Genetic Polymorphisms in *ACTN3* Contribute to the Etiology of Bruxism in Children

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Objective: *Bruxism is a condition defined as a masticatory muscle activity with an unexplored genetic background. The aim of this study was to evaluate the association between genetic polymorphisms in ACTN3 and bruxism.* ***Study design:*** *A total of 151 biological-unrelated children, aged 7–12 years were included in a case control ratio of 1:1.5. The data collection was performed during interview and clinical examination. Saliva samples were collected from all children and 3 genetic polymorphisms in the ACTN3 (rs678397, rs1671064 and rs1815739) were selected for genotyping using real time PCR. Pearson chi-square calculation was used to assess Hardy-Weinberg equilibrium and to evaluate the association between genotypes and alleles frequencies for each genetic polymorphism in the co-dominant and recessive models. An alpha of 5% was used.* ***Results:*** *The genetic polymorphisms rs678397, rs1671064 and rs1815739 were associated with bruxism in the co-dominate model and in the recessive model ($p<0.05$). Allele distribution was also associated with bruxism for the polymorphisms rs678397 and rs1671064 ($p<0.05$).* ***Conclusion:*** *The genetic polymorphisms rs678397, rs1671064 and rs1815739 in ACTN3 are associated with bruxism and can contribute to the etiology of this condition in children.*

KEYWORDS: *Genes, polymorphism, children, bruxism, muscle and ACTN3*

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INTRODUCTION

Bruxism is a condition defined as a masticatory muscle activity, which clinical consequences can be risk factors to the stomatognathic system[1]. This condition presents two circadian manifestations; either occurring during sleep, called sleep bruxism or during wakefulness, called awake bruxism[2,3]. Sleep bruxism is characterized by rhythmic (phasic) or non-rhythmic (tonic) movement and awake bruxism could be described as a repetitive movement of the masticatory muscle, a prolonged and continued contact of the tooth and/or by bracing or thrusting of the mandible[1]. It is generally considered that sleep bruxism is more common during childhood, although it is not unusual in adulthood and is uncommon in the elderly[4]. The prevalence of sleep bruxism in children is high and can reach 40.6%[5].

A previous study performed a systematic analysis of the literature, which indicated that bruxism has a genetic component[3]. Recent articles revised many aspects of the bruxism, including the etiology of this condition, and suggested that bruxism is likely to be a complex condition with a multifactorial etiology[1,6]. Bruxism occurs due to contraction of the masseter, temporalis and other jaw muscles[6]. Most jaw muscles activities occur during light phases of sleep and have been observed to take place in connection with body movement[7].

Genes related to muscle activity have been associated with craniofacial morphology phenotypes[8-10]. *ACTN3* (alpha-actinin-3) gene codifies myofibril anchor proteins expressed in muscle fibers that influences the contractile properties, muscle performance and fiber type amounts[9,11,12]. It is possible to hypothesize that variations in *ACTN3* can be involved in the etiology of bruxism. Therefore, this study aimed, for the first time, to explore the association between genetic polymorphisms in *ACTN3* and bruxism in children.

MATERIALS AND METHOD

The present research was approved by the Ethics Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, Brazil, number (CAAE 35323314.7.0000.5419), and was conducted in accordance with the Declaration of Helsinki. Informed consent and age appropriate assent were obtained from all participating individuals or parents/legal guardians.

Sample

The sample size calculation was performed based on the genotypes/alleles differences of 25% among bruxers and non-bruxers. The calculation predicted a minimum of 125 children with a power of 0.80% and alpha of 0.05 in 1.5 times more control (http://clincalc.com). Thus, a total of 151 biological-unrelated children, aged 7–12 years were included in this study. Children with bruxism (bruxers) were recruited from the Service of Bruxism and Temporomandibular Disorder in Childhood (SABDI) in the University of São Paulo, Ribeirão Preto Dental School, and the control children (without bruxism) were recruited from the Pediatric Dentistry Clinic of the same School. Both, bruxers and non-bruxers children, were consecutively included during their follow-up visits, in a case control ratio of 1:1.5, from March 2016 to December 2017.

Non-syndromic unrelated children, with no dental pain, and without history of facial trauma were included. Children undergoing medical treatment using corticosteroids or immunosuppressant; anti-inflammatories, analgesics, antihistaminic, anxiolytic, homeopathic or other drugs with suppressive action on the central nervous system that could interfere with neuromuscular activities; children with history of a surgical intervention within twelve months before the beginning of the research, were excluded.

The data collection was performed in two phases: interview and physical/clinical examination. The same researcher conducted the interviews, the collection of the questionnaires and clinical examination. Patients were considered bruxers when there was parents' report referring to audible tooth grinding sounds or tooth clenching during sleep, frequent headaches and orofacial pain on awakening, chewing and/or opening the mouth and showed signs and symptoms related to bruxism, such as dental wear facets and fractures of restorations; dental impressions on the cheek mucosa and tongue, tenderness and pain on the temporal and masseter muscles during bilateral palpation.

