

Meta-Analysis of Observational Studies on the Most Commonly Missing Permanent Dentition (Excluding the Third Molars) in Non-Syndromic Dental Patients or Randomly-Selected Subjects, and the Factors Affecting the Observed Rates

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Purpose: The aim of this study was to summarize the literature on the most frequently missing permanent teeth excluding the third molars. **Study design:** A search was conducted to find all the available literature (in various scientific and general databases) regarding the most commonly missing teeth with respect to ethnicity and time, as well as factors biasing this outcome. Quality assessment was done to exclude studies with inconsistent information, poor designs, or data pertaining to syndromic cases or the third molars. The role of biasing factors was as well quantitatively assessed using statistical analyses [Q-test, Egger regression, Spearman correlation coefficient, multiple linear regression, Welch t-test] ($\alpha=0.05$). **Results:** A total of 81 reports was included. The meta-sample was heterogeneous ($P=0.000$, Q-test). No significant publication bias was detected ($P>0.1$, Egger regression). The mandibular second premolar was reported as the most commonly missing tooth in most studies, followed by the maxillary lateral (the most commonly missing in the rest). In terms of the missing share of each tooth percent of all missing teeth, the mandibular second premolar and incisors are more likely to be absent, followed by the maxillary second premolar and lateral. The absence of different teeth can be affected by the ethnicity, sample types (epidemiological or dental patients), sample sizes (only in the case of bimaxillary second premolars), and the minimum ages of pooled subjects (only in the case of the maxillary lateral and the mandibular second premolar). **Conclusions:** Since enrolling younger patients can bias the results, older patients should be sampled.

Key words: Hypodontia; Congenitally Missing Teeth; Permanent Dentition; Most Commonly Missing Teeth; Occurrence Pattern; Risk Factors.

INTRODUCTION

Congenital missing of teeth (CMT) or dental agenesis is a very frequent dental anomaly in the permanent dentition¹⁻¹³. It can lead to esthetic and functional complications^{2-4,10-23}, which might need costly and challenging treatments^{13,14,24,25}. Therefore, it is a significant clinical issue^{1-9,14,24-26}, and of interest in various fields of dentistry and public health^{27,28}.

By aggregating different studies, meta-analyses can be valuable in understanding which teeth are most frequently missing, and which factors affect their missing frequency. Nevertheless, to the best of the author's knowledge, many aspects of the most commonly missing teeth are not analyzed before. Therefore, the author aimed to analyze the most frequently missing teeth. The questions to be addressed were: which teeth are usually missing? What are their missing rates? And which variables might affect/bias the observed missing frequencies?

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MATERIALS AND METHOD

Search strategy

During September 2012 till June 2013, the author searched the Internet for the relevant keywords, using advanced search settings including Boolean operators and double quotations. The search keywords were: Variations of "congenitally missing teeth", hypodontia, anodontia, oligodontia, with or without the variations of the word "prevalence". The keywords were searched using three Internet search engines (Pubmed, ISI Web of Science [WoS], and Google Scholar). The databases MeSH, MEDLINE, EMBASE, Scopus, and CINAHL were later searched; their results had been already covered by Google Scholar and/or Pubmed. Google was as well searched to make sure no grey literature was missing. Not only study titles, but also their texts were searched. About 2500 non-repeated study titles were initially found²⁸.

There were no limitations over the type of reports to be included (original articles, short communications, grey literature, online-only publications, dissertations, etc.). Each readable / translatable full text or abstract on the agenesis of permanent teeth excluding the third molars was read for at least twice (many were investigated at least 3 or 4 times). The data were re-calculated whenever possible from studies' texts, figures, and tables²⁸. There was no limitation over the selected language (as long as the author could comprehend or translate the text / abstract). The reference lists and summarizations of the full articles were as well searched

for new studies. Some studies cited and/or summarized previous papers that were not primarily available. In such cases, the second-hand information provided/summarized by the later articles was used. The primary articles that had been summarized in a later study were sought for on the Internet. The authors who had email addresses were contacted. Of all the corresponded authors, only one responded and sent their article.

