Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2023; 23:e210244
https://doi.org/10.1590/pboci.2023.063
ISSN 1519-0501 / eISSN 1983-4632

ORIGINAL ARTICLE

Single Nucleotides Polymorphisms in *COX2 Gene* and their Association with Signs and Symptoms of Teething – A Pilot Study

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Academic Editor: Alidianne Fábia Cabral Cavalcanti

Received: 29 December 2021 / **Review:** 27 September 2022 / **Accepted:** 01 December 2022

How to cite: Mauta AG, Reis CLB, Teodoro VB, Madalena IR, Kirschneck C, Proff P, et al. Single nucleotide polymorphisms in *COX2 gene* and their association with signs and symptoms of teething – a pilot study. Pesqui Bras Odontopediatria Clín Integr. 2023; 23:e210244. https://doi.org/10.1590/pboci.2023.063

ABSTRACT

Objective: To investigate the association between single nucleotide polymorphisms in *the COX2 gene* (rs689466 and rs5275) and local and systemic signs and symptoms of teething. **Material and Methods:** Forty-four pairs of mothers-babies/toddlers were included. Erupted primary teeth were evaluated during clinical examination. Local and systemic signs and symptoms of teething were obtained from mothers' reporting via anamnesis. Samples of buccal cells were retrieved for DNA genotyping using real-time PCR. The T-test, Chi-square test, logistic regression, and haplotype analyses were applied. **Results:** Almost all mothers (95.5%) reported at least one local or systemic sign and symptom of teething. The most common was increased salivation (79.5%), diarrhea (72.3%), and fever (70.5%). The mean number of signs and symptoms per child was higher in boys than girls (mean = 5.1; SD= 1.5; p=0.008). Sleep disturbance (p=0.03) and loss of appetite (p=0.05) were more reported in boys. The rs689466 and rs5275 were not associated with signs and symptoms of teething (p>0.05). **Conclusion:** The single nucleotide polymorphisms in *the COX2 gene* (rs689466 and rs5275) were not associated with local and systemic signs and symptoms of teething.

Keywords: Cyclooxygenase 2; Genes; Tooth Eruption.

Introduction

Tooth eruption, or teething, is the process of movement of primary or permanent teeth from the intraosseous to their final position in the oral cavity [1,2]. This physiological mechanism is finely regulated in the human body, and it involves the movement of the dental germ and follicle through the bone by a mechanism of bone resorption and formation [3] until teeth emerge through the gum to their final position in the dental arch [4,5]. The expression of many genes strongly regulates this physiological process, but the exact mechanisms involved in primary tooth eruption are still not completely understood [4,6].

Most parents and health professionals report that the process of primary teeth eruption is a delicate time for the baby [7,8]. Primary tooth eruption can be associated with local and systemic signs and symptoms such as increased salivation, diarrhea, fever, increased crying, sleep disturbance, loss of appetite, a runny nose and cough, vomiting, and others [3,9,10]. Some researchers postulated that these signs and symptoms occur coincidentally with the decrease in maternal antibodies in the baby and the consequently increased susceptibility to infections by microorganisms, which are not exclusively related to teething [3,11,12]. Other studies reported that releasing inflammatory mediators during teething could explain commonly reported signs and symptoms [13].

Cyclooxygenase-2 (COX-2) is an enzyme within the arachidonic acid pathway responsible for producing prostaglandins (PGs) and induced by acute inflammation. COX-2 is encoded by the Prostaglandin-Endoperoxide Synthase 2 (*PTGS2*) gene, which can also be called the *COX2 gene*. COX-2 is related to pain sensitivity, local inflammatory reactions, bone metabolism, and febrile response [5,14,15]. Two single nucleotide polymorphisms (SNPs) in the *COX2 gene* (rs689466 and rs5275) have been associated with changes in the periradicular tissues of teeth in different populations [16,17]. In view of the functionality of the periodontal ligament during the process of tooth eruption and gingival emergence, it can be assumed that SNPs in COX2 may be involved in the susceptibility of the baby to signs and symptoms of teething. The rs689466 polymorphism regulates the transcription levels of COX-2 [18], while the rs5275 polymorphism may determine the stability of COX-2 mRNA and translation efficiency [19].

Therefore, these two SNPs emerge as candidates to test the association with signs and symptoms of teething. So, the main goal of this study was to investigate if rs689466 and rs5275 in *the COX2 gene* are associated with systemic and local signs and symptoms of teething in Brazilian babies.

