ANKH Polymorphisms and Clicking of the Temporomandibular Joint in Dental Residents

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Abstract

Aim This study aimed to carry out a case-control research study to assess occurrence of clicking of the temporomandibular joint (TMJ) in order to establish the relationship between TMJ clicking and the genotype of “ANKH inorganic pyrophosphate transport regulator” (ANKH) polymorphisms.

Materials and Method A sample of 41 first-year dental residents was selected. Each was examined using standard clinical procedures and genotyping techniques.

Results The participation rate was 91.8 %. The prevalence of TMJ clicking was 51.2 % (95 % CI: 35.7–66.7 %). Occurrence of TMJ clicking was not related to age, gender and genotypes of ANKH-OR as well as ANKH-TR polymorphisms ($p \geq 0.165$).

Conclusion A similar distribution of ANKH genotypes in TMJ clicking and asymptomatic individuals has been demonstrated by this study. A high percentage of TMJ

clicking has been confirmed. Future investigations are indicated.

Keywords Temporomandibular disorders · Clicking · ANKH · Polymorphism · Oral surgeon

Introduction

Clicking of the temporomandibular joint (TMJ) has been observed in one-third of the population [1, 2]. Generally this noise resulted from anterior disc displacement with reduction [3] which produced “a sound as a result of the impact of the condyle against the central portion of the meniscus” during mandibular movements [4]. Although TMJ clicking did not predispose severe temporomandibular disorders (TMD) [5], such a manifestation could be a precursory sign of closed lock [6], a presenting symptom of condylar hyperplasia [7], or an indication of destructive changes in chronic polyarthritis [8]. Therefore, clicking of the TMJ is a noticeable medical condition. Management options for this disorder included observation [1], therapeutic exercise [9], splint [10], surgery [11] and botulinum toxin injection into the lateral pterygoid muscle [12].

Epidemiological papers have demonstrated a relationship between occurrence of TMJ clicking and psychological stress [13], impaired masticatory functions [2], parafunctions [13, 14] as well as dental caries [14]. A cephalometric study has found a shorter sagittal midface amongst those patients who displayed the joint sound [15]. From a biomedical viewpoint, clicking and normal TMJs could be differentiated with levels of cytokines [16] and nitric oxide [17] in the synovial fluid. Overexpression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and “tumor necrosis factor receptor superfamily,

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member 10b” (TNFRSF10B, previously known as DR5) was also identified in TMJ discs sustaining anterior displacement with reduction [18]. Even though, to interpret any of these as a causal relationship requires further investigation.

On the other hand, TMJ clicking more often appeared in patients suffering from genetic disorders including Marfan syndrome [19], Ehlers-Danlos syndrome [19], and/or rheumatoid arthritis [20]. Literatures have also reported that occurrence of some TMDs was related to genetic polymorphisms of ANKH inorganic pyrophosphate transport regulator (ANKH) [21], catechol-*O*-methyltransferase (COMT) [22] and matrix metalloproteinase 1 (MMP1) [23]. This implied a potential genetic influence on TMJ clicking, which has not been fully understood.

Among the genes relevant to TMD, ANKH has been suggested as a determinant of ankylosing spondylitis [24], cuff tear arthropathy [25], craniometaphyseal dysplasia [26] and calcium pyrophosphate dihydrate disease [27]. The ANKH protein is a key factor in transporting pyrophosphate ions (PPi) across the plasma membrane and it has shown a rescue effect on some phenotypes of the above diseases [28]. Of further note, existence of ANKH-OR allele 1 and ANKH-TR allele 7 is a genetic marker for ankylosing spondylitis [24]. Although homozygotes of ANKH-OR polymorphisms sustained TMJ closed lock more often than clicking [21], this finding could not be readily interpreted as a protective effect of the heterozygous genotype on TMD. A comparison of ANKH polymorphisms between individuals with TMJ clicking and without any TMD symptoms was thereby indicated.

Hence, the main purpose of this study was to investigate the aetiology of TMJ clicking, using a sample of first-year dental residents in Japan. The aim was to identify the relationship between TMJ clicking and the genotype of ANKH polymorphisms.