Eligibility criteria

The inclusion and exclusion criteria in the order of PICOS/PECOS items were:

- **Population:** Reports had to include the agenesis of individual teeth in the permanent dentition excluding the third molars in radiographs of non-syndromic people. The samples could consist of the radiographs of randomly selected subjects in epidemiological studies (e.g., school-children) or dental/orthodontic patients. Reports dealing with patients having craniofacial syndromes or developmental disorders were excluded. Also, if the information regarding the missing of permanent-only dentition excluding third molars was not provided or was not extractable, the study would be excluded. The usage of radiographs was confirmed by reading the full texts and/or abstracts. Regarding the studies not available but cited in other articles, if the citing article stated that the included articles were based on radiographic evaluation, or if there was no mention of another examination method in the citing article, the second-hand information of the cited report would be used.
- **Exposure:** There were no limitations regarding the exposures (ethnicities and time of publication).
- **Comparator groups:** There was no limitation over study designs in terms of having a comparator group.
- **Outcome:** The outcome was the order and the prevalence of the agenesis of individual teeth. As far as at least the missing rate or the missing rank of one tooth was reported by a study (or it could be extracted from the recovered data), it would be included, even if the needed information was not analyzed/reported by the original authors.
- **Settings:** Any available designs would be included (e.g., epidemiological samples or samples consisting of dental patients' radiographs).

Method errors and quality assessments

Three months after the data collection, 15 studies were re-evaluated thoroughly (regarding every collected variable) by this author. The results of the new evaluation were 100% consistent with the previous records in terms of all the variables. About six months later, all the found articles were re-read by the author and their information regarding the most commonly missing teeth were re-abstracted. There was 100% intra-rater agreement between the two meta-sample.

The quality of the studies was assessed subjectively by evaluating their methods of diagnosis and data collection, their statistics, and any potential inconsistencies in their tables/figures and texts.

Also, whenever the provided information allowed, their raw data were recovered, and their analyses were re-done. Their quality was as well evaluated quantitatively using statistical analyses, searching for potential sources of bias.

Statistical analysis

The most commonly missing teeth were summarized in different ethnicities and population types. Also their missing frequencies were summarized from the available reports on the missing prevalence of the first four frequently missing tooth types.

The existence of publication bias and heterogeneity in the meta-sample was assessed using an Egger regression and a Q-test, respectively.

The roles of the year of publication and ethnicities (as the exposures) were evaluated using the regression / Spearman correlation analyses. The level of significance was set at 0.05.

As part of the quality assessment, the roles of samples' types, sample sizes, gender imbalances among recruited test subjects, journal reputation (defined as 0 if not indexed in Pubmed or WoS, 1 if indexed in one of them, and 2 if indexed in both of them), and the minimum age of included patients in each study were assessed using a Spearman coefficient and when needed, using a multiple linear regression. Also a Welch t-test was used to identify the minimum subject ages that might bias the results, if chosen as inclusion criteria.

RESULTS

There were no interventions assessed. No quasi-experimental designs (such as cohort or case-control) or experimental studies regarding the types of missing teeth existed in the literature. There were no comparator groups in any of the studies available in the literature. All the existing designs were cross-sectional on previously taken radiographs, or radiographs prospectively collected (in certain ones, together with dental casts / clinical findings).

A total of 81 reports or recoverable data pertaining to the most prevalent missing teeth were included for analysis (from 80 studies, Table 1)^{1,3-13,15-19,21-23,26,29-87}.

In the study of Endo *et al*⁵, the most prevalent CMT was incorrectly reported which was corrected in this report according to their data (Table 1). An interesting finding was observed in the study of Sheikh *et al*⁸⁶ where all the patients had either missing of third molars or missing of other teeth, but no patient had concurrent missing of a third molar and another tooth.

Meta-sample heterogeneity and publication bias

The included percentages for the lower second premolar (L5) were heterogeneous according to the Q test ($Q=265.1$, $I\text{-squared}=90.2$, $P=0.000$). A similar result was observed for the maxillary lateral ($Q=352.9$, $I\text{-squared}=92.8$, $P=0.000$).

The information regarding the agenesis of L5 was free of publication bias, according to the Egger regression ($P=0.941$). As well, no significant publication bias was detected for the upper lateral incisor (U2) ($P=0.155$) and the upper second premolar (U5) ($P=0.131$).

The order of missing teeth

The teeth which were mostly reported as the most often missing tooth and as the second most frequently missing tooth in different studies are demonstrated in Figures 1 and 2. The summary

Commonly Missing Teeth in the Permanent Dentition of Non-Syndromic Individuals

Table 1. The included studies and the most commonly missing teeth.