Material and Methods

Ethical Clearance

Babies and toddlers were included in this study after the mothers returned the informed consent form. Ethical approval (Ethical Committee N° 3.316.91807) was provided by the Ethical Committee of Positivo University, Curitiba, Brazil, and was performed following the ethical principles for medical research involving human subjects of the Declaration of Helsinki.

Pilot Study and Sample

This pilot cross-sectional nested case-control study included 44 biologically unrelated children (one child per family) of both genders aged between 9 to 48 months. The sample was selected for convenience. Children using any medication or diagnosed with systemic diseases or syndromes were omitted. They were recruited at the Baby's Clinic at Positivo University.

Data Collection

The mothers answered an anamnesis form designed to collect data on systemic and local signs and symptoms that they perceived to be associated with teething. Signs and symptoms were recorded based on those ascribed by parents in a similar previous study [20], including increased salivation, diarrhea, fever, increased crying, sleep disturbance, loss of appetite, runny nose and cough, and vomiting.

Trained examiners using the knee-to-knee technique performed the oral examination. Children were positioned across the dentist's and the guardian's laps, and the clinical examination and sampling of epithelial buccal cells were performed with a sterile wooden spatula.

The collected material was placed into a tube with a glucose solution of 3%. After that, the material was centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded, and the pelleted cells were resuspended in 1.300 mL extraction buffer [10 mM Tris-HCl (pH 7.8), 5 mM EDTA, 0.5% SDS]. Ten µL proteinase K (20 mg / mL) were added to the solution and left overnight at 65°C. DNA was purified by adding 10 M ammonium acetate, isopropanol-precipitated, and resuspended with 50 µl 10 mM Tris (pH 7.6) and 1 mM EDTA [21]. Spectrophotometry was performed to determine DNA concentration and purity using NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA).

We used the websites www.ncbi.nlm.nih.gov and https://www.thermofisher.com to identify candidate SNPs in *the COX2 gene* according to their possible function regulation and alleles frequency. A total of 2 SNPs in the *COX-2 gene*: rs689466 (flanking sequence in the context GACAG[C/T]TGGA; global minor allele frequency= 0.217) and rs5275 (flanking sequence in the context AAAAT[A/G]ACCA; global minor allele frequency = 0.4000) were selected. The polymorphisms rs689466 and rs5275 were selected based on their frequency in the population, location, and previous demonstration of association with dental phenotypes [16,17]. Genotyping assays were performed according to Ranade et al. [22]. Taqman™ method for real-time PCR was used in the Mastercycler® ep realplex-S thermocycler (Eppendorf AG, Hamburg, Germany).

Data Analysis

Gender, genotype, and allele differences were evaluated for each sign and symptom of teething, categorized as 'Yes' (for the case group) or 'No' (for the control group), using Chi-square or Fisher's exact tests and regarding the mean number of signs and symptoms reported per child using T-tests. The Hardy-Weinberg equilibrium was determined using a Chi-square test within each SNP. A multivariate analysis was performed, adjusted by gender. GraphPad Prism 5.0 (Graph-Pad, San Diego, CA, USA) was used for these tests. Haplotype analysis was performed by Plink® software (Massachusetts General Hospital, Boston, MA, USA).

