Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio



Mandibular molar C-shaped root canals in 5th millennium BC China



Hui Ying Ren^{a,1}, Yong Sheng Zhao^{b,1}, Yeon-Jee Yoo^c, Xiao Wen Zhang^d, Hui Fang^d, Fen Wang^d, Hiran Perinpanayagam^e, Kee-Yeon Kum^c, Yu Gu^{f,*}

- ^a School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University & Shandong Key Laboratory of Oral Tissue Regeneration & Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, Shandong Province, PR China
- ^b Institute of Cultural and Heritage, Shandong University, Qingdao, PR China
- ^c Department of Conservative Dentistry, Dental Research Institute, National Dental Care Center for Persons with Special Needs, Seoul National University Dental Hospital, Seoul National University School of Dentistry. Seoul. Republic of Korea
- ^d School of History and Culture, Shandong University, Jinan, PR China
- ^e Division of Restorative Dentistry, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Canada
- ^f Department of Endodontics, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University & Shandong Key Laboratory of Oral Tissue Regeneration & Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, Shandong Province, PR China

ARTICLE INFO

ABSTRACT

Keywords:
C-shaped root canal
Cone-Beam computed tomography
Craniofacial bone remains
Mandibular second molar

Objective: The aim of this study was to analyze the occurrence and variations in C-shaped canals in ancient Chinese teeth and compare the differences of these features between ancient and age-matched modern populations.

Design: Approximately 5000-year-old craniofacial bone remains were collected from the fossils of 38 individuals (total: 68 mandibular second molars) excavated from the Jiaojia site. The control group comprised of an equal number of randomly selected modern samples. We used cone-beam computed tomography to scan the mandible along the apex-crown axis and analyzed the canal morphology, based on Fan's categorization criterion, at 2 mm, 5 mm, and 8 mm to the apical level. Grooves on the lingual and buccal sides were also recorded.

Results: The proportion of C-shaped roots among ancient samples on the left and right sides were 48.57% (17/35 teeth) and 54.55% (18/33 teeth), respectively, and 51.47% (35/68 teeth) in the total sample. Conversely, in the control group, 44.12% (15/34) and 38.24% (13/34) occurred on the left and right sides, respectively, and 41.18% (28/68) in the total sample. Among the C-shaped canals from the Jiaojia site samples, the classification type changed between two adjacent levels in 84.31% of samples. Approximately 35 (51.5%) teeth had a fused root, 20 (29.41%) had one shallow buccal and one deep lingual groove. The occurrence of C-shape variation was not significantly correlated with time (p > 0.05).

Conclusions: This study identified a high rate of C-shaped root canals among individuals of Jiaojia who lived approximately 5000 years ago.

1. Introduction

Root canal systems with an anatomical configuration described as "C-shaped" are most often seen in mandibular second molars, followed by maxillary second molars, particularly within the dentition of Asian ethnic groups (Cooke & Cox, 1979; Sidow, West, Liewehr, & Loushine, 2000). Mandibular second molars with C-shaped root canal systems

may be present in almost half the Chinese population (Wang, Guo, Yang, Han, & Yu, 2012), as opposed to only 2.7%–5% of Caucasians (von Zuben, Martins, & Berti, 2017; Roy et al., 2019). For these and other characteristics of tooth morphology, genetics appears to play a major role (Turner, 1987; Scott, 1997). Usually, the genetics for tooth form is selectively neutral, so that root shape is evolutionarily conserved and less affected by environmental factors (Shields & Jones,

Abbreviations: micro-CT, microcomputed tomography; CBCT, cone-beam computed tomography; C1, Category 1; C2, Category 2; C3, Category 3; C4, Category 4; C5, Category 5

^{*} Corresponding author at: Department of Endodontics, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University & Shandong Key Laboratory of Oral Tissue Regeneration & Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, 44-1, Wenhua Xi road, Jinan 250002, Shandong Province, PR China.

E-mail address: guyu@sdu.edu.cn (Y. Gu).

¹ These authors (HuiYing Ren & Yong Sheng Zhao) contributed equally to this study.

1996). Consequently, variations in tooth anatomy could be significant for studying prehistoric human populations in anthropology (Ceperuelo, Lozano, Duran-Sindreu, & Mercadé, 2014). Furthermore, teeth are the most resilient tissues among skeletal remains; they can resist destruction and are preserved over thousands of years to provide evidence of an individual's dietary habits (Martínez et al., 2020; Frank et al., 2019) and physical condition (G. Goude, Dori, Sparacello, Starnini, & Varalli, 2020), as well as taxonomic relationships (Hanegraef et al., 2018). Thus, several studies have been able to utilize prehistoric dentition to perform detailed analyses of their morphological features (Kasai & Kawamura, 2001; Shi, Duan, Shao, Duan, & Peng, 2013; Ramírez-Salomón et al., 2014; Zilberman, Milevski, Yegorov, & Smith, 2019).

The C-shaped root canal is anatomically characterized by fins or webs that interconnect individual canals within a fused root. In cross-sectional images, the canal orifice appears ribbon-shaped with an arc of 180° or greater (Jafarzadeh & Wu, 2007). The surrounding root structure is usually conical or cuboidal and has a longitudinal groove on the lingual or buccal surfaces, which are typical of fused roots (Jerome, 1994; von Zuben et al., 2017).

The origin of C-shaped root canals has been ascribed to a developmental failure of Hertwig's epithelial root sheath to fuse on the root surface (Manning, 1990). Additionally, their root anatomy could be formed by a coalescence of cementum deposition over time (Fan, Cheung, Fan, Gutmann, & Bian, 2004). Several reports have described variations in the number and shape of these roots and their canal morphologies. These variations appear to be genetically predetermined and can be traced to the ethnic origins of a population and the classification of hominids.