Country	Year	Type	1st t	2nd t	Country	Year	Type	1st t	2nd t
Switzerland	1936	SC	L5	U5	Japan	1995	PeDP	L5	U5
–	1939	–	U2	L5=U5	China	1998	–	L1/2	–
USA	1943	–	L5	U2=U5	S Arabia	1999	DP	2	5
Japan	1949	St	L2	–	Brazil	1999	OP	U2	L5
Japan	1951	SC	U2	L2	Japan	1999	OP	L5	U5
Japan	1954	SC	L5	U2	Kenya	2001	OP	L5	U5
Japan	1955	DS	U2	L1	Hungary	2001	OP	U2	L5
Sweden	1956	SC	L5	–	Korea	2001	SC	L5	L1/L2
USA	1956	DP	L5	U2	Norway	2002	PuDP	L5	U5
Sweden	1959	–	L5	–	S Arabia	2002	PeDP	L2	U5
Austria	1963	SC	L5	U5	Mexico	2003	OP	U2	L5
Japan	1963	SC	L2	L5	Slovenia	2005	OP	U2	U5
UK	1966	OP	L5	U2	Japan	2006	OP	L5	U5
USA	1970	St	U2	L5	Jordan	2006	DP	L5	U2
USA	1970	St	U2	L5	Hungary	2006	OP/PeDP	U2	L5
Finland	1971	SC	L5	–	Hungary	2006	PeDP	U2	L5
–	1971	–	L5	U2	Turkey	2007	OP	U2	L5
Israel	1973	–	U2	5	Turkey	2007	OP	U2	L5
Norway	1973	SC	L5	U5	Italy	2008	OP	L5	U2
Sweden	1973	SC	L5	U5	Japan	2008	PeDP	L5	U2
UK	1974	SC	L5	–	Brazil	2008	PeDP	L5	U5
Norway	1974	SC	L5	–	Korea	2008	OP	L2	L5
Canada	1974	SC	L5	U2	Iraq	2009	DP	U2	L5
Sweden	1976	SC	L5	U6=U5	Turkey	2009	DP	U2	L5
Iceland	1977	SC	L5	U5	Pakistan	2010	OP	L5	U2
Sweden	1977	SC	L5	U2	India	2010	DP	U2	L1
USA	1979	SC	L5	U2	Iran	2010	DP	L5	L1
Denmark	1980	SC	L5	U5	Turkey	2010	OP	U2	L5
Hong-Kong	1987	SC	L1/L2	–	Spain	2010	PHS	L5	U5
Japan	1988	OP	L5	U5	Iran	2010	OP	U2	U5
Malaysia	1989	SC	U2	L2	S Arabia	2010	OP	U2	L5
Australia	1989	DP	L5	–	Turkey	2010	DP	L5	U2
Italy	1989	DP	L5	2	Korea	2011	OP	L5	L2
S Arabia	1990	SC	L5	U2	Korea	2011	DP	U4/5	L4/5
Japan	1990	PeDP	U5	U2	India	2011	DP	U2	L1
Thailand	1990	OP	U2	U5	Iran	2012	OP	U2	L5
Australia	1990	DF	U2	–	India	2012	SC	U2	L1
Ireland	1990	OP	L5	–	Portugal	2012	DP	L5	U2
Italy	1991	DF	U2	L5	Iran	2012	DP/OP	L5	U5
–	1992	OP	U2	L5	Venezuela	2012	OP	U1	L5
Norway	1993	SC	L5	U5					

DS, dental student; **OP**, orthodontic patients; **SC**, schoolchildren; **PuDP**, public dental patients; **PeDP**, pediatric dental patients; **DF**, defense force recruits, **PHS**, attendees to the primary health services; **DP**, dental patients; **1st t**, the most commonly missing tooth; **2nd t**, the second most commonly missing tooth; **U**, upper; **L**, lower; **Tooth numbers (1-7)**, the tooth number according to the Palmer notation.

demonstrates that most studies of different types and ethnicities reported L5 as the most often missing tooth. U2 was as well reported as the most commonly missing tooth in many studies. In dental patients, the number of studies reporting this tooth as the most frequently missing tooth was close to those studies reporting L5 (Figure 1).

The second most commonly missing tooth in epidemiological samples was U5. The same held true in Mongoloids. In dental samples and also in Caucasians, the second most commonly missing teeth were L5 followed by U5 and U2 (Figure 2).

The association between sample types and the most frequently missing teeth

When three types of samples (epidemiologic, orthodontic patients, and non-orthodontic dental patients) were assessed, the Spearman coefficient showed a significant correlation between sample types and the missing teeth ($n=68$, $Rho=0.245$, $P=0.044$) such that epidemiologic samples tended to have more premolar agenesis while orthodontic samples tended to have more lateral missing. When two types of samples (epidemiologic, orthodontic patients) were assessed, the Spearman coefficient showed that there was a significant correlation between sample types and the missing teeth ($n=50$, $Rho=0.287$, $P=0.043$) such that epidemiologic samples

tended to have more premolar ageneses while orthodontic samples had more lateral ageneses. However, there were no associations when only epidemiologic samples and dental patients were assessed ($n=45$, $Rho=0.082$, $P=0.592$).

