Determinants of the Number of Main Canals in a Tooth: Deciphering Potential Mechanisms *

Determinantes del número de conductos principales en un diente: descifrando posibles mecanismos

Determinantes do número de canais principais em um dente: decifrando mecanismos potenciais

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ABSTRACT

Background: Although millions of root canal treatments are performed globally on a daily basis, factors that determine the number of main root canals in a tooth have not yet been elucidated. Variations in the number of root canals in different teeth is of utmost importance in clinical practice. However, clinicians are not aware about the determinants of such number, let alone these determinants have been approached in the literature, to the best of our knowledge. **Purpose:** This narrative review aimed to integrate the potential mechanisms involved in determining the number of main canals in a permanent tooth. **Methods:** We used the search terms "root canal number," "root canal morphology," "tooth morphology," "root development," and "root formation" to identify articles from the PubMed and Scopus databases. **Results:** 57 articles and 2 books were obtained. A multifactorial basis is plausible considering the influence of anthropological, demographic, environmental, genetic, epigenetic, tooth size related mechanisms and the pivotal role of Hertwig's epithelial root sheath. Live-cell imaging techniques, mathematical models, quantitative genetics and dental phenomics could provide insightful information in the near future. **Conclusions:** Overall, it seems that the potential mechanisms determining the number of main canals in a tooth have a multifactorial basis. The orchestrating role of the Hertwig's epithelial root sheath seems pivotal, although the specific regulatory signals that induce or repress its diaphragmatic processes remain unknown. However, there is a dire need for molecular studies that help unveil these and other potential mechanisms involved.
Keywords: dental anatomy; dentistry; endodontics; Hertwig's epithelial root sheath; root canal morphology; root canal number; root canal system; tooth root development; tooth root formation

RESUMEN

Antecedentes: Aunque diariamente se realizan millones de tratamientos de conducto radicular en todo el mundo, aún no se han dilucidado los factores que determinan el número de conductos radiculares principales en un diente. Las variaciones en el número de conductos radiculares en diferentes dientes son de suma importancia en la práctica clínica. Sin embargo, los médicos no conocen los determinantes de tal número, y mucho menos estos determinantes han sido abordados en la literatura, según nuestro conocimiento. **Objetivo:** Esta revisión narrativa tuvo como objetivo integrar los posibles mecanismos involucrados en la determinación del número de conductos principales en un diente permanente. **Métodos:** Utilizamos los términos de búsqueda "número de conducto radicular", "morfología del conducto radicular", "morfología dental", "desarrollo

radicular" y "formación radicular" para identificar artículos de las bases de datos PubMed y Scopus. **Resultados:** Se obtuvieron 57 artículos y 2 libros. Una base multifactorial es plausible considerando la influencia de los mecanismos antropológicos, demográficos, ambientales, genéticos, epigenéticos, relacionados con el tamaño del diente y el papel fundamental de la vaina radicular epitelial de Hertwig. Las técnicas de imagen de células vivas, los modelos matemáticos, la genética cuantitativa y la fenómica dental podrían proporcionar información reveladora en un futuro próximo. **Conclusiones:** En general, parece que los posibles mecanismos que determinan el número de conductos principales en un diente tienen una base multifactorial. El papel orquestador de la vaina radicular epitelial de Hertwig parece fundamental, aunque las señales reguladoras específicas que inducen o reprimen sus procesos diafragmáticos siguen sin conocerse. Sin embargo, existe una gran necesidad de estudios moleculares que ayuden a revelar estos y otros posibles mecanismos involucrados.
Palabras clave: anatomía dental; desarrollo de la raíz del diente; endodoncia; formación de la raíz del diente; morfología del conducto radicular; número de conductos radiculares; odontología; sistema de conductos radiculares; vaina radicular epitelial de Hertwig