As C-shaped canals occur in various configurations, Melton, Krell, and Fuller (1991)) proposed a classification based on their cross-sectional shape. However, their shape can vary along the length of the root canal. Therefore, the coronal outline of the canal on the pulp chamber floor, which is observed clinically during endodontic treatment, may not be representative of deeper canal configurations (Fan, Chen, & Fan, 2001). Therefore, Fan et al. (2001) proposed a more comprehensive classification of canal type, as follows: C1 have a continuous C-shaped cross-section; C2 have discrete C-shaped cross-sections and at least one arc $\geq 60^\circ$; C3 have 2–3 separate canals with arcs $< 60^\circ$; C4 have only a single round/oval canal; and C5 have no visible canal lumen. The canal in 11 root-sample cross sections were classified as C-shaped because one or more cross-sections were in categories 1–3 (Fan et al., 2004). This classification accounts for the variation in C-shaped canal anatomy along the length of the root.

These root canal configurations can be examined clinically through radiographs, which are limited by their two-dimensional interpretations. More detailed three-dimensional information can be obtained from extracted teeth *in vitro* by tooth clearing, dye staining, grinding, and splitting, which unfortunately destroy the specimens. In contrast, microcomputed tomography (micro-CT) and cone-beam computed tomography (CBCT) can provide accurate and detailed digital three-dimensional reconstructions of complex root canal anatomical configurations, without damaging the sample (Patel, 2009). Micro-CT

provides details of the internal structures in a limited field-of-view, which is usually confined to a single tooth, and is limited to *in vitro* applications. However, CBCT produces hundreds of cross-sectional images in a larger field-of-view that can accommodate larger specimens such as the skeletal remains from archaeological sites. Furthermore, CBCT is routinely used as an *in vivo* technique for clinical applications. Therefore, CBCT may be an ideal technique for analyzing intricate three-dimensional structures within the root canal systems of ancient skeletal remains that warrant preservation.

The morphological features of human mandibular teeth can differ significantly among populations of different ethnicities and geographic regions (Martins, Ordinola-Zapata, Marques, Francisco, & Caramês, 2018), and these features have been the subject of numerous anatomical and paleontological studies (Ramírez-Salomón et al., 2014; Turner, 1987, 1990). However, most of the archaeological studies have focused on external tooth crown morphologies (Gómez-Robles, de Castro, Martinón-Torres, Prado-Simón, & Arsuaga, 2015; Peretz & Smith, 1993), and only a very few have examined the internal root structure of ancient fossilized teeth or discussed the possibility of evolutionary changes in root canals. These include Keith (1913) who identified these roots in Neanderthals, but ascribed them to be an extinct collateral species that were not predecessors of modern humans. Then, Pederson (1949) observed C-shaped roots and canals in East Greenland Eskimos, and subsequently Cooke and Cox (1979) determined an 8% prevalence rate for C-shaped root canal among Neanderthals.

Given the higher prevalence of C-shaped roots and canals in modern Chinese, a similar predominance among their prehistoric populations may have occurred. However, few studies have analyzed the root canals of ancient Chinese teeth, and there are none from the Neolithic era. Recently, Hominid fossils were excavated from the Jiaojia site, which belong to the 5000-year-old Neolithic Age. The Jiaojia site is in Jinan, Shandong Province, China, and is associated with the Dawenkou culture. Therefore, the purpose of this study was to analyze these ancient Chinese skeletal remains and identify the prevalence of C-shaped canals and their variants in mandibular second molar teeth. At the same time, we compared the difference of these features between ancient and agematched contemporary clinical cases.

2. Materials and methods

2.1. Prehistoric skeletal remains

Craniofacial skeletal remains were collected from 38 individuals (68 mandibular second molars) that had been excavated from the Jiaojia site in Shandong Province, China. The individuals were identified as male (21), female (14), or of unknown sex (3), and were approximately 5000 years old (Table 1). Their ages ranged from 15 to 40 years, and their mean age was 28.82 years. These prehistoric samples were divided into the following age groups: 15–25 years, 26–35 years, and 36–40 years, for comparisons with age-matched controls obtained from current clinical cases.

Table 1

The prevalence of C-shaped canals in the mandibular second molars of prehistoric skeletal remains according to their sex, age, and location.

	Sex ^a		Age (years)			Location		
	Male (n = 38)	Female (n = 25)	15–25 (n = 23)	26–35 (n = 40)	36-40 (n = 5)	Left (n = 35)	Right (n = 33)	Total (n = 68)
No. of teeth Frequency (%)	18 47.37	13 52.00	13 56.52	18 45.00	4 80.00	17 48.57	18 54.55	35 51.47

There was a higher prevalence of C-shaped canals in ancient females than in males (p = 0.002).

There was no correlation between age and the prevalence of C-shaped canals in fossil skulls (p = 0.125).

^a The sex of the individual could not be determined for five teeth.

Table 2

The prevalence of C-shaped canals in the mandibular second molars of clinical cases according to their sex, age, and location.

	Sex		Age (years)			Location		Total $n = 68$
	Male (n = 37)	Female (n = 31)	15–25 (n = 20)	26–35 (n = 44)	36-40 (n = 4)	Left (n = 34)	Right (n = 34)	
No. of teeth Frequency (%)	16 43.24	12 38.71	11 68.75	16 36.36	1 25.00	15 44.12	13 38.24	28 41.18

The prevalence of C-shaped canals in prehistoric remains and in modern clinical cases was not significantly different (p = 0.391). There was no correlation between sex and the prevalence of C-shaped in modern clinical cases (p = 0.46).