What percentage of all missing teeth is one the following types?

The ageneses of second premolars, upper laterals, and lower incisors account for what fraction of all missing teeth?

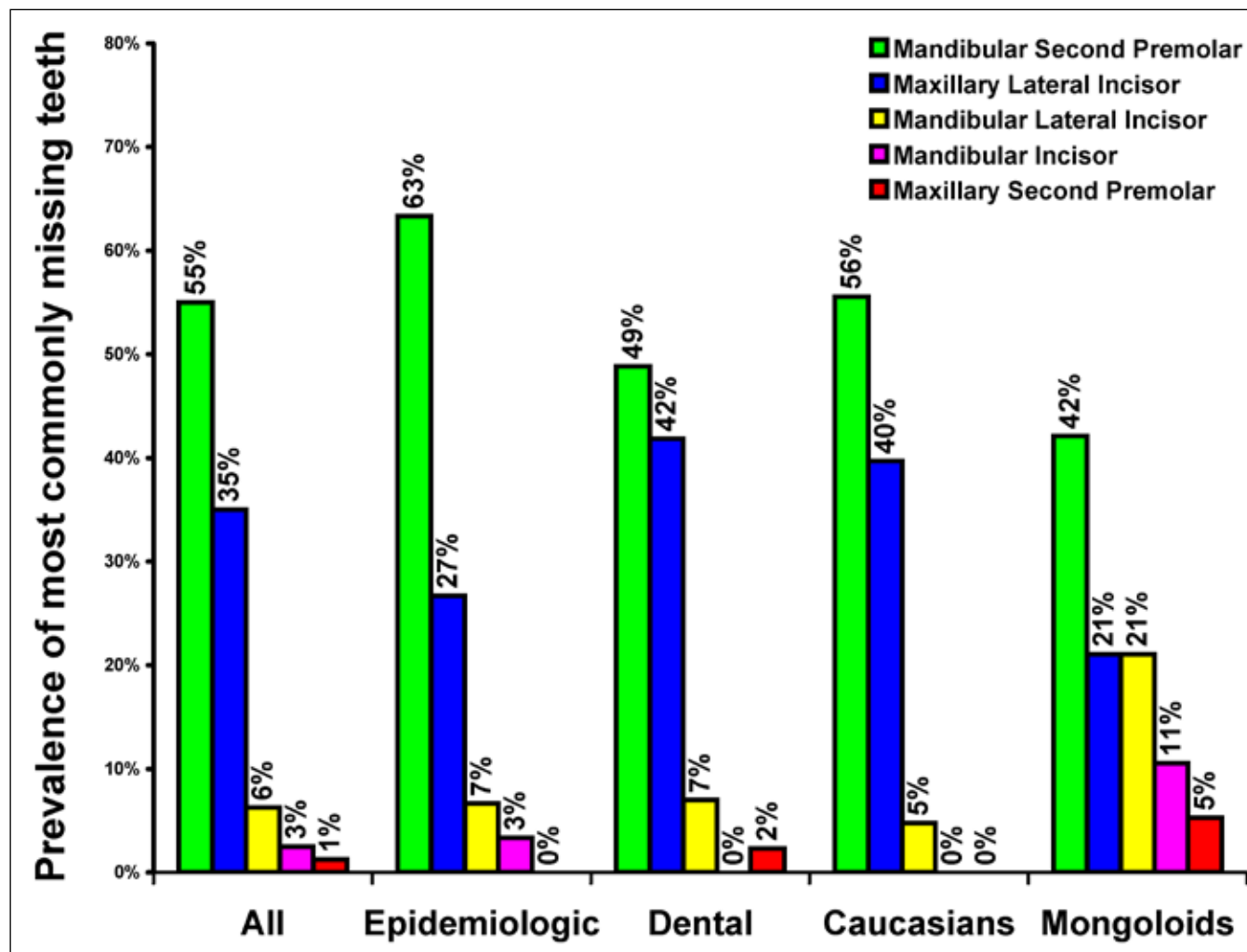
– The mandibular second premolar

This tooth had the greatest share of missing among all the missing teeth reported in the total meta-sample, in the epidemiological studies, and in Caucasians (Figures 3 and 4). However, in dental patients, it had the second highest share of agenesis among other teeth. In Mongoloids, it had the highest rate of missing together with the mandibular central/lateral incisors (Figures 3 and 4).

– The maxillary lateral

The mean missing rate of U2 percent of all missing teeth ranked as second in the total meta-sample and in Caucasians. In Epidemiological samples and Mongoloids, it had the third rank. In dental patients, it had the highest mean percentage of missing (Figures 3 and 4).