Results

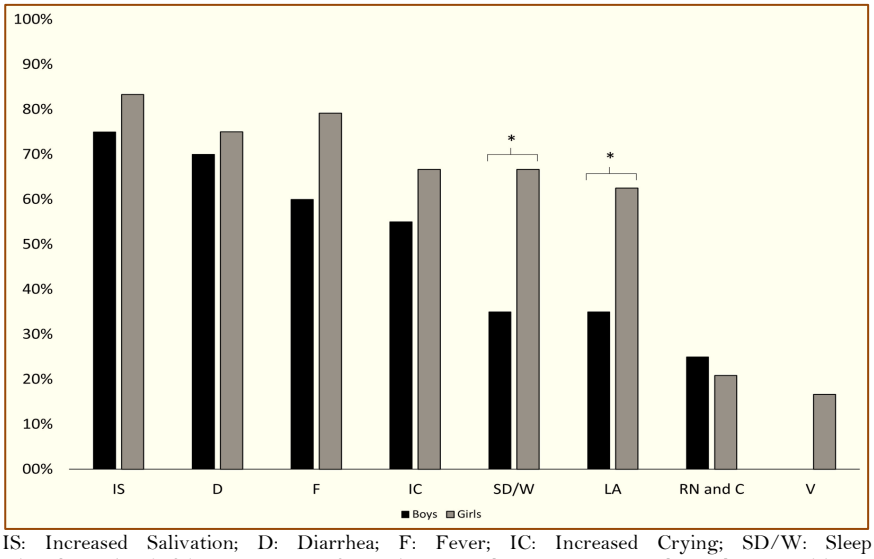
A total of 44 babies and toddlers were included. The sample characteristics are presented in Table 1. Twenty-four (56.6%) were boys, and 20 (45.4%) were girls. Almost all mothers reported at least one sign and symptom of teething (95.5%). The most commonly reported were increased salivation (79.5%), followed by diarrhea (72.3 %) and fever (70.5 %). The mean number of signs and symptoms per child was 4.2 (SD=2.1). Thirty-three mothers (75 %) reported that primary incisor eruption caused more signs and symptoms of teething than other primary teeth. Age and number of erupted teeth were not associated with signs and symptoms of teething.

Table 1. Characteristics of the studied population.

Variables	N (%)
Gender	
Male	24 (54.6)
Female	20 (45.4)
Age in Months (Mean and SD)	32.1 + 10.7
Number of Erupted Primary Teeth	
Minimum-Maximum	1-20
Mean and SD	17.5 (5.0)
Signs and Symptoms of Teething	
Increased salivation	35 (79.5)
Diarrhea	32 (72.3)
Fever	31(70.5)
Increased crying	27 (61.3)
Sleep disturbance/wakefulness	23 (52.3)
Loss of appetite	22 (50.0)
Runny nose and cough	10 (22.7)
Vomiting	4 (9.1)

Note: None of the mothers reported convulsion.

Signs and symptoms of teething according to gender are presented in Figure 1. Sleep disturbance/wakefulness was more commonly reported in boys than in girls (p=0.03). Loss of appetite was also more common in boys (p=0.05). The mean number of signs and symptoms of teething according to gender was significantly (p=0.008) higher in boys (mean = 5.1, SD= 1.5) than in girls (mean = 3.5, SD = 2.1).



IS: Increased Salivation; D: Diarrhea; F: Fever; IC: Increased Crying; SD/W: Sleep Disturbance/Wakefulness; LA: Loss of Appetite; RN and C: Runny Nose and Cough; V: Vomiting.

Figure 1. Signs and symptoms of teething according to gender. Sleep disturbance/wakefulness was more commonly reported in boys than in girls (p=0.03). Loss of appetite was also more common in boys (p=0.05).

SNP analysis demonstrated that rs689466 and rs5275 were within the Hardy-Weinberg equilibrium ($HW_{rs689466}$ Chi-square = 1.13; HW_{rs5275} Chi-square = 1.14). Table 2 shows the genotype (in co-dominant, dominant, and recessive models) and allele distribution of the investigated SNPs.

Neither any genotype nor genotype distribution was associated with any signs and symptoms of teething (Table 3 and Figure 2). Haplotype analysis revealed no statistically significant association (Table 4).

Table 2. Distribution of the genetic polymorphisms rs689466 and rs5275 according to teething signs and symptoms.