RESUMO

Antecedentes: Embora milhões de tratamentos de canal radicular sejam realizados diariamente em todo o mundo, os fatores que determinam o número de canais radiculares principais em um dente ainda não foram elucidados. Variações no número de canais radiculares em diferentes dentes são de extrema importância na prática clínica. No entanto, os clínicos não têm conhecimento dos determinantes desse número e muito menos esses determinantes foram abordados na literatura, até onde sabemos. **Objetivo**: Esta revisão narrativa teve como objetivo integrar os potenciais mecanismos envolvidos na determinação do número de canais principais em um dente permanente. **Métodos**: Utilizamos os termos de pesquisa “número do canal radicular”, “morfologia do canal radicular”, “morfologia dentária”, “desenvolvimento radicular” e “formação radicular” para identificar artigos das bases de dados PubMed e Scopus. **Resultados**: foram obtidos 57 artigos e 2 livros. Uma base multifatorial é plausível, considerando a influência de mecanismos antropológicos, demográficos, ambientais, genéticos, epigenéticos, relacionados ao tamanho dos dentes e o papel central da bainha epitelial da raiz de Hertwig. Técnicas de imagem de células vivas, modelos matemáticos, genética quantitativa e fenômica dentária poderiam fornecer informações esclarecedoras em um futuro. **Conclusões**: No geral, parece que os potenciais mecanismos que determinam o número de canais principais num dente têm uma base multifatorial. O papel orquestrador da bainha epitelial da raiz de Hertwig parece fundamental, embora os sinais regulatórios específicos que induzem ou reprimem seus processos diafragmáticos permaneçam desconhecidos. No entanto, há uma extrema necessidade de estudos moleculares que ajudem a desvendar estes e outros potenciais mecanismos envolvidos.
Palavras-chave: anatomia dentária; bainha radicular epitelial de Hertwig; desenvolvimento da raiz dentária; endodontia; formação de raiz dentária; morfologia do canal radicular; número de canais radiculares; odontologia; sistema de canais radiculares

INTRODUCTION

A deep knowledge of the root anatomy and configuration of the root canal system (RCS) of each dental group is essential to conduct an adequate root canal treatment (1,2). Existing canal configuration classifications according to Vertucci (3), Gulabivala (4), and Sert &Bayirli (5) are based on the pulp space configurations and number of main canals in the tooth. The clinician must have sufficient skills and knowledge to treat teeth that present a complex RCS (6-10), which sometimes involves precisely analyzing the number of main canals. However, although Carabelli provided the first detailed description of the number of canals in teeth by 1842 (11) and millions of root canal treatments are performed globally on a daily basis (12), the factors that determine the number of main root canals in a tooth have not yet been elucidated (Thesleff, I. personal communication).

The Hertwig's epithelial root sheath (HERS) plays a critical role in root formation (13,14), determining the shape, size and number of dental roots (15). Genetic studies have identified that several molecules, such as Nfic, Osterix, β-catenin, and sonic hedgehog, constitute key factors in root formation (13). Since these molecules regulate root formation, they could help to understand the mechanisms determining the number of canals. However, the precise factors determining the number of canals in a tooth are still unknown, since there are case reports of single-rooted teeth with four canals (9), molars with a single root and single canal (16), and maxillary (17) and mandibular molars (18) with eight canals.

However, it has been postulated that root morphology results from the complex interaction of genetic, epigenetic, and environmental factors (19-23). Demographic and ethnic factors are interrelated with root anatomy, root number, and RCS configuration (1,2,7,24,25); therefore, variations can be considered as specific traits that occur with varying prevalence in certain populations (7,24,25).

In the last decade, a series of investigations have been conducted in various populations using cone-beam computed tomography (CBCT) (1,2,7,24-27) or microcomputed tomography (µCT) (8,10,28,29) to determine variations in both root anatomy and RCS configurations. However, to the best of our knowledge, no publications have adequately explained the mechanisms or factors that determine the number of main root canals in a permanent tooth. This narrative review aimed to integrate the potential mechanisms involved in determining the number of main canals in a permanent tooth. In fact, skilled clinicians should be aware about the fundamentals behind the determinants of the number of main canals in different teeth, as they deal with such variations on a daily basis. Additionally, future perspectives are also included in the final section of the manuscript.

MATERIALS AND METHODS

Available literature was searched in the PubMed and Scopus databases to identify relevant articles, from the first reports until May 25, 2022, using the search terms "root canal number," "root canal morphology," "tooth morphology," "root development," and "root formation". In this study, we included only articles published in English. The reference lists of the selected articles were manually searched to complement the electronic search.

The search strategy was specific for each database. Experimental and non-experimental studies were included, in order to address various aspects such as concepts, definitions, methodologies, and available scientific evidence. In most cases, articles that did not explicitly link to the search terms used were removed. Then, the search results were merged, proceeding to remove duplicates. After the initial selection of titles and abstracts, only articles and books of greatest relevance to the topic under review were selected. In the end, 57 articles and 2 books were obtained. The information was categorized according to the sections of the present narrative review.