2.2. Contemporary clinical cases

Approval for this study was obtained from the Institutional Review Board of the School of Stomatology at Shandong University (IRB number: 20,190,705). The CBCT images of 68 mandibular second molars from 39 patients who had undergone implant or oral surgical procedures at the Shandong University Stomatology Hospital were randomly selected to provide an age-matched control group. Their ages ranged from 15 to 40 years, and the distribution of ages for these clinical cases were similar to those of the prehistoric skeletal remains (Table 2).

2.3. CBCT image analysis

Each prehistoric individual was examined by a CBCT scanner (Newtom, Verona, Italy) according to the manufacturer's recommended protocol. A 12-inch by 12-inch field-of-view was used, and the scanner operated at 85 kV and 21 mA with a 14-second exposure time, a 0.3-mm voxel size, and a 0.5-mm slice thickness.

Axial, coronal, and sagittal two-dimensional images were displayed on a monitor and examined using the NNT software (New Net Technologies, Ltd., Hertfordshire, England). The data sets in Digital Imaging and Communications in Medicine format were transferred to Mimics 20.0 software (Materialise NV, Leuven, Belgium) for three-dimensional reconstruction using a threshold-based segmentation approach and odontometric analysis. CBCT images that are representative of the prehistoric remains (Fig. 1) and the contemporary clinical cases (Fig. 2), are shown.

2.4. Classification of C-shaped canals

C-shaped root canal show a broad spectrum of morphological variants, and different classification systems have been used in prior studies (Manning, 1990; Melton, 1991). The classification proposed by Fan et al. (2004) was used, which includes the following five categories:

Category 1 (C1), the root has a continuous C-shaped cross-section with no separation or division;

Category 2 (C2), the root has a discontinuous C-shaped cross-section with at least one arc that is no less than 60°;

Category 3 (C3), the root has 2–3 separate canals and no arc is more than 60° :

Category 4 (C4), the root has only one round or oval canal in the cross-section:

Category 5 (C5), the root canal has no lumen but the canal usually occurs near the apex.

Along the length of each root from the apex to the crown, three levels were selected for the cross-sectional analysis of canal shape as follows: (1) A + 8 (8 mm from the apex), (2) A + 5 (5 mm from the apex), and (3) A + 2 (2 mm from the apex) (Fig. 3). At each of these levels the cross-sectional shape of the canal was identified according to Fan's method.

2.5. Statistical analysis

The prevalence of C-shaped canals in the prehistoric remains and contemporary clinical cases was compared by Chi-square test. The correlation between sex and the prevalence of C-shaped canals in each

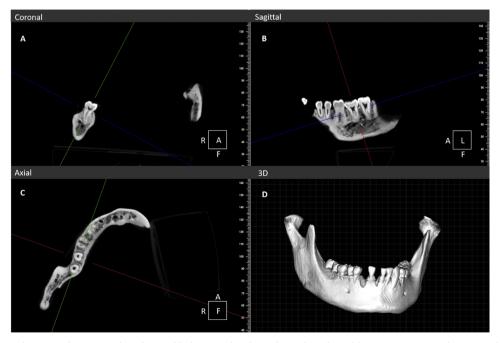


Fig. 1. Cone beam computed tomography images of a right mandibular second molar with a C-shaped canal from an ancient sample as viewed in the coronal, sagittal, and axial directions using NNT software. A, coronal view; B, sagittal view; C, axial view; D, 3D reconstructed image.

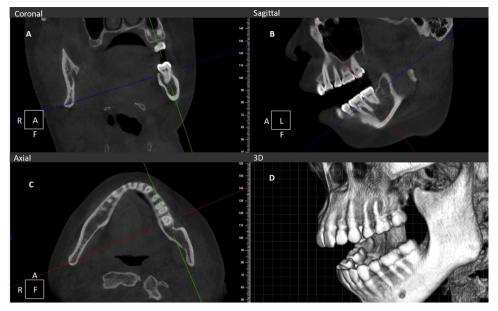


Fig. 2. Cone beam computed tomography images of a right mandibular second molar with a C-shaped canal from a current sample as viewed in the coronal, sagittal, and axial directions using NNT software. A, coronal view; B, sagittal view; C, axial view; D, 3D reconstructed image.

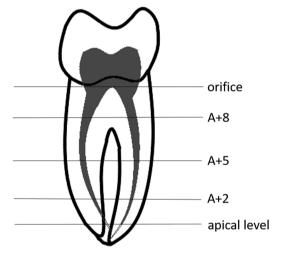


Fig. 3. Measurement locations. A+8,8 mm from the apex; A+2,2 mm from the apex.

sample, and the association between age and the prevalence of C-shaped canals, was evaluated by Spearman rank correlation coefficient. The statistical significance threshold was set at p < 0.05. All statistical analyses were performed using IBM SPSS Statistics 23 software (IBM; Armonk, NY, USA).

3. Results

The percentage of C-shaped roots in the ancient craniofacial bones on the left and right sides were 48.57 % (17/35) and 54.55 % (18/33), respectively, and 51.47 % (35/68) overall in the samples. In contrast, the modern clinical cases had a lower percentage of C-shaped roots, which were 44.12 % and 38.24 % on the left and right sides, respectively, and 41.18 % overall. The age- and sex-specific frequencies of C-shaped root canals are presented in Tables 1 and 2.

