on the eruption time of the first primary tooth, where it was early eruption time among the breastfeeding group. The data also revealed that breastfeeding may have a genetic effect related to early eruption time among this group.

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## Conflicts of interest
There are no conflicts of interest.



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