RESULTS

We found the following mechanisms to be potentially responsible for main canal determination in a tooth: anthropological and demographic mechanisms, environmental mechanisms, genetic mechanisms, epigenetic mechanisms, HERS mechanisms, and tooth size-related mechanisms.

Anthropological and Demographic Mechanisms

Root canal information of many *hominin taxa* is rarely available because of the limitations in historical methodologies (22). Nevertheless, valuable information concerning canal variations in hominin fossils have been documented using µCT (22,30-32), thus elucidating potential developmental influences on root morphology in modern humans and their fossil relatives (22).

Among the extant and extinct hominoid species, the premolar root morphology is a useful phylogenetic indicator since its root number, root size, and canal number vary the most compared to other tooth types (31-33). In South African hominin fossils attributed to *Australopithecus africanus* (2-3 million years ago) and *Paranthropus robustus* (1-2 million years ago), the canal numbers in maxillary premolars (1-3 roots) varied between one (rarely) and two or three (more commonly), while in mandibular premolars (1-3 roots) it varied between one and three, and even four canals in the case of double plate-like mesial and distal roots (22). Both hominins exhibited reduced maxillary premolar root/canal numbers from the inferred ancestral great ape condition; however, *A. africanus* showed more reduction than *P. robustus* (22). Figure 1 shows a CBCT axial view of a mandibular first premolar with four double-plate-like canals in a patient, similar to the aforementioned type. It is possible that this case reflects an atavic trait.

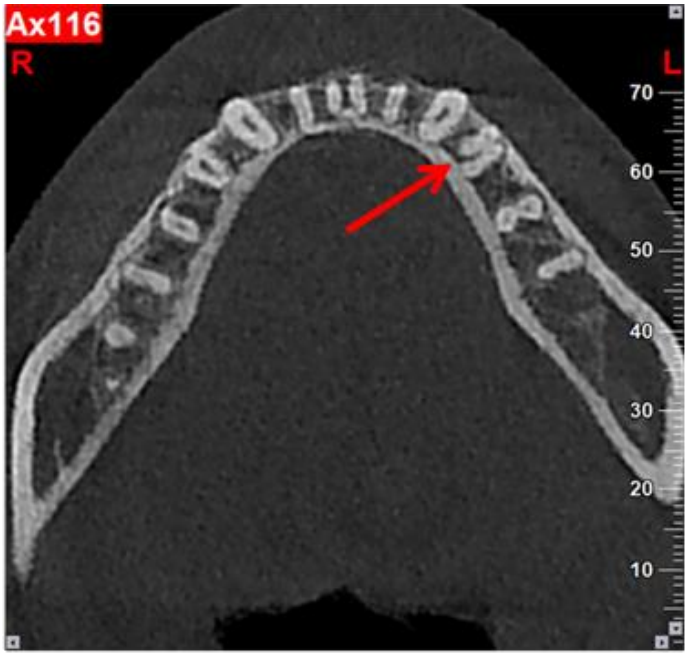

FIGURE 1

CBCT axial view of the left mandibular first premolar (red arrow) of a female patient that had four canals (author's personal database- JLAV), reflecting the double plate-like type mandibular premolars described in South African hominin fossils.

Source: the authors.

The genus *Homo*, particularly the lineage leading to modern humans, has been characterized by a reduction in root/canal number, while the genus *Paranthropus* has displayed an elaboration in the form and number of mandibular premolars (22). However, a study on root and root canal diversity in human premolars and molars from two historical periods found an increase in the diversity in the number of roots and shape of root canals in the modern period compared to the late medieval period (34).

In contrast, *Homo floresiensis*, an extinct diminutive hominin species (found on an Indonesian island) with unique physical characteristics, has shown primitive canine-premolar (comparable to *H. erectus)* and advanced molar morphologies (even compared to those of modern humans), a combination of dental traits unknown to any other hominin species (35). However, to the best of our knowledge, no studies have addressed root canal variations in this species, and such investigations could help us understand the influence of anthropological factors on root/canal morphology, especially since teeth are one of the most informative elements in hominin evolutionary studies (36).

Finally, anthropological studies allow us to link the variations and discrepancies found in the different populations due to prehistoric colonization and their division into the three main branches of the phenotypes. In this sense, each population was exposed to different environmental factors, genetic and demographic changes, and natural pressures, thus generating the phenotypes of each population, which determined variations both in the maxillary bones and in the teeth (22-25). This clearly shows that demographic and ethnic factors are interrelated with root anatomy, root number, and RCS configuration.