Figure 1. The most commonly missing teeth in the meta-sample and different groups.



– *The maxillary second premolar*

This tooth was in the third or fourth place in all the groups, with no considerable intergroup variation (Figures 3 and 4).

– *The mandibular incisors (central and lateral together)*

Since many studies had reported these two teeth together, this author as well did not separate them in order to avoid losing many such studies. The missing of these teeth accounted for a considerable proportion of all agenesis rates in epidemiological samples (second place) and in Mongoloids (first rank). In the remainder, they had the smallest share of missing compared to the other three predominant teeth (Figure 4).

The effect of ethnicities on the missing percentage of each tooth

The correlations between the ethnicities (Caucasians vs. Mongoloids) and two types of teeth were significant. The L5 missing was more prevalent in Caucasians, while the agenesis of mandibular incisors was more common in Mongoloids (Tables 1 and 2).

When the effect of sample types was controlled for using multiple linear regression analyses, none of the missing percentages of these four teeth were correlated with the ethnicity, while the effect of sample type was significant or marginally significant for L5 and U2 (Table 3).

The association between the year of publication and the missing of each tooth percent of all teeth missing

Only the L5 missing was significantly associated with the year of publication (Table 2). Since orthodontic and dental samples form most of the recent literature, it might be an artifact of study types. A multiple linear regression analysis (n=41) indicated that when sample types were controlled for (beta=0.418, P=0.025), there was no significant association between the year of publication and the mean prevalence of L5 aplasia (beta=-0.268, P=0.142).

Biasing factors

– *The biasing effect of sample types on the missing share of each tooth*

The Spearman coefficient showed significant and marginally significant associations between samples types (epidemiological vs. dental) and the agenesis of the four studied tooth types (Tables 1 and 2).

Figure 2. The second most commonly missing teeth. L1/L2, mandibular incisors.