DNA extraction and ACTN3 genetic polymorphisms genotyping

For saliva collection, children were instructed to provide unstimulated saliva in a 50 mL propylene tube and additionally to rinse the mouth with 10 mL of saline. Therefore, the genomic DNA was extracted from buccal epithelial cells from saliva samples as previously described[13]. Quantification of the concentration and purity of the DNA was determined by spectrophotometer (Nanodrop 1000; Thermo Scientific, Wilmington, DE, USA).

Three genetic polymorphisms in the *ACTN3* (rs678397, rs1671064 and rs1815739) were analyzed. The characteristics of the studied genetic polymorphisms are presented in the Table 1. Polymerase chain reactions (PCR) using end-point analysis and TaqMan technology on a real-time PCR system (Applied Biosystems®, Prism QuantStudio 6 Flex PCR System, Thermo Fisher Scientific Inc., Foster City, CA, USA) was blinded performed following an established protocol [14].

Table 1. The characteristics of the studied genetic polymorphisms

Reference sequence	Base changed	MAF	Alteration	Function
rs678397	C>T	0.42	-	Intron
rs1671064⊤	G>A	0.41	R [Arg] ⇒ Q [Gln]	Missense
rs1815739#	C>T	0.40	R [Arg] ⇒Ter[*]	Stop Gain

Note: MAF means minor allele frequency; https://www.ncbi.nlm.nih.gov. #Also known as R577X. ⊤Also known as R523Q.

Statistical Analysis

For the statistical analysis, PLINK 1.9 package (http://zzz.bwh.harvard.edu/plink/)[15] was used. Bruxers group was compared with non-bruxers group. Pearson chi-square calculation was used to assess Hardy-Weinberg equilibrium and to evaluate the association between genotype and allele frequencies for each genetic polymorphism in the co-dominant and recessive models. The odds ratio (OR) and confidence interval 95% (CI) were calculated for all models and polymorphisms. Haplotype analysis was also performed. The established alpha was 5%.

RESULTS

One hundred fifth-one children were included (mean age was 9.34 years), 81 males and 70 females. Of this total, 61 were bruxers (33 males and 28 females) and 90 individuals were non-bruxers (48 males and 42 females). There was no statistical difference in the gender distribution between the groups (p=0.46). The characteristics of the bruxers group are presented in the Table 2. Seven (11.47%) children presented teeth grinding when awake. Fifteen (24.59%) children presented clenching when sleep, while 30 (49.18%) presented clenching when awake.

Table 2. Bruxism sample characteristics

Bruxism group characteristics	n (%)
Teeth grinding when sleep	61 (100%)
Teeth grinding when awake	7 (11.47%)
Clenching when sleep	15 (24.59%)
Clenching when awake	30 (49.18%)

The genotypes and alleles distributions are presented in the Table 3. In the polymorphism rs678397, to carry the CC genotype and the C allele increased the chance to present bruxism. In the polymorphism rs1671064 to carry the GG (RR) genotype and the G (R) allele, increased the chance to present bruxism. In the polymorphism rs1815739, to carry the TT (XX) genotype increased the chance to present bruxism in comparison with the heterozygotes and in the recessive model.

The haplotype analysis is presented in the Table 4. Although the haplotype TGT was overrepresented and the haplotype CAC was underrepresented in the Bruxers group, none of the haplotype analysis demonstrated statistical significant association (p>0.05).

Table 4. Haplotype frequency among the groups in the haplotype order rs678397, rs1671064 and rs1815739

Haplotype	Haplotype frequency %		p-value
	Non-bruxers	Bruxers	
TGT	0.364	0.469	0.071
CAT	0.017	0	0.156
CGC	0.003	0.021	0.408
TAC	0.014	0.004	0.127
CAC	0.601	0.504	0.101

Table 3. Genotypic and allele distribution in the co-dominant and recessive models and allele distribution.