For instance, the maxillary (17) and mandibular molars (18) with eight canals, which are currently the teeth with the greatest reported number of main canals, are from Indian patients.

Environmental Mechanisms

Although the influence of environmental factors on dental morphology is not yet clearly understood (37), environmental disruption caused by non-genetic factors, such as malnutrition and systemic diseases during tooth development, has been found to influence the final phenotype (37). Malnutrition and systemic diseases, such as cystic fibrosis, human immunodeficiency virus infection, and leukemia, negatively affect both the dentine and enamel during tooth development. These environmental factors, seen as stress markers, could have a significant effect on the morphological variations of teeth, for instance, increasing variability in the dental cusps (37,38). Additionally, diet is an environmental factor that alters genetic expression and changes in DNA methylation patterns and can affect the final phenotype of the offspring (37-40).

Studies based on primates have shown an increase in the surface area of the dental root in those consuming hard food, such as seeds, thus evidencing a link between dental root morphology and diet. However, masticatory loads significantly influence the dental root development (23). Species of primates that eat hard or fibrous food exhibited an increase in root curvature compared to species that eat fruits and barks. This adaptive process can be transmitted by species and thus influence dental root morphology (23), in turn potentially affecting the number and/or disposition of canals.

During pregnancy, environmental factors, such as alcohol consumption, smoking habits, and obesity, have proven to be negative effects and caused asymmetry in children's teeth (37), but there are no studies concerning root development or the number of canals. On the other hand, it is surprising how monozygotic twins show differences in shape, number, and teeth size, while in appearance they are almost identical (37,41,42). This supports the potential role of environmental factors in tooth morphology; indeed, certain transplacental teratogens and diseases can cause differences in monozygotic twin phenotypes (39). In addition, scientific research has underlined that tooth development is not only controlled by genetic factors, but also by environmental factors (37,38,43) and they equally affect the developmental changes in teeth morphology (37).

The increased variability in the expression of morphological traits produced by an environmental stressor can be explained by a buffering mechanism (37). The genetic buffering mechanism controls different phenotypes that can be expressed in each individual (44,45). Therefore, it reduces the probability of mutation of a specific gene (44,45). During normal development, the presence of hidden genetic variability can be canalized by the buffering mechanism; however, if a stressful factor exceeds the threshold, the buffering mechanism cannot be canalized, and it appears as new genetic variation (37,44). Nonetheless, the mechanisms or factors that produce this new genetic variability are not clear (37).

Finally, tooth development corresponds to a morpho dynamic mechanism in which morphogenesis and induction processes play significant interdependent roles (37,46). Therefore, if any error occurs during the developmental cascade and affects the morpho dynamic process, it can generate changes in the final morphology (37). Environmental stressors can affect both genetic and cellular interactions, which in turn control tooth development. These stressors can downregulate cellular metabolism and changes in epithelial and mesenchymal growth and differentiation rates (37); nonetheless, their effect on the number of canals in teeth have not been explored yet.

Genetic Mechanisms

Genetic expression plays a pivotal role in variations in dental morphology, with more than 300 genes playing roles in different stages of the dental morphogenetic process (39,43,46-48). In this regard, an increase in the expression of only one gene in the DNA sequence can lead to significant changes in dental development, such as increased tooth number, altered tooth shape, disturbed tooth size, increased cusp numbers, and new variations (41,47).

Gene mutations can appear at different stages of odontogenesis, generating changes in normal tooth development and tooth morphology that can be translated to offsprings (40,47). When an error occurs during the transcription process of evolutionary genes, it leads to morphological diversity, especially when the homeobox genes are involved (40,49); Cis regulatory elements (CREs) of genes are specific non-coding DNA sites that regulate the transcription process of neighboring genes (40) and play paramount roles in controlling gene expression in specific cell types, conditions, and developmental stages (50). Disruption of these regions are considered as the most prevalent cause of phenotypic changes, especially morphological (50,51), so it could be that CREs have a role in determining the number of main canals.

Interestingly, the effect of gene mutation on the dentition depends on the gene type and the region of the affected gene (40). There are many genes that are strongly related to dental development, such as Msx1, Pax9, and Lef1, and genes that have less influence on developmental processes, such as Dlx1, Dlx2, Gli2, and Gli3 (40). In addition, the downstream targets of tooth-specific transcription factors and their role in regulatory mechanisms are poorly characterized (49,52); therefore, there is still a need to elucidate the role of genetics in odontogenesis, and specifically its involvement in main canal number determination.