Materials and Methods

Appropriate ethics approval for this study has been received from the Institutional Review Board of Kyoto University (approved ID Number: G86). This study hypothesised that those residents who carried a homozygous ANKH polymorphic genotype had a higher risk of TMJ clicking. However, the zygosity distribution of the ANKH polymorphisms in the general population has never been reported. Without an appropriate estimation of the percentage of controls carrying a homozygous ANKH polymorphism, sample size determination according to the statistical power to hypothesis testing was unattainable. To provide a satisfactory sample size for an adequate statistical power, all dental residents at the Department of Oral

and Maxillofacial Surgery, Kyoto University Hospital, from 2006 to 2009, were invited to participate in their first year of residence. Before inclusion, informed consent was sought from all participants. Each was checked for the history of TMD and was assessed with standard clinical procedures including evaluation of mandibular range of motion, joint pain and joint sounds [29]. As TMJ clicking accompanying with other joint symptoms indicated different types of TMD and/or medical conditions [6–8], participants appearing with any joint symptoms but clicking were excluded from this study. Based on clinical diagnoses, subjects were considered TMJ clicking or asymptomatic. The clicking group was composed of cases appearing with TMJ clicking and being free of any other types of TMD. Contrarily, the asymptomatic group only included subjects that have never experienced TMJ clicking and any other types of TMD. Clinical and demographic data of all residents, including the type and the history of TMD, age and gender, were collected.

Blood samples of the residents were obtained and then prepared for DNA isolation. Total genomic DNA from peripheral leukocytes was extracted with a QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany). Genotyping techniques used in this study have been reported earlier [21, 24]. In brief, with the techniques of polymerase chain reaction (PCR), sequencing and purification, TCGCCCCG (Allele-1) and/or TCGCCCCGTCGCCCCG (Allele-2) located in the putative 5′-noncoding region of the ANKH gene were identified as the two genomes of ANKH-OR polymorphisms [21, 24]. Similarly, 7 (Allele-7) and/or 8 (Allele-8) copies of GGC repeats located in the promoter region of the ANKH gene were detected and defined as the two genomes of ANKH-TR polymorphisms [21, 24]. According to combinations of alleles, the variables of genotypes studied included ANKH-OR (homozygotes or heterozygotes) and ANKH-TR (homozygotes or heterozygotes) polymorphisms. All procedures were carried out according to the manufacturers’ instructions.

Data entry and statistical analysis were implemented with the IBM SPSS Statistics (version 20.0, IBM Corporation, Somers, NY, USA). Data analysis included descriptive statistics (frequency distribution and cross tabulation). A univariate logistic regression method was used to assess the individual (unadjusted) contribution of explanatory variables including genotypes of ANKH polymorphisms, gender and/or age [30]. The variables were then arranged to enter a multivariate logistic regression model to examine the collective (adjusted) effect [30]. As highly correlated variables included in a model together might lead to overestimation or underestimation of their significance [30], correlated ANKH-OR and ANKH-TR [24] were separately added into two different models. The level of two-sided significance was set at 5 %.

Table 1 Frequency distribution of TMJ clicking by age, gender and genotypes of ANKH polymorphisms in the sample of the study (n = 41)

	Clicking [n (%)]	No symptom [n (%)]	All [n (%)]	Unadjusted OR ^a (95 % CI)	<i>p</i> values	Adjusted OR ^b (95 % CI)	<i>p</i> values	Adjusted OR ^c (95 % CI)	<i>p</i> values
Genotypes of ANKH-OR polymorphisms									
Heterozygotes	5 (45.5 %)	6 (54.5 %)	11 (26.8 %)	1				1	
Homozygotes	16 (53.3 %)	14 (46.7 %)	30 (73.2 %)	1.4 (0.3–5.5)	0.655			1.4 (0.3–5.9)	0.665
Genotypes of ANKH-TR polymorphisms									
Heterozygotes	4 (40.0 %)	6 (60.0 %)	10 (24.4 %)	1		1			
Homozygotes	17 (54.8 %)	14 (45.2 %)	31 (75.6 %)	1.8 (0.4–7.8)	0.417	2.3 (0.5–10.9)	0.294		
Gender									
Female	9 (64.3 %)	5 (35.7 %)	14 (34.1 %)	1		1		1	
Male	12 (44.4 %)	15 (55.6 %)	27 (65.9 %)	0.4 (0.1–1.7)	0.232	0.4 (0.1–1.5)	0.165	0.4 (0.1–1.7)	0.221
Age									
	27.6 ± 3.3 ^d	27.4 ± 3.4 ^d	27.5 ± 3.3 ^d	1.0 (0.8–1.2)	0.867	1.0 (0.8–1.2)	0.845	1.0 (0.8–1.2)	0.843

^a Results based on univariate logistic regression statistics^b Results based on multivariate logistic regression statistics when excluding genotypes of ANKH-OR polymorphisms^c Results based on multivariate logistic regression statistics when excluding genotypes of ANKH-TR polymorphisms^d Mean ± standard deviation (years of age)

Results

Forty-nine new dental residents were employed at the Department of Oral and Maxillofacial Surgery, Kyoto University Hospital, during the 4 years from 2006 to 2009. Each was approached in his/her first year of residence. Four out of 49 expressed a negative consent to participation. This contributed to a participation rate of 91.8 % in the study. Excluding 4 participants showing TMD symptoms other than clicking, a total of 41 subjects were included in the final sample. The dental residents' age ranged from 24 to 37 years and the mean age was 27.5 ± 3.3 years. Fourteen (34.1 %) out of 44 were females. The prevalence of TMJ clicking was 51.2 % (95 % CI 35.7–66.7 %) in this sample (Table 1).