Regarding the cross-sectional shape of the C-shaped canals at different root levels, most ancient teeth had root canals longer than 8 mm, whereas the sample numbered "JM-M125" had a root canal of only 6 mm. Table 3 shows different two-dimensional types at 2 mm, 5 mm,

Table 3Cross-sectional canal shapes of C-shaped canals at different levels.

Location		C1	C2	C3	C4	C5
Orifice level	Ancient Modern	12 (34.29) 20 (68.97)	7 (20.00) 7 (25.00)	10 (28.57) 1 (3.45)	6 (17.14) 0	0
A + 8 (%)	Ancient	7 (20.00)	9 (25.71)	12 (34.29)	6 (17.14)	0
A + 5 (%)	Modern Ancient	14 (50.00) 7 (20.00)	12 (42.86) 10 (28.57)	2 (7.14) 14 (40.00)	0 4 (11.43)	0
A + 2 (%)	Modern Ancient	7 (25.00) 3 (8.57)	17 (60.71) 7 (20.00)	4 (14.29) 20 (57.14)	1 (3.45) 5 (14.29)	0
	Modern	6 (21.4)	12 (42.86)	8 (28.57)	1 (3.45)	0

A + 2, 2 mm from apex; A + 5, 5 mm from apex; A + 8, 8 mm from apex. *Note*: The C1 type has a continuous C-shaped cross-section; the C2 type has discrete C-shaped cross-sections and at least one of the arcs is at least 60° ; the C3 type has 2–3 separate canals with arcs $<60^\circ$; the C4 type has only a single round/oval canal; and the C5 type has no visible canal lumen.

and 8 mm over the apical level. C1 most frequently occurred at the orifice level (34.29 %), and C3 most frequently occurred at other levels (A8, 34.29 %; A5, 40.00 %; A2, 57.14 %). However, in the modern group, C1 was the most predominant type of root canal (50 %) at 8 mm over the apical level. C2 accounted for a large proportion of C-shaped canals at 5 mm (60.71 %) and 2 mm (42.86 %) over the apical levels. Fig. 4 shows representative axial CBCT images of a continuous C-shaped canal configuration.

Variations along the apex-crown axis was apparent from the changes in the root canal cross-sectional classification at different levels. Changes in the classification of the C-shape type occurred between two adjacent levels in 84.31 % of the samples in the ancient group. In contrast, classification changes occurred in only 55.26 % of the samples in the control group (Table 4).

Facial appearance reflected the extent to which the C-shaped root was fused. In mandibular second molars, a complete failure in the division on the buccal side, associated with a partial division on the lingual side, creates roots with a horseshoe-shaped cross-sectional appearance (Keith, 1913). As shown in Table 5, among the 68 teeth examined, 35 (51.5 %) had fused roots. Most (13, 19.12 %) of these teeth had only one longitudinal groove on the lingual root surface; 20 (29.41 %) teeth had one shallow buccal and one deep lingual groove; and 2 (2.94 %) teeth had no groove. However, in the modern group, 13 (19.12 %) teeth with fused roots had only one longitudinal groove on

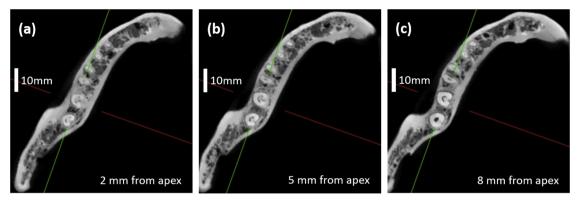


Fig. 4. Example of cone beam computed tomography axial images of a tooth with a C-shaped canal configuration. (a) 2 mm from apical level; (b) 5 mm from apical level; (c) 8 mm from apical level.

Table 4
The number of C-shaped canals showing a change in classification at adjacent levels.

	Number of teet	h
	Ancient	Modern
Configuration remained unchanged Configuration changed apically	8	11
Orifice to A + 8	9	7
A + 8 to A + 5	12	9
A + 5 to A + 2	22	5

A+2, 2 mm from the apex; A+5, 5 mm from the apex; A+8, 8 mm from the apex.

Table 5Frequency distribution of root morphology in the mandibular second molar.

Root morphology	Mandibular second molar, $\%$; (n = 68)		
	Ancient	Modern	
All roots separate	33 (48.53)	40 (58.82)	
Fused roots			
With buccal groove	0 (0)	0 (0)	
With lingual groove	13 (19.12)	13 (19.12)	
With both buccal and lingual groove	20 (29.41)	15 (22.06)	
No groove	2 (2.94)	0 (0)	
Total	68 (100)	68 (100)	

the lingual root surface; 15 (22.06 %) teeth had one shallow buccal and one deep lingual groove.

In the whole sample, the ancient and modern samples had comparable prevalence of C-shaped canal configurations (p = 0.391). The differences in the prevalence of C-shaped canal configurations by sex were significant in the ancient group (p = 0.002), but they were not significant in the modern clinical cases (p = 0.46). There was no correlation between age and prevalence of C-shaped canal in fossil skulls (p = 0.125).

4. Discussion

This is the first study to have analyzed the prevalence of C-shaped canals and their variants in the mandibular second molars of a prehistoric Chinese population from the Neolithic era. The study found a high prevalence of C-shaped canals in the mandibular second molars of prehistoric skeletal remains from the Jiaojia archaeological site in Shandong Province, China. Although other studies have reported on the prevalence and morphological variations among C-shaped root canals in archaeological samples including Neanderthal hominid fossils (Yang et al., 1988), these have not extended back to the Neolithic age in

China.