Polymorphism	Model	Genotype or Allele	Groups n(%)		Statistical results	
			Non-bruxers	*Bruxers*	p-value	OR (CI95%)
rs678397	Co-dominant	CC	11 (12.5%)	18 (30%)	Reference	Reference
		CT	45 (51.13%)	25 (41.66%)	0.016*	2.9 (1.2-7.2)
		TT	32 (36.36%)	17 (28.33%)	0.018*	3.0 (1.1-7.9)
	Recessive	CC	11 (12.5%)	18 (30.0%)	0.008*	3.0 (1.3-3.9)
		CT+TT	77 (87.5%)	42 (70.0%)		
	Allele	C	59 (35.11%)	61 (47.65%)	0.029*	1.68 (1.0-2.6)
		T	109 (64.88%)	67 (52.34%)		
rs1671064	Co-dominant	GG (RR)	6 (7.23%)	14 (25.45%)	Reference	Reference
		GA (RQ)	44 (53.01%)	24 (43.64%)	0.005*	4.2 (1.4-12.5)
		AA (QQ)	33 (39.76%)	17 (30.90%)	0.006*	4.5 (1.4-13.8)
	Recessive	GG	6 (7.23%)	14 (25.45%)	0.002*	4.3 (1.5-12.2)
		GA+AA	77 (92.77%)	41 (74.54%)		
	Allele	G	56 (33.73%)	52 (47.27%)	0.024*	1.7 (1.0-2.8)
		A	110 (66.26%)	58 (52.72%)		
rs1815739	Co-dominant	TT (XX)	11 (12.22%)	15 (25.42%)	Reference	Reference
		CT (RX)	48 (53.33%)	26 (44.06%)	0.044*	2.5 (1.0-6.2)
		CC (RR)	31 (34.44%)	18 (30.50%)	0.080	2.3 (0.8-6.2)
	Recessive	TT	11 (12.22%)	15 (25.42%)	0.037*	2.4 (1.0-5.7)
		CT+CC	79 (87.77%)	44 (74.57%)		
	Allele	T	70 (38.88%)	56 (47.45%)	0.139	1.2 (0.7-1.9)
		C	110 (61.11%)	62 (52.54%)		

Note: * indicates statistical significant difference (p<0.05)

DISCUSSION

There is a consensus in the literature that bruxism has a multifactorial etiology. However, the influence of the genes on this condition is still unclear[16]. Our present study presents an interesting result and sheds some light on the contribution of genetic factors involved in muscle metabolism and bruxism.

The alpha-actinins are a family of actin-binding proteins that have been identified in a diverse range of organisms[17]. The *ACTN3* is expressed only in fast glycolytic skeletal muscle fibers[18]. A study with knockout animal of *Actn3* demonstrated the important metabolic functions that this protein has on muscles.[17] In fact, although the etiology of bruxism involves many factors[19], including psychosocial aspects such as behavioral problems, personality traits, stress or anxiety[20,21], (respiratory disorders[22,23], gastroesophageal reflux[24-25], and central or pathophysiological causes involving brain neurotransmitters[19], the muscles activity presents an important role in its condition[26], in which masticatory muscles contraction is involved in the bruxism manifestation[6].

Our study demonstrated that the 3 evaluated genetic polymorphism in *ACTN3*- rs678397, rs1671064 and rs1815739- increased the chance of the children presents bruxism. Although was not statistically significant, our results also suggest that the TGT haplotype might be a marker for bruxism manifestation in children. The genetic polymorphism rs678397 is locate in an intronic region. This polymorphism has been associated with craniofacial patterns[9], which indicates that this polymorphism has an important function in oral facial muscles.

The rs1671064 is a functional polymorphism changing a Glutamine (CAG) to an Arginine (CGG) at residue 523 (Q523R). We observed that this polymorphism was associated with bruxism in children, in which, children that carry at least one A allele (Q or QQ) had the chance 4 times higher to present bruxism. In a previous study, this polymorphism was also associated with muscles phenotypes, more specifically aerobic capacity, in a previous study[27].

One interesting genetic polymorphism is the rs1815739. Two decades ago, this polymorphism was described as a common single-base change (C>T) in exon 16 of the *ACTN3* gene that converts an arginine residue (R) to a stop codon (X) at amino acid position 577[28]. Approximately 16% of the world population is completely deficient in alph-actinin-3 protein due to homozygosity for the R577X stop codon (*ACTN3* 577XX genotype)[29]. In our population we observed 12% in the non-bruxers, while in bruxers 25.2% presented the XX-null form. A large number of human studies that have been performed show that the *ACTN3* R577X polymorphism represents an important genetic factor associated with variations in muscle performance in humans[17]. Although ACTN3 deficiency is associated with poorer muscle strength, loss of alpha-actinin-3 (XX-null genotype) seems to favor endurance athletes[30]. This polymorphism was also associated with variation in alpha-actinin-3 expression in human masseter, and smaller diameters of fast type II fibers in masseter muscles[9]. Mouse knockout for *Actn3* have also demonstrated reduced diameter for type II fibers, but without changes in fiber numbers[17]. Therefore, it is possible that the polymorphic variant that is associated with endurance increases the risk for bruxism.

Although a previous systematic review had already strongly suggested that bruxism is partly genetically determined[16], to the best of our knowledge, this is the first study that demonstrates that polymorphisms in genes involved in muscle metabolism could be a marker for bruxism. It is probable that bruxism does indeed have a significant genetic cause with many genes playing a role together, although this may not be the sole cause.

CONCLUSION

The genetic polymorphisms rs678397, rs1671064 and rs1815739 in *ACTN3* are associated with bruxism and can contribute to the etiology of this condition in children.

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