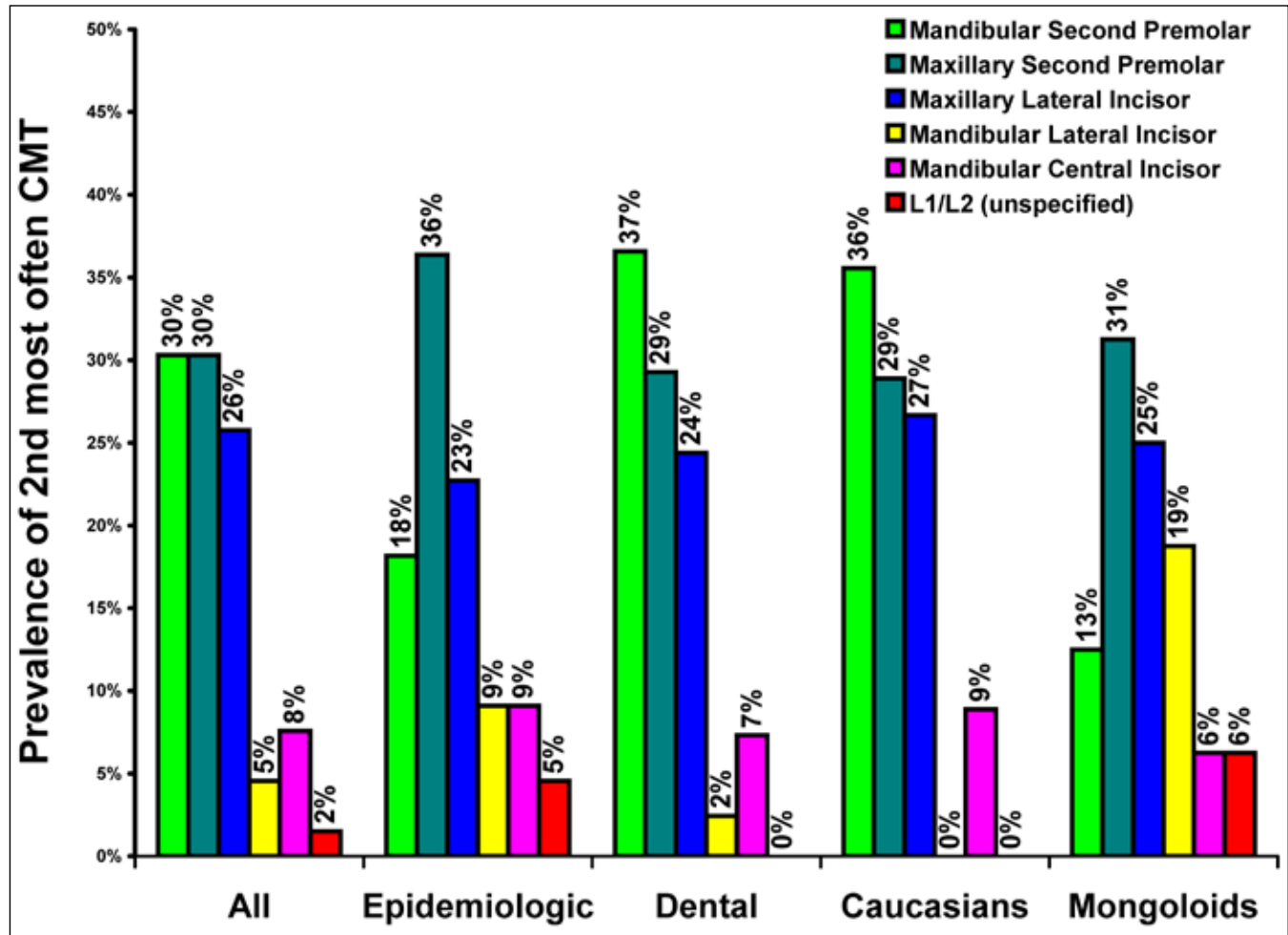


Figure 3. Forest plots for the reported proportion (%) of missing of the mandibular second premolar (A), maxillary lateral (B), and maxillary second premolar (C) in different countries and years.

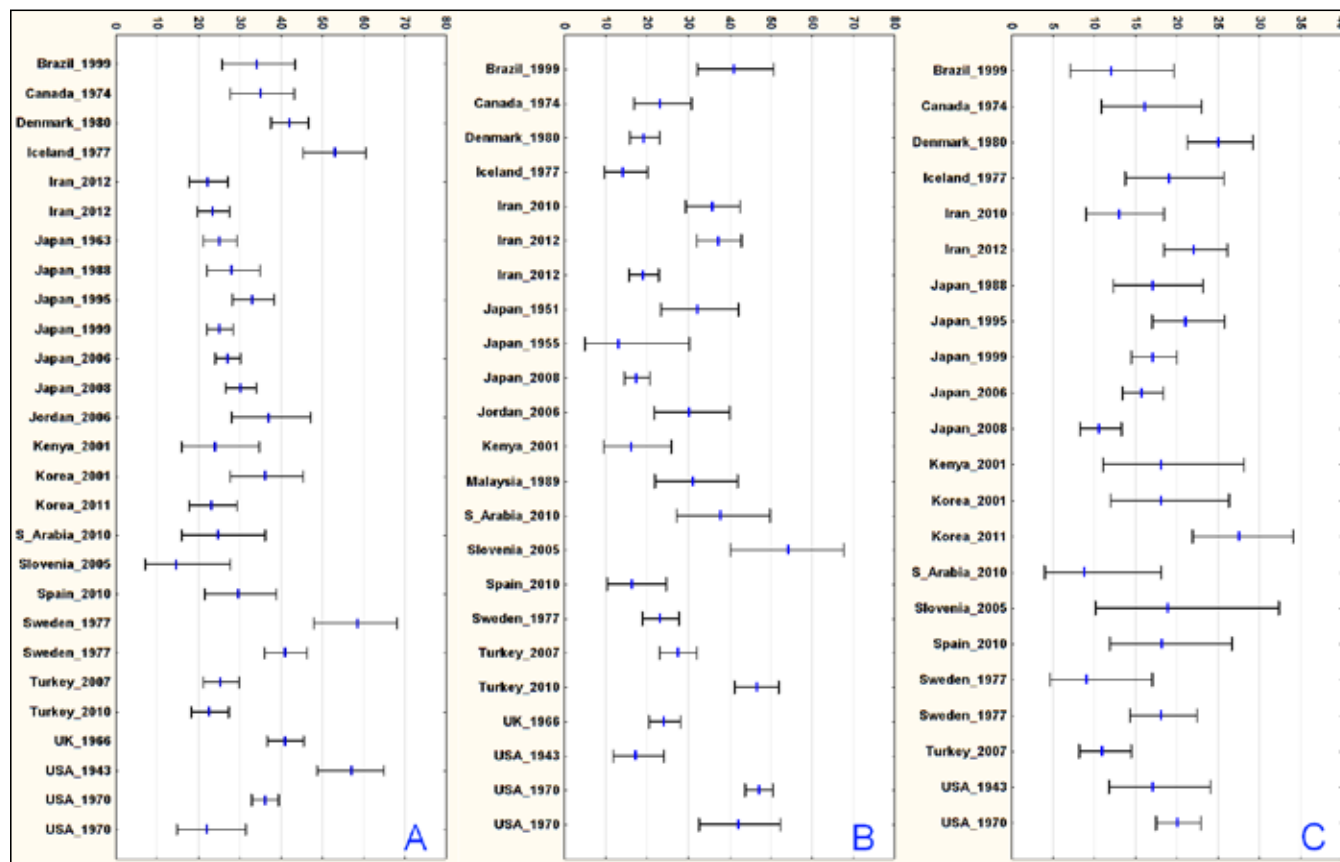


Table 2. Associations between potentially biasing factors and the percentage of missing.