	Signs/Symptoms		Models											
			Co-dominant				Recessive			Dominant			Alleles		
			TT N (%)	CT N (%)	CC N (%)	p-value	TT + CT N (%)	CC N (%)	p-value	TT N (%)	CT + CC N (%)	p-value	C N (%)	T N (%)	p-value
rs689466	Increased salivation	No	4 (57.1)	3 (42.9)	0 (0.0)	0.36	7 (100.0)	0 (0.0)	0.44	4 (57.1)	3 (42.9)	0.41	3 (21.4)	11 (79.6)	0.72
		Yes	19 (73.1)	5 (19.2)	2 (7.7)		24 (92.3)	2 (7.7)		19 (73.1)	7 (26.9)		9 (17.3)	43 (82.7)	
	Diarrhea	No	6 (66.7)	3 (33.3)	0 (0.0)	0.55	9 (100.0)	0 (0.0)	0.37	6 (66.7)	3 (33.3)	0.81	3 (16.7)	15 (83.3)	0.84
		Yes	17 (70.8)	5 (20.8)	2 (8.3)		22 (91.6)	2 (8.3)		17 (70.8)	7 (29.1)		9 (18.7)	39 (81.3)	
	Fever	No	6 (60.0)	4 (40.0)	0 (0.0)	0.28	10 (100.0)	0 (0.0)	0.33	6 (60.0)	4 (40.0)	0.42	4 (20.0)	16 (80.0)	0.80
		Yes	17 (63.9)	4 (17.4)	2 (8.7)		21 (91.3)	2 (8.7)		17 (73.9)	6 (26.1)		8 (17.4)	38 (82.6)	
	Increased crying	No	8 (61.5)	5 (38.5)	0 (0.0)	0.19	13 (100.0)	0 (0.0)	0.23	8 (61.5)	5 (38.5)	0.41	5 (19.2)	21 (80.8)	0.85
		Yes	15 (75.0)	3 (15.0)	2 (10.0)		18 (90)	2 (10.0)		15 (75.0)	5 (25.0)		7 (17.5)	33 (81.5)	
	Sleep disturbance	No	9 (64.3)	5 (35.7)	0 (0.0)	0.23	14 (100.0)	0 (0.0)	0.21	9 (64.3)	5 (35.7)	0.56	5 (17.9)	23 (82.1)	0.95
		Yes	14 (73.7)	3 (15.8)	2 (10.5)		17 (89.5)	2 (10.5)		14 (73.7)	5 (26.3)		7 (18.4)	31(82.6)	
	Loss of appetite	No	10 (58.8)	6 (35.3)	1 (5.9)	0.30	16 (94.1)	1 (5.9)	0.96	10 (58.8)	7 (41.2)	0.16	8 (23.5)	26 (76.5)	0.06
		Yes	13 (81.2)	2 (12.5)	1 (6.3)		15 (93.8)	1 (6.2)		13 (81.2)	3 (18.8)		2 (6.7)	28 (93.3)	
	Runny nose and cough	No	17 (73.9)	7 (26.9)	2 (7.7)	0.54	24 (92.3)	2 (7.7)	0.44	17 (65.4)	9 (34.6)	0.29	11 (21.1)	41(78.9)	0.23
		Yes	6 (85.7)	1 (14.3)	0 (0.0)		7 (100.0)	0 (0.0)		6 (85.7)	1 (14.3)		1 (7.1)	13 (92.9)	
	Vomiting	No	6 (18.2)	10 (30.3)	17 (51.5)	0.13	16 (48.5)	17 (51.5)	0.54	6 (18.2)	27 (81.8)	0.06	44 (66.7)	22 (33.3)	0.10
		Yes	2 (66.7)	0 (0.0)	1 (33.3)		2 (66.7)	1 (33.3)		2 (66.7)	1 (33.3)		2 (33.3)	4 (66.7)	