The prevalence of C-shaped root canals in 68 mandibular second molars from the 5000 year old remains of 38 individuals at the Jiaojia site in Shandong (51.47 %), was higher than in an age-matched control group of current clinical cases at Shandong University (41.18 %). This prevalence was also higher than in modern Chinese populations, as reported by Yang, Yang, Lin, Shay, and Chi (1988)) (33.6 %), Wang et al. (2012) (34.64 %), and Zheng et al. (2011) (39.9 %), respectively. The prevalence in this study was higher than that reported in a study conducted on ancient Homo sapiens from the El Mirador Cave (12.5 %) (Ceperuelo et al., 2014). Interestingly, Ceperuelo and colleagues reported a higher percentage of C-shaped canals in historic samples than contemporary populations, which is similar to the findings of the current study. However, the limited sample size and scarcity of recorded evidence makes it difficult to ascertain whether the occurrence of Cshaped canals has become rarer over the course of human evolution. This study also found that the ancient mandibular second molars had a high frequency of fused roots with only a lingual groove or with both buccal and lingual grooves. In contrast, the control group of clinical cases had fewer aberrations on their root surfaces, which are consistent with a cohort of modern Chinese, as reported by Zheng et al. (2011).

There was a transformation in the cross-sectional shape of these C-shaped root canals, which proceeded from the pulp chamber canal orifice to the root apex, as previously reported. This study found that the prevalence of C1 and C2 categories decreased from the crown to the apex, whereas the prevalence of C4 and C3 increased, in both the ancient molars and the contemporary clinical cases. These findings agree with those previously reported by Fan et al. (2004). These transformations of their cross-sectional shapes indicate that the continuous C1 type canals and the semicolon-shaped C2 type may often divide into two or three canals within the middle and apical regions of the root (Zheng et al., 2011). These results confirm that the three canals in mandibular second molars tend to form only two apical foramina, as reported by Vertucci (1984).

Root canal morphology is also affected by the age of the individual. Gani, Boiero, and Correa (2014)) demonstrated that mandibular first molar root canals become simpler, more sharply defined, and narrower over the years. Children and teenagers tend to have single large canals, young adults (20–40 years) normally experience changes in their canals from dentine deposition and calcifications, and older individuals tend to have narrower canals (Gani et al., 2014). Therefore, this study selected age-matched clinical cases for the control group, to account for any effects of age on the C-shaped canals.

For root development, the formation of the pulp chamber floor and the bifurcation or trifurcation of multi-rooted teeth has been ascribed to Hertwig's epithelial root sheath (Vertucci, 1984). Influenced by dietary customs and other environmental factors, the development and calcification of Hertwig's epithelial root sheath may induce variations in the frequency and morphological distribution of C-shaped canals in

Table 6
Prior reports on the prevalence of C-shaped roots/canals in mandibular second molars.

Study	Population	Sample size	Number of C-shaped roots $^{R}/$ canals C	Prevalence (%)
Al-Fouzan, 2002	Saudi Arabian	151	16 ^C	10.6
Gaby Y.	Lebanese	94	18 ^C	19.1
Weine et al., 1988	Chicago area	75	2^{c}	2.7
Yang et al., 1988	Hong Kong and Taiwan	581	183 ^R	31.5
Walker, 1988	Hong Kong Chinese	100	55 ^R	55
Zheng et al., 2011	Chinese	528	204 ^C	38.6
Jun-Beom Park	Korean	660	281 ^R	42.6
Zhang et al., 2011	Chinese	157	46 ^C	29

mandibular second molars (Martins et al., 2018). Additionally, the individual's sex was proposed to be another factor that influenced the morphology and configuration of teeth in a study of root canal configuration in a Turkish population by Sert and Bayirli (2004). In this study, the prevalence of C-shaped canals in prehistoric men and women was 47.37 % and 52.00 % respectively, and the corresponding prevalence in modern clinical cases was 43.24 % and 38.71 % respectively. There was no significant correlation between sex and the prevalence of the C-shaped variant in mandibular second molars (p > 0.05). Similarly, Zheng et al. (2011) reported that there was no correlation between sex and the prevalence of C-shaped canals.

Root canal morphologies have a genetic influence, and ethnic variations may occur. Although there are very few studies that have reported on the morphological variants in ancient mandibular second molars (Keith & Knowles, 1911, 1913), there are multiple reports on differences in the prevalence of C-shaped canals for modern populations of varying ethnicity. The high prevalence of C-shaped canals (51.47 %) in these ancient skeletal remains from the Jiaojia archaeological site is consistent with their belonging to the Mongolian ethnic groups. Similarly, prior studies on the root canal anatomy of Chinese (Yang et al., 1988; Zhang et al., 2011), Hong Kong Chinese (Walker, 1988), and Korean (Park, Kim, Park, Kim, & Ko, 2013) populations have reported high prevalence of 29-55 % for C-shaped configurations in mandibular second molars. In contrast, C-shaped root canals were sparsely prevalent among Neanderthals, and. studies of Saudi Arabian (Al-Fouzan, 2002), Lebanese (Haddad, Nehme, & Ounsi, 1999), and American populations (Chicago area) (Weine, Pasiewicz, & Rice, 1988) reported a low-frequency of C-shaped variants (Table 6).