Epigenetic Mechanisms

Epigenetics involves processes that occur in the cell nuclei and generate modifications in gene expression while still maintaining the same DNA sequence (42,43,53). Epigenetic influence can be explained as an autonomous developmental system with self-organization (39,43); therefore, even in monozygotic twins that share the same genotype, great differences can occur in their phenotype (39). External influences on epigenetic processes can occur, such as the effects of diet in cancer, and thus epigenetic mechanisms would allow an organism to respond to the environment through changes in gene expression (53).

It is important to highlight that epigenetic processes are crucial for development and differentiation, even in mature humans. Much epigenetic research has focused on two molecular mechanisms that mediate this phenomenon: DNA methylation and histone modifications. DNA methylation signals can repress transcription through the exclusion of proteins involved in histone modifications, through their DNA binding sites, and cause long-term silencing of gene expression (53). Odontogenesis requires the participation of many transcription factors (48,54,55), and repression through DNA methylation may be involved in the regulation of the number of main canals.

It has been postulated that epigenetic factors may explain dental differences in monozygotic twin pairs, such as variations in the expression of missing or extra teeth (39) and can also influence the number or position of the teeth (39,42). Moreover, differential placental implantation and nutrition, differential transplacental teratogens, and infections have been cited as possible environmental factors (56).

Finally, it has been regarded that dental phenotype variations within species may respond to epigenetic mechanisms, like minor variations in the timing of interactions between cells and their position, acting at a local tissue level (39). In this regard, studies on epigenetic influences in monozygotic twin pairs could help elucidate the mechanisms involved in root canal number determination.

HERS Mechanisms

HERS is known to play a critical role in root formation (13,14) and determining the shape, size, and number of dental roots (15). The number of roots is determined by the number of inward-bending horizontal processes, called interradicular or diaphragmatic processes (DP) that are emitted from the epithelial diaphragm shortly after crown formation; if two DP are extended, two roots will be formed, with three DP, three roots will be formed, and so on; if no DP is formed, then a single-rooted tooth will be the result. The root structure (single- or multi-rooted) is thus determined by the shape and folding of the HERS (19,21,32,57).

As for the number of canals, each formed root has a minimum of one, so inherently multi-rooted teeth have more canals. Nevertheless, single-rooted teeth with multiple canals, which are common in non-human extant ape genera such as Hylobates/Pan maxillary first premolars and Gorilla/Pongo mandibular premolars, demonstrate a tendency for DP suppression of the outer surface but not in the expression of individual canals (31). Similarly, the above mentioned South African hominin fossils attributed to *A. africanus* and *P. robustus* exhibit multiple canals within one root body in both maxillary and mandibular premolars (22). Nonetheless, the cause of this “selective” suppression has not yet been elucidated, and the specific molecular signals that govern root size and induce the invagination of HERS remain unknown in hominids and hominins (30). It is known that the timing and rate of constriction of the HERS regulate root length, that is, if the HERS narrows rapidly, the root will be shorter; if not, the root will be longer (19).

It is worth mentioning taurodont molars, which show enlarged pulp chambers and apically positioned root furcations, resulting in an enlarged root body with short root branches and their corresponding terminal canals (30). Taurodontism is common in modern human deciduous and permanent dentitions, both bilaterally and in more than one tooth type (58), and its extreme form is hypertaurodont or pyramidal molars, which have been described in modern humans (59). However, there are no known cases in any fossil member of the genus Homo, other than Neanderthals (at a high frequency) (30). Pyramidal molars have a bifurcation either positioned at the apical fourth of the root or almost completely missing and seem to be the result of a complete failure of DP formation (30).

Additionally, crown pattern formation in a tooth may depend on the spatial and temporal expression of activating and inhibiting molecules derived from enamel knots (60). In an analogous comparison, it could be hipothesized that there are regulation centers at the DP level that determine the number of canals and generate a spatiotemporal expression of activating and inhibitory molecules (figure 2); however, future investigations are necessary to study this hypothesis.

FIGURE 2
Apical view of a histology-based 3D reconstruction of a mouse upper first molar on day 6. Three HERS processes, that is, diaphragmatic processes (DP) are in contact at two regions (arrows), giving rise to three root-canal spaces (asterisks). It could be hipothesized that there are regulation centers at the DP level, determining the number of main root canals, by generating a spatiotemporal expression of activating and inhibitory molecules. M, mesial; D, distal; B, buccal; P, palatal. Adapted from Shimazu, *et al.*, 2009 (57).