	Signs/Symptoms		Models											
			Co-dominant				**Recessive**			**Dominant**			**Alleles**		
			AA N (%)	AG N (%)	GG N (%)	p-value	AA + AG N (%)	GG N (%)	p-value	AA N (%)	AG + GG N (%)	p-value	A N (%)	G N (%)	p-value
rs5275	Increased salivation	No	5 (55.6)	1 (11.1)	3 (33.3)	0.14	6 (66.7)	3 (33.3)	0.12	5 (55.6)	4 (44.4)	0.70	11 (61.1)	7 (38.9)	0.56
		Yes	13 (48.2)	11 (40.7)	3 (11.1)		24 (88.9)	3 (11.1)		13 (48.2)	14 (51.8)		37 (68.5)	17 (31.5)	
	Diarrhea	No	4 (40.0)	4 (40.0)	2 (20.0)	0.75	8 (80.0)	2 (20.0)	0.73	4 (40.0)	6 (60.0)	0.45	12 (60.0)	8 (40.0)	0.45
		Yes	14 (53.8)	8 (30.8)	4 (15.4)		22 (84.6)	4 (15.4)		14 (53.8)	12 (46.2)		36 (69.2)	16 (30.8)	
	Fever	No	5 (45.5)	5 (45.5)	1 (9.0)	0.51	10 (90.9)	1 (9.1)	0.28	5 (45.5)	6 (54.5)	0.29	15 (68.2)	7 (31.8)	0.88
		Yes	13 (52.0)	2 (28.0)	5 (20.0)		15 (75.0)	5 (25.0)		13 (65.0)	7 (35.0)		28 (70.0)	12 (30.0)	
	Increased crying	No	9 (64.3)	4 (28.6)	1 (7.1)	0.31	13 (92.9)	1 (7.1)	0.22	9 (60.0)	6 (40.0)	0.25	22 (78.6)	6 (21.4)	0.08
		Yes	9 (40.9)	8 (36.4)	5 (22.7)		17 (77.3)	5 (22.7)		9 (40.9)	13 (59.1)		26 (59.1)	18 (40.9)	
	Sleep disturbance	No	9 (50.0)	8 (44.4)	1 (5.6)	0.13	17 (94.4)	1 (5.6)	0.07	9 (50.0)	9 (50.0)	0.99	26 (72.2)	10 (27.8)	0.31
		Yes	9 (50.0)	4 (33.3)	5 (27.8)		13 (72.2)	5 (27.8)		9 (50.0)	9 (50.0)		22 (61.1)	14 (38.9)	
	Loss of appetite	No	9 (50.0)	8 (44.4)	1 (5.6)	0.28	17 (94.4)	1 (5.6)	0.14	9 (50.0)	9 (50.0)	0.99	26 (72.2)	10 (27.8)	0.44
		Yes	9 (50.0)	5 (27.8)	4 (22.2)		14 (77.8)	4 (22.2)		9 (50.0)	9 (50.0)		23 (63.9)	13 (36.1)	
	Runny nose and cough	No	15 (51.7)	9 (30.0)	5 (17.3)	0.83	24 (82.8)	5 (17.2)	0.85	15 (51.7)	14 (48.3)	0.67	39 (67.2)	19 (32.8)	0.83
		Yes	3 (42.9)	3 (42.9)	1 (14.2)		6 (85.7)	1 (14.3)		3 (42.9)	4 (57.1)		9 (64.3)	5 (35.7)	
	Vomiting	No	17 (51.5)	10 (30.3)	6 (18.2)	0.40	27 (81.8)	6 (18.2)	0.41	17 (51.5)	16 (48.5)	0.54	44 (66.7)	22 (33.3)	0.98
		Yes	1 (33.3)	2 (66.7)	0 (0.0)		3 (100.0)	0 (0.0)		1 (33.3)	2 (66.7)		4 (66.7)	2 (33.3)	