As genes may be important determinants of root development in different populations (Ahmed, Abu-bakr, Yahia, & Ibrahim, 2007), and traits in dental morphology may be mostly regulated by genes and minimally affected by environmental factors (Scott & Dahlberg, 1982). Therefore, variations in human dental morphology may be due to random genetic drift (Scott & Turner, 1997). Thus, the variability observed between the ancient Jiaojia inhabitants and current residents could be due to population influx over the past 5000 years. Similarly, Przesmycka et al. (2020) reported increased morphological variability in the number of roots and in the configuration of root canals when comparing populations from the late medieval era and modern times in Radom, Poland.

The different findings in some of these studies could be attributed to the techniques used. This study used CBCT, which is an extraoral imaging system that was developed in the late 1990's to generate three-dimensional images of the maxillo-facial skeleton (Mozzo, Procacci, Tacconi, Martini, & Andreis, 1998; Arai, Tammisalo, Iwai, Hashimoto, & Shinoda, 1999). It has only recently become more widely available for clinical applications and research. CBCT has been found to be extremely accurate and capable of providing two- and three-dimensional measurements irrespective of the skull's orientation (Lascala, Panella, & Marques, 2004). The exact location and anatomy of the root canal systems can be assessed by CBCT for the provision of surgical and non-surgical root canal treatments and the clinical management of complications (Cotton, Geisler, Holden, Schwartz, & Schindler, 2007).

Although Micro-CT techniques can provide an even higher resolution for the identification of intricate canal anatomy, they are limited to in vitro applications and small areas, usually a single tooth. CBCT techniques are routinely utilized for in vivo clinical applications and they scan larger viewing areas of the maxilla and mandible for the analyses of craniofacial skeletal remains. However, the CBCT technique used in this study does have some limitations. Although CBCT scans provide clear images of internal root canal structure, they may conceal intricate anatomical details such as fine branches and interconnecting fins, especially in calcified canals, which can lead to misidentification of root types (Degerness & Bowles, 2010; Lee et al., 2011). Indeed, Wang et al. (2012) have reported that a combination of both radiographic and clinical examinations was able to detect more C-shaped canals in 1146 lower second molars, than either technique alone. In addition, the small sample size limited the effectiveness of the investigations to identify more differences between hominids' and modern human's root and canal morphologies. More samples need to be identified to ascertain the trends in evolution of the human tooth.

5. Conclusions

This CBCT study found a high prevalence of C-shaped root canals in the mandibular second molars of ancient humans at the Jiaojia site, 5000 years ago. Their prevalence was higher than in an age-matched control group of current clinical cases from the local population. Additional studies are needed to decipher the various genetic influences on human root canal morphology.

Declarations of conflicting interests

None.

Acknowledgements

This research was supported by a grant from Shandong University multidisciplinary research and innovation team of young scholars (2020QNQT018), the Fundamental Research Funds of Shandong University (2018HW019), and the National Social Science Fund of China (19BKG048).

References

Ahmed, H. A., Abu-bakr, N. H., Yahia, N. A., & Ibrahim, Y. E. (2007). Root and canal morphology of permanent mandibular molars in a Sudanese population. *International Endodontic Journal*, 40, 766–771. https://doi.org/10.1111/j.

Al-Fouzan, K. S. (2002). C-shaped root canals in mandibular second molars in a Saudi Arabian population. *International Endodontic Journal*, 35, 499–504. https://doi.org/ 10.1046/j.1365-2591.2002.00512.x.

Arai, Y., Tammisalo, E., Iwai, K., Hashimoto, K., & Shinoda, I. T. (1999). Development of a compact computed tomographic apparatus for dental use. *Dentomaxillofacial Radiology*, 28, 245–248. https://doi.org/10.1038/sj/dmfr/4600448.

Ceperuelo, D., Lozano, M., Duran-Sindreu, F., & Mercadé, M. (2014). Root canal morphology of chalcolithic and early bronze age human populations of El Mirador Cave (Sierra De Atapuerca, Spain). The Anatomical Record, 297(12), 2342–2348. https://doi.org/10.1002/ar.22958

Cooke, H. G., & Cox, F. L. (1979). C-shaped canal configurations in mandibular molars.

- The Journal of the American Dental Association, 99, 836–839. https://doi.org/10.14219/jada.archive.1979.0402.
- Cotton, T. P., Geisler, T. M., Holden, D. T., Schwartz, S. A., & Schindler, W. G. (2007). Endodontic applications of cone-beam volumetric tomography. *Journal of Endodontics*, 33, 1121–1132. https://doi.org/10.1016/j.joen.2007.06.011.
- Degerness, R. A., & Bowles, W. R. (2010). Dimension, anatomy and morphology of the mesiobuccal root canal system in maxillary molars. *Journal of Endodontics*, 36(6), 985–989. https://doi.org/10.1016/j.joen.2010.02.017.
- Fan, B., Chen, W. X., & Fan, M. W. (2001). Configuration of C-shaped canals in mandibular molars in Chinese population. *Journal of Dental Research*, 80, 704.
- Fan, B., Cheung, G. S., Fan, M., Gutmann, J. L., & Bian, Z. (2004). C-shaped canal system in mandibular second molars: Part I—Anatomical features. *Journal of Endodontics*, 30, 899–903. https://doi.org/10.1097/01.don.0000136207.12204.e4.
- Gani, O. A., Boiero, C. F., Correa, C., et al. (2014). Morphological changes related to age in mesial root canals of permanent mandibular first molars. Acta Odontológica Latinoamericana, 27, 105–109. https://doi.org/10.1590/S1852-482401400020001
- Gómez-Robles, A., de Castro, J. M., Martinón-Torres, M., Prado-Simón, L., & Arsuaga, J. L. (2015). A geometric morphometric analysis of hominin lower molars: Evolutionary implications and overview of postcanine dental variation. *Journal of Human Evolution*, 82, 34–50. https://doi.org/10.1016/j.jhevol.2015.02.013.
- Goude, G., Dori, I., Sparacello, V. S., Starnini, E., & Varalli, A. (2020). Multi-proxy stable isotope analyses of dentine microsections reveal diachronic changes in life history adaptations, mobility, and tuberculosis-induced wasting in prehistoric Liguria (Finale Ligure, Italy, northwestern Mediterranean). *International Journal of Paleopathology*, 28, 99–111. https://doi.org/10.1016/j.ijpp.2019.12.007.
- Haddad, G. Y., Nehme, W. B., & Ounsi, H. F. (1999). Diagnosis, classification, and frequency of C-shaped canals in mandibular second molars in the Lebanese population. *Journal of Endodontics*, 25, 268–271. https://doi.org/10.1016/S0099-2399(99) 80157-5.
- Hanegraef, H., Martinón-Torres, M., Martínez de Pinillos, M., Martín-Francés, L., Vialet, A., Arsuaga, J. L., ... Bermúdez de Castro, J. M. (2018). Dentine morphology of Atapuerca-Sima de los Huesos lower molars: Evolutionary implications through three-dimensional geometric morphometric analysis. American Journal of Physical Anthropology, 166(2), 276–295. https://doi.org/10.1002/ajpa.23428.
- Jafarzadeh, H., & Wu, Y. N. (2007). The C-shaped root canal configuration: A review.