Tooth Size-Related Mechanisms

A developmental model called the root “size/number continuum” (SNC) has been proposed for premolars, which correlates increasing root number and, in turn root canals, with tooth size. This model predicts that tooth germ size, inferred from tooth size, predisposes the number and expression of DP, that is, smaller premolars are predisposed to fewer roots and larger premolars to greater numbers of roots (21). Tooth germ size is linked to the final phenotype (61). It affects the number of molecular signals and the distance between signaling centers, such as the number of enamel knots and/or DP (21). However, studies using the SNC model and the cross-sectional cervix area as a proxy for tooth size in hominoids (31,32) and hominins (22) have provided equivocal support; thus, further investigation is necessary.

In addition, size and shape of teeth are determined by cellular and apoptotic interactions during dental development (38,46). Ectodysplasin A (EDA) is an important signaling pathway involved in the regulation of tooth signaling centers. Inhibition of the EDA pathway generates a reduction in signaling centers, creating small teeth buds, and an increase in EDA signaling results in larger teeth buds and extra teeth (46). In this regard, the EDA pathway may be involved in the regulation of signaling centers in the DP, thus having a potential role in root canal number determination. Additionally, hyaluronic acid has been shown to affect cellular decisions regarding tooth size and number by controlling proliferation, cell orientation, and migration in the developing tooth (62), and thus could have a role as well.

Many studies have demonstrated the relationship between tooth size, tooth number, and individual sex; thus, studies in women have shown a reduction in tooth size and an increase in missing teeth, while men have shown an increase in tooth size and a major number of extra teeth (39,42,46). However, a study of four extant apes taxa showed that male and female individuals within genera differ in tooth size but not in canal/root form and number (31).

Finally, an investigation analyzed the variability and patterning of permanent tooth size in four human ethnic groups (Southern Chinese, North Americans of European ancestry, Modern British of European ancestry, and Romano-British) evidencing that the different patterns of tooth size observed between the study groups reflect different genetic and environmental influences to dental development (63). This adds to the information on the potential mechanisms involved in root canal number determination.

CONCLUSIONS AND FUTURE PERSPECTIVES

Overall, it seems plausible that the potential mechanisms determining the number of main canals in a tooth have a multifactorial basis, considering the influence of anthropological, demographic, environmental, genetic, epigenetic, and tooth size-related mechanisms. The orchestrating role of the HERS in root development seems pivotal, although the specific molecular signals that govern the induction or repression of DP remain unknown until now. Furthermore, the latest findings on odontogenesis could help to decipher these potential mechanisms, such as the role of the transcription factor Nfic in root patterning and growth through the regulation of mesenchymal cell proliferation, which may interact with HERS to guide the size, shape, and number of tooth roots (64).

In the near future, live-cell imaging techniques could be useful tools to elucidate the mechanisms involved in odontogenesis, such as the number of main canals, because this innovative technique allows direct observation of cell movement and morphological changes (65,66). Further, mathematical models have been developed to demonstrate how large morphological changes can be produced by small epigenetic events (67), thus these models can help provide evidence for factors that determine the number of main canals in a tooth. Quantitative genetics may also provide insight into these mechanisms because it has proven useful in outlining the contribution of genes to tooth form, how genetic effects determine dental phenotypes, and the potential for pleiotropy (68). Additionally, dental phenomics provides acquisition of high-dimensional phenotypic data on a large scale and has been used to quantify phenotypic variations in craniofacial development (69). Hence, it can help to elucidate the roles of genetic, epigenetic, and environmental factors in the variations in root canal number because such interactions account for variations in tooth number, size, and shape within and among species (20).

Finally, it must be emphasized that variations in the number of root canals in different teeth are of utmost importance in clinical practice. Skilled clinicians should be aware of the fundamentals behind the determinants of the number of main canals in different teeth, as they deal with such variations on a daily basis. In this regard, endodontists, biologists, and embryologists should intensify their understanding of the complex development of the RCS in order to support accurate endodontic therapy (70) and unveil potential mechanisms involved in determining the number of main canals in a tooth.

RECOMMENDATIONS

Due to the scarce information found, in the near future it would be important to conduct more research concerning studies related to the biological and molecular aspects of the potential mechanisms that determine the number of main canals in a tooth. In addition, in future studies we recommend including studies in other languages, since the present study only included literature in English.

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