Table 3. Multivariate analysis using signs and symptoms as co-variant.

Phenotype	SNP	Reference Genotype	Genotype	Coefficient	Unadjusted OR (CI 95%)	p-value	Coefficient	Adjusted OR (CI 95%)	p-value
Increased Salivation	rs689466	TT	CT	-1.14	0.31 (0.05–1.90)	0.20	-0.91	0.39 (0.05–3.01)	0.37
			CC	8.71	6111.73 (#)	0.88	7.96	2874.82 (#)	0.86
	rs5275	AA	AG	1.70	5.50 (0.60–50.44)	0.13	2.02	7.58 (0.62–93.31)	0.11
			GG	-1.38	0.25 (0.04–1.56)	0.13	-16.44	0.0 (#)	0.67
Diarrhea	rs689466	TT	CT	-0.64	0.52 (0.10–2.88)	0.45	-0.21	0.80 (0.08–8.01)	0.85
			CC	8.80	6667.35 (#)	0.87	8.86	7093.88 (#)	0.83
	rs5275	AA	AG	-0.40	0.66 (0.15–3.03)	0.59	-0.21	0.80 (0.09–7.18)	0.84
			GG	-0.31	0.72 (0.11–4.77)	0.73	-1.54	0.21 (0.01–3.47)	0.27
Fever	rs689466	TT	CT	-1.15	0.31 (0.06-1.66)	0.17	-0.17	0.83 (0.04–16.23)	0.90
			CC	8.85	6984.84 (#)	0.86	7.45	1731.99 (#)	0.83
	rs5275	AA	AG	-0.76	0.46 (0.11–2.04)	0.31	-0.96	0.38 (0.02–6.35)	0.50
			GG	0.91	2.50 (0.26–24.38)	0.43	-23.62	0.00 (#)	0.69
Increased Crying	rs689466	TT	CT	-1.26	0.28 (0.05–1.48)	0.13	-1.61	0.19 (0.01–3.51)	0.27
			CC	9.00	8148.98 (#)	0.84	7.97	2895.46 (#)	0.80
	rs5275	AA	AG	0.35	1.42 (0.34–6.08)	0.62	1.51	4.53 (0.33–62.48)	0.25
			GG	1.34	3.82 (0.40–36.83)	0.24	16.09	731743.17 (#)	0.68
Sleep Disturbance	rs689466	TT	CT	-1.08	0.33 (0.06–1.75)	0.19	1.57	4.83 (0.16–143.16)	0.36
			CC	9.06	8628.33 (#)	0.83	7.82	2500.83 (#)	0.79
	rs5275	AA	AG	-1.02	0.35 (0.08–1.52)	0.16	-1.93	0.14 (0.01–2.55)	0.18
			GG	1.87	6.53 (0.68–62.99)	0.10	24.26	723527.31 (#)	0.68
Loss of Appetite	rs689466	TT	CT	-1.33	0.26 (0.04–1.56)	0.14	-2.40	0.09 (0–1.99)	0.12
			CC	0.06	1.06 (0.06–18.62)	0.96	-0.69	0.50 (0.01–19.55)	0.71
	rs5275	AA	AG	-1.02	0.35 (0.08–1.52)	0.16	-0.81	0.44 (0.05–3.78)	0.45
			GG	1.87	6.53 (0.68–62.99)	0.10	1.43	4.21 (0.08–222.40)	0.47
Runny Nose and Cough	rs689466	TT	CT	-0.79	0.45 (0.05–4.46)	0.49	1.20	3.32 (0.13–85.88)	0.46
			CC	-8.71	0.0002 (#)	0.88	-9.41	0.0001 (#)	0.86
	rs5275	AA	AG	0.51	1.66 (0.31–9.04)	0.55	0.70	2.01 (0.17–24.14)	0.58
			GG	-0.22	0.80 (0.08–8.19)	0.85	-7.76	0.0004 (#)	0.77
Vomiting	rs689466	TT	CT	-10.19	0.00 (#)	0.91	-11.24	0.00 (#)	0.89
			CC	-8.85	0.002 (#)	0.92	-9.07	0.0001 (#)	0.91
	rs5275	AA	AG	1.52	4.60 (0.37–56.75)	0.23	1.99	7.34 (0.36–148.80)	0.19
			GG	-9.69	0.0001 (#)	0.91	-8.94	0.0001 (#)	0.91

Each sign and symptom was adjusted by gender; The # symbol means it was impossible to describe the present data.

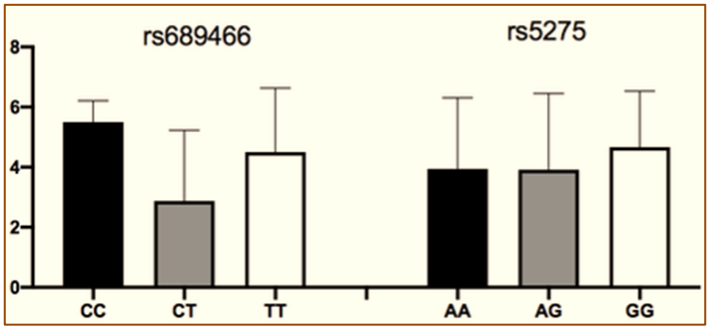


Figure 2. The mean number of signs and symptoms of teething according to the children's genotypes.

Table 4. Haplotype analysis among the groups in the haplotype order rs689466 – rs5275.

Phenotype	Haplotype	Fa[1]	Fu[2]	p-value
Increased Salivation	C – A	0.20	0.21	0.93
	T – G	0.25	0.42	0.20
	T– A	0.54	0.35	0.21
Diarrhea	C – A	0.21	0.18	0.82
	T – G	0.28	0.31	0.84
	T– A	0.50	0.50	1.00
Fever	C – A	0.20	0.22	0.84
	T – G	0.30	0.27	0.86
	T– A	0.50	0.50	1.00
Increased Crying	C – A	0.20	0.20	0.98
	T – G	0.35	0.20	0.23
	T– A	0.44	0.58	0.28
Sleep Disturbance	C – A	0.23	0.17	0.60
	T – G	0.33	0.25	0.48
	T– A	0.43	0.57	0.29
Loss of Appetite	C – A	0.14	0.26	0.24
	T – G	0.32	0.26	0.64
	T– A	0.53	0.46	0.59
Runny Nose and Cough	C – A	0.08	0.23	0.23
	T – G	0.41	0.26	0.29
	T– A	0.50	0.50	1.00
Vomiting	C – A	0.00	0.22	0.28
	T – G	0.5	0.27	0.34
	T– A	0.50	0.50	1.00

Discussion

Local and systemic signs and symptoms of primary tooth eruption are a subject that draws the attention of clinicians and researchers in different areas. Thus, this is probably the first study investigating the association between candidate SNPs in *COX2* with signs and symptoms of teething.