 Journal of Endodontics, 33, 517–523. https://doi.org/10.1016/j.joen.2007.01.005.
- Jerome, C. E. (1994). C-shaped root canal systems: Diagnosis, treatment, and restoration. General Dentistry, 42, 424.
- Kasai, K., & Kawamura, A. (2001). Correlation between buccolingual inclination and wear of mandibular teeth in ancient and modern Japanese. Archives of Oral Biology, 46, 269–273. https://doi.org/10.1016/s0003-9969(00)00106-0.
- Keith, A. (1913). Problems relating to the teeth of the earlier forms of prehistoric man. Proceedings of the Royal Society of Medicine, 6, 103–124. https://doi.org/10.1177/ 003591571300601018.
- Keith, A., & Knowles, F. H. (1911). A description of teeth of Palaeolithic man from Jersey.
 Journal of Anatomy and Physiology, 46, 12. https://doi.org/10.1038/086414a0.
 Lascala, C. A., Panella, J., & Marques, M. M. (2004). Analysis of the accuracy of linear
- Lascala, C. A., Panella, J., & Marques, M. M. (2004). Analysis of the accuracy of linear measurements obtained by cone beam computed tomography (CBCT-NewTom). *Dentomaxillofacial Radiology*, 33, 291–294. https://doi.org/10.1259/dmfr/25500850.
- Lee, J. H., Kim, K. D., Lee, J. K., Park, W., Jeong, J. S., Lee, Y., ... Baek, S. H. (2011). Mesiobuccal root canal anatomy of Korean maxillary first and second molars by cone-beam computed tomography. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 111(6), 785–791. https://doi.org/10.1016/j.tripleo. 2010.11.026.
- Manning, S. A. (1990). Root canal anatomy of mandibular second molars: Part II. C-shaped canals. *International Endodontic Journal*, 23, 40–45. https://doi.org/10.1111/i.1365-2591.1990.tb00801.x.
- Martínez, L. M., Estebaranz-Sánchez, F., Ferràndez-Cañadell, C., Romero, A., Ribot, F., Galbany, J., ... Pérez-Pérez, A. (2020). Buccal dental-microwear and feeding ecology of Early Pleistocene Theropithecus oswaldi from Cueva Victoria (spain). Journal of Human Evolution. 142, 102736. https://doi.org/10.1016/j.jhevol.2019.102736.
- Martins, J. N., Ordinola-Zapata, R., Marques, D., Francisco, H., & Caramês, J. (2018). Differences in root canal system configuration in human permanent teeth within different age groups. *International Endodontic Journal*, 51, 931–941. https://doi.org/ 10.1111/jei.12896
- Melton, D. C., Krell, K. V., & Fuller, M. W. (1991). Anatomical and histological features of C-shaped canals in mandibular second molars. *Journal of Endodontics*, 17, 384–388. https://doi.org/10.1016/S0099-2399(06)81990-4.
- Mozzo, P., Procacci, C., Tacconi, A., Martini, P. T., & Andreis, I. B. (1998). A new volumetric CT machine for dental imaging based on the cone-beam technique: Preliminary results. *European Radiology*, 8, 1558–1564. https://doi.org/10.1007/s003300050586.
- Park, J. B., Kim, N., Park, S., Kim, Y., & Ko, Y. (2013). Evaluation of root anatomy of permanent mandibular premolars and molars in a Korean population with cone-beam computed tomography. European Journal of Dentistry, 7, 94. https://doi.org/10.4103/ 1305-7456.115413.