	Rho (correlation coefficient)				P value				Sample size (n)			
	L5	U2	U5	L1-2	L5	U2	U5	L1-2	L5	U2	U5	L1-2
Sample types	0.584	-0.288	0.417	0.434	0.000	0.088	0.016	0.093	41	36	33	16
Race	-0.358	-0.189	0.120	0.658	0.017	0.249	0.486	0.006	44	39	36	16
Sample Sizes	0.375	-0.174	0.475	-0.321	0.012	0.288	0.003	0.226	44	39	36	16
Sex composition (M:F)	0.418	-0.171	0.186	0.267	0.019	0.395	0.352	0.488	31	27	27	9
Publication year	-0.517	0.146	-0.204	-0.277	0.000	0.374	0.232	0.298	44	39	36	16
Journal Credit	0.017	0.016	-0.113	-0.032	0.910	0.921	0.511	0.906	44	39	36	16
Minimum Age	-0.191	0.400	0.223	0.119	0.278	0.026	0.245	0.727	34	31	29	11

L5, mandibular second premolar; U2, maxillary lateral; U5, maxillary second premolar; L1-2, mandibular incisors.

Significant P values in bold font.

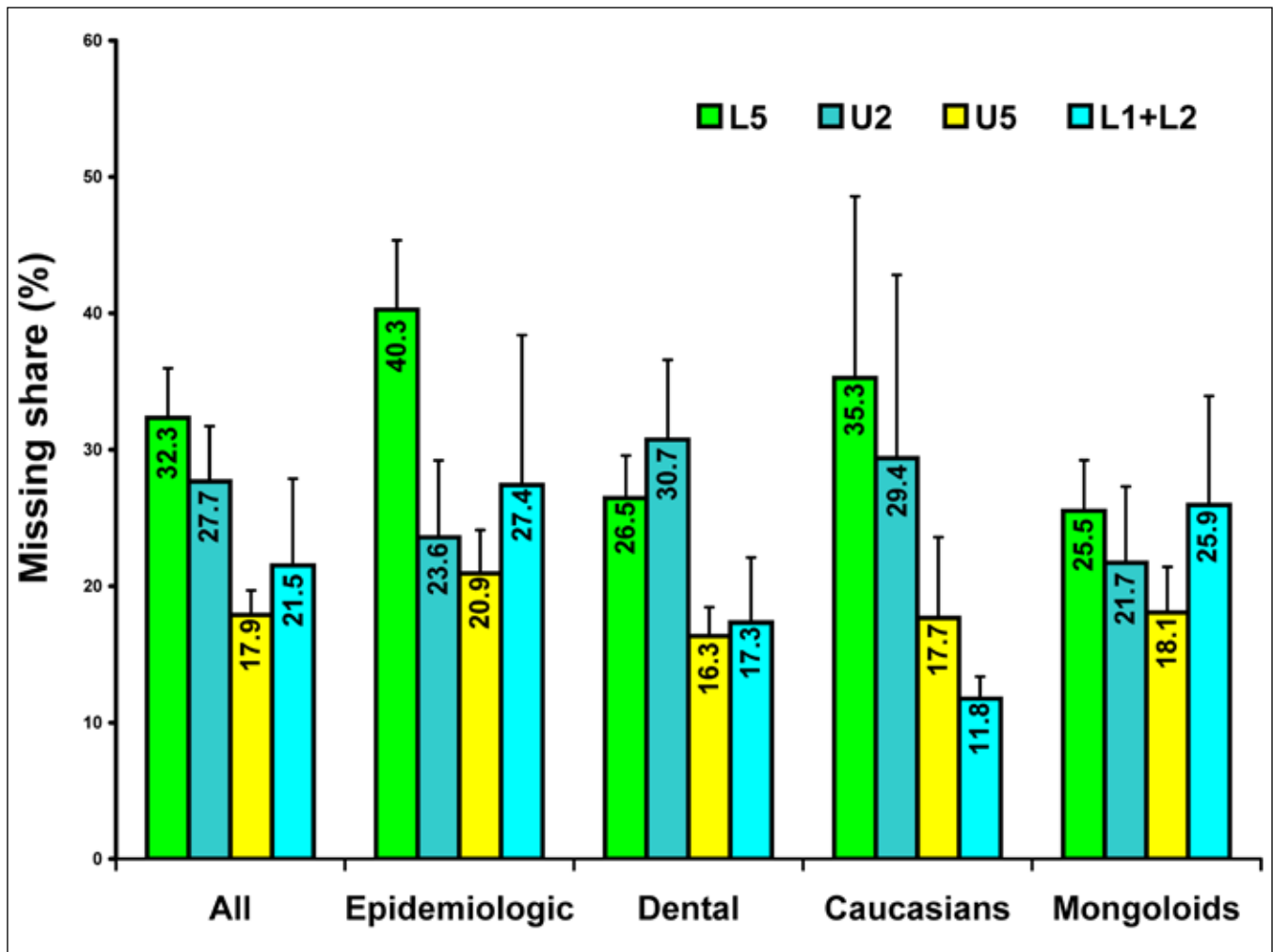
Table 3. The regression results regarding the effects of sample types and ethnicity on the percentage of missing of each tooth.