COX-2 is an enzyme expressed in low levels in most tissues [14] and is the main cyclooxygenase, which plays a vital role in inflammation [23]. Its decoder gene, *COX2*, is under the control of the NF-κB/Rel transcription factor family, a protein complex wherein pro-inflammatory signaling of different types of cells regulates COX-2 expression, such as interleukin β, tumor necrosis factor-alpha, epidermal growth factor and others [24]. Thus, COX-2 is released by fibroblasts, macrophages, and vascular endothelial cells in local inflammation [25]. Clinical and *in vitro* studies also demonstrated that COX-2 is expressed by periodontal ligament cells [17,26], which are involved in orthodontic tooth movement via alveolar bone remodeling [27]. For this reason, in our study, we focused on the evaluation of SNPs rs689466 and rs5275 in *COX-2* to evaluate if the individual genetic background is involved in the susceptibility of signs and symptoms of teething.

Although we did not find an association between the rs689466 and rs5275 and the studied teething signs and symptoms, it is essential to highlight that COX-2 is a crucial mediator of local inflammatory response and is involved in fever and pain [28]. COX-2 was also found in large amounts within the dental follicle [5,13,29]. Thus, it was plausible to assume that local and systemic signs and symptoms of teething could be due to the release of COX-2, and other SNPs in COX-2 may still be involved in these signs and symptoms, which merits further investigation.

In this study, an increase in salivation was the most common sign reported by mothers. This sign could be due to gum irritation caused by these pro-inflammatory agents, as the child inserts objects more frequently into the oral cavity, which stimulates greater saliva production and, consequently, increases the frequency of coughing, a sign also observed by some parents [10,30].

Fever is also often reported. Two systematic reviews with meta-analyses demonstrated that fever is the most evident symptom of teething [4,6]. COX-2 regulates PGE_2 synthase in acute inflammation. PGE_2 is transported by the bloodstream to the ventromedial preoptic area of the anterior hypothalamus, starting the feverish state [5,31]. This whole set of factors prompts episodes of irritation, anxiety, loss of appetite, and sleep disturbances [7].

Gastrointestinal disorders, such as diarrhea and vomiting, were also reported by parents during teething. These symptoms, however, could be related to the contamination of the baby's hands and objects [32]. Previous studies related these disorders with the release of certain interleukins [9,13], but the mechanism still needs to be fully understood.

In our study, we observed that signs and symptoms were more commonly reported in boys than in girls. Some studies also showed a gender preference in signs and symptoms during teething [10,20]. This gender preference is noteworthy. Sexual dimorphism is a product of both genetics and environmental factors. It is thus plausible to assume that other genes play a role in the manifestation of signs and symptoms of teething.

Investigating SNPs involved in the signs and symptoms of teething is essential to improve the understanding of the molecular processes involved in teething, and it may hold the possibility to use these as biomarkers for the diagnosis and identification of risk groups. Therefore, we investigated two SNPs, rs689466 and rs5275, which alter the transcriptional efficiency and possibly modify the inflammatory response [33]. These SNPs, however, were not associated with signs and symptoms of teething. The lack of association could be due to some limitations of our study. The demand for dental care in early childhood is still limited. The prevalence of dental babies treated at the Baby's Clinic at Positivo University stands out. This justifies the impossibility of collecting oral samples before tooth eruption. The sample size, potential confounding factors, and the fact that the phenotypes were self-reported are also evidence of the limitations of this study. Some mothers may have reported the signs and symptoms inaccurately. Therefore, although our study raised an interesting issue, future studies should be performed with a prospective design including other candidate SNPs and genes.

Conclusion

The single nucleotide polymorphisms in the *COX2 gene* (rs689466 and rs5275) were not associated with local and systemic signs and symptoms of teething.

Authors' Contributions

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All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

Financial Support

None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

Acknowledgments

We thank everyone involved in this work.

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