According to the combinations of the ANKH-OR polymorphisms, there were 11 (26.8 %) Allele-1 homozygotes (Genotype 1/1), 19 (46.4 %) Allele-2 homozygotes (Genotype 2/2) and 11 (26.8 %) Allele-1/Allele-2 heterozygotes (Genotype 1/2) in this sample. As for combinations of the ANKH-TR polymorphisms, 12 (29.2 %) Allele-7 homozygotes (Genotype 7/7), 19 (46.4 %) Allele-8 homozygotes (Genotype 8/8) and 10 (24.4 %) Allele-7/Allele-8 heterozygotes (Genotype 7/8) were identified. Matches of Genotype 1/1 for Genotype 7/7, Genotype 2/2 for Genotype 8/8, and Genotype 1/2 for Genotype 7/8 were observed in 10 out of 11, 19 out of 19, and 9 out of 11 subjects, respectively. Age, gender and genotypes of ANKH-OR as well as ANKH-TR polymorphisms were not related to occurrence of TMJ clicking ($p \geq 0.165$) (Table 1).

Discussion

This study demonstrated that those who sustained TMJ clicking did not differ from asymptomatic subjects in the genotypes of ANKH polymorphisms. Since an earlier study has reported a higher risk of closed lock over TMJ clicking in ANKH-OR homozygotes [21], this study added a resemblance of ANKH-OR zygosity between clicking and asymptomatic individuals to understanding of TMD aetiology. The findings in this and the previous studies can be jointly used as a benchmark for future investigations in genetic distinction of closed lock, TMJ clicking and other types of TMD. Moreover, this study implied that TMJ clicking might be more similar to a healthy condition from a genetic viewpoint although a widely adopted classification scheme has categorised TMJ clicking and closed lock into the same diagnostic group [31]. Some biochemical markers which have distinguished closed lock from clicking and/or asymptomatic subjects failed to show a difference between clicking and healthy TMJs [32, 33]. These included concentration of glycosaminoglycans in the TMJ disc [32] and MMP2 as well as MMP9 expression in synovial fluid [33]. In addition, electromyographic tracing patterns of the lateral pterygoid muscle in patients with clicking were identical with those of normal controls, whilst severer TMD cases displayed a different tracing pattern of the muscle [34]. Thus, the biochemical and biomechanical similarity between clicking and asymptomatic TMJs could be a reason for the lack of differentiability in this study.

On the other hand, past studies applying a magnetic resonance imaging method have found anterior disc

displacement in 20 % of clinically asymptomatic individuals [29, 35]. As a clinical assessment method was used to detect TMJ conditions in this study, the potential mixture of asymptomatic anterior disc displacement and normal subjects in the control group could result in a similar distribution of the ANKH genotypes between the cases and the controls.

Occurrence of TMJ clicking was not associated with age in this sample. This confirmed the findings reported in earlier studies [1, 2, 14]. Furthermore, gender was not a key factor for clicking joints in this study. This agreed with a previous paper [14], whilst some articles reported a higher prevalence of TMJ clicking amongst females [1, 2]. Because all subjects were from a specific profession background, a potential effect of gender on the condition might be overwhelmed by the professional particularity of this sample.

Approximately 50 % of subjects appeared with TMJ clicking in this sample. The prevalence was higher than that in the general population [1, 2]. Since psychological stress was associated with TMJ clicking [13] and junior hospital dentists of the oral surgery specialty sustained larger stress [36], the high percentage of such a manifestation among the first-year dental residents at the Department of Oral and Maxillofacial Surgery is anticipatable.

Conclusions

This study has manifested a similar distribution of ANKH genotypes in TMJ clicking and asymptomatic individuals. A high percentage of TMJ clicking has been confirmed. In addition, age and gender were not associated with occurrence of TMJ clicking.

TMJ clicking has become a relevant issue in public health and dental medicine. A genetic approach to this manifestation can provide a new viewpoint toward prevention and treatment of TMD. Future investigations in genetic distinction among closed lock, TMJ clicking and other types of TMD are indicated.

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