- Patel, S. (2009). New dimensions in endodontic imaging: Part 2. Cone beam computed tomography. *International Endodontic Journal*, 42, 463–475. https://doi.org/10.1111/ i.1365-2591.2008.01531.x.
- Peretz, B., & Smith, P. (1993). Morphometric variables of developing primary mandibular second molars. Archives of Oral Biology, 38, 745–749. https://doi.org/10.1016/0003-006023200669.
- Przesmycka, A., Jędrychowska-Dańska, K., Masłowska, A., Witas, H., Regulski, P., & Tomczyk, J. (2020). Root and root canal diversity in human permanent maxillary first premolars and upper/lower first molars from a 14th–17th and 18th–19th century Radom population. Archives of Oral Biology, 110, 104603. https://doi.org/10.1016/j.archoralbio.2019.104603.
- Ramírez-Salomón, M., Vega-Lizama, E., Tiesler, V., Alvarado-Cárdenas, G., López-Villanueva, M., Sierra-Sosa, T., ... Cucina, A. (2014). The C-shaped canal molar: An Endodontic-Archaeological study of the relationships between Mayan pre-Hispanic and contemporary population of Yucatán. *International Endodontic Journal*, 47(11), 1084–1089. https://doi.org/10.1111/jej.12255.
- Roy, A., Astekar, M., Bansal, R., Gurtu, A., Kumar, M., & Agarwal, L. K. (2019). Racial predilection of C-shaped canal configuration in the mandibular second molar. *Journal of Conservative Dentistry: JCD*, 22, 133–138. https://doi.org/10.4103/JCD.JCD_369 18.
- Scott, G. R., & Dahlberg, A. A. (1982). Microdifferentiation in tooth crown morphology among indians of the american southwest. teeth: Form. Function and evolution. New York, NY, USA: Columbia University Press259–291.
- Scott, G. R., & Turner, C. G. (1997). Anthropology of modern human teeth. Cambridge, UK: Cambridge University Press.
- Sert, S., & Bayirli, G. S. (2004). Evaluation of the root canal configurations of the mandibular and maxillary permanent teeth by gender in the Turkish population. *Journal* of Endodontics, 30, 391–398. https://doi.org/10.1097/00004770-200406000-00004.
- Shi, S., Duan, X., Shao, J., Duan, Q., & Peng, S. (2013). Dens invaginatus in ancient Chinese teeth of 2,000 years ago. The Anatomical Record, 296, 1628–1633. https://doi.org/10.1002/ar.22738.
- Shields, E. D., & Jones, G. (1996). Heterochronic quantitative microevolution: Dental divergence in aboriginal Americans. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists, 100(3), 355–365. https://doi.org/10.1002/(SICI)1096-8644(199607)100:3<355::AID-AJPA4>3.0.CO'2-T.
- Sidow, S. J., West, L. A., Liewehr, F. R., & Loushine, R. J. (2000). Root canal morphology of human maxillary and mandibular third molars. *Journal of Endodontics*, 26. https:// doi.org/10.1097/00004770-200011000-00011 675-658.
- Turner, C. G. (1987). Late Pleistocene and Holocene population history of East Asia based on dental variation. American Journal of Physical Anthropology, 73, 305–321. https://doi.org/10.1002/ajpa.1330730304.
- Turner, C. G. (1990). Major features of Sundadonty and Sinodonty, including suggestions about East Asian microevolution, population history, and late Pleistocene relationships with Australian aboriginals. *American Journal of Physical Anthropology*, 82, 295–317. https://doi.org/10.1002/ajpa.1330820308.
- Vertucci, F. J. (1984). Root canal anatomy of the human permanent teeth. Oral Surgery, Oral Medicine, and Oral Pathology, 58, 589–599. https://doi.org/10.1016/0030-4220(84)90085-9.
- von Zuben, M., Martins, J. N. R., Berti, L., et al. (2017). Worldwide prevalence of mandibular second molar C-shaped morphologies evaluated by cone-beam computed tomography. *Journal of Endodontics*, 43, 1442–1447. https://doi.org/10.1016/j.joen. 2017.04.016.
- Walker, R. T. (1988). Root form and canal anatomy of mandibular first molars in a southern Chinese population. *Dental Traumatology*, 4, 19–22. https://doi.org/10. 1016/S0099-2399(88)80192-4.
- Wang, Y., Guo, J., Yang, H.-B., Han, X., & Yu, Y. (2012). Incidence of C-shaped root canal systems in mandibular second molars in the native Chinese population by analysis of clinical methods. *International Journal of Oral Science*, 4(3), 161–165. https://doi.org/ 10.1038/ijos.2012.42.
- Weine, F. S., Pasiewicz, R. A., & Rice, R. T. (1988). Canal configuration of the mandibular second molar using a clinically oriented in vitro method. *Journal of Endodontics*, 14, 207–213. https://doi.org/10.1016/S0099-2399(88)80171-7.
- Yang, Z. P., Yang, S. F., Lin, Y. C., Shay, J. C., & Chi, C. Y. (1988). C-shaped root canals in mandibular second molars in a Chinese population. *Dental Traumatology*, 4, 160–163. https://doi.org/10.1111/j.1600-9657.1988.tb00315.x.
- Zhang, R., Wang, H., Tian, Y. Y., Yu, X., Hu, T., & Dummer, P. M. H. (2011). Use of cone-beam computed tomography to evaluate root and canal morphology of mandibular molars in Chinese individuals. *International Endodontic Journal*, 44, 990–999. https://doi.org/10.1111/j.1365-2591.2011.01904.x.
- Zheng, Q., Zhang, L., Zhou, X., Wang, Q., Wang, Y., & Tang, L. (2011). C-shaped root canal system in mandibular second molars in a Chinese population evaluated by cone-beam computed tomography. *International Endodontic Journal*, 44, 857–862. https://doi.org/10.1111/j.1365-2591.2011.01896.x.
- Zilberman, U., Milevski, I., Yegorov, D., & Smith, P. (2019). A 3000-year-old case of an unusual dental lesion: Pre-eruptive intracoronal resorption. Archives of Oral Biology, 97, 97–101. https://doi.org/10.1016/j.archoralbio.2018.10.015.