	Beta (regression coefficient)				P value				Sample size (n)			
	L5	U2	U5	L1-2	L5	U2	U5	L1-2	L5	U2	U5	L1-2
Sample type	0.574	-0.282	0.035	0.307	0.000	0.082	0.800	0.231	41	36	33	16
Race	-0.206	-0.211	0.216	0.398	0.111	0.203	0.274	0.127	41	36	33	16

L5, mandibular second premolar; U2, maxillary lateral; U5, maxillary second premolar; L1-2, mandibular incisors.

Significant P values in bold font.

Figure 4. The mean (and 95% CI) share of missing of individual teeth percent of the total number of missing teeth in all studies. L5, mandibular second premolar; U2, maxillary lateral; U5, maxillary second premolar; L1+L2, mandibular incisors.



– *The biasing effect of sample sizes on the missing share of each tooth*

A significant bivariate association was observed between larger samples and greater rates of detecting missing bimaxillary premolars (Table 2).

When the effects of sample type and race were controlled for, there was a significant result for the variable sample size in the case of U5 (n=33, sample size [beta=0.345, P=0.039]; sample type [beta=0.411, P=0.023]; race [beta=0.280, P=0.101]). Regarding L5 (n=41), only sample type had a significant role (beta=0.5, P=0.000) and the other two variables were non-significant (both P values between 0.15 to 0.2).

– *The effect of imbalances in samples' sex compositions on the missing percentage of each tooth*

Only the L5 agenesis was significantly associated with the ratio of the number of males divided by the number of females. It was positively correlated with the number of males (Table 2). It has been suggested that females might outnumber males in studies on orthodontic records but not in studies on randomly selected people. Therefore, the significant association between the lower premolar and the ratio of the number of males and females might be an artifact.

In order to control for the effect of sample type, a multiple linear regression analysis was employed (n=31). The model indicated that when sample type had been adjusted for (beta=0.646, P=0.000), no correlation existed between the L5 missing and sex imbalances of samples (beta=0.102, P=0.526).

– *The association between journal reputation (being indexed in Pubmed/WoS or none) and the agenesis percentage of each tooth*

No associations were found between the percentage of missing of any of the teeth and journals' scientific credit (Table 2).

– *The association between the minimum age of enrolled subjects and the percent of missing*

The Spearman coefficient indicated a significant association only with the U2 missing percentage. It was possible that other factors such as ethnicity and sample types might be associated with the minimum age, and thus bias the results. In order to control for the effect of sample types and ethnicity, a multiple regression was used.

The model (n=28) showed that the minimum age was correlated with the percentage of U2 missing (beta=0.403, P=0.027), while the effect of race (beta=-0.190, P=0.281) and sample type (beta=-0.310, P=0.085) were controlled for.

The Welch t-test was used to assess the minimum ages 6 to 15 years as cutoff ages. It identified certain significant changes in the reported missing share of the L5 and U2 at ages around 10 years old (Table 4).

DISCUSSION

The findings of this study indicated that the meta-sample was heterogeneous; hence, other sources of bias existed besides variations due to sampling from different populations (e.g., the ethnicity). These biasing factors might include the minimum age of the enrolled subjects, the size of samples, their types, and imbalances in the numbers of males and females. Although the ethnicity might affect the results, the year of publication was unlikely an influencing factor.

Can sample types bias the results?

It is suggested that orthodontic patients might tend to show higher missing rates of the maxillary laterals which can affect the esthetics^{12,20}. The maxillary lateral was the most frequent missing tooth in many studies on dental patients^{1,12,14,17,21,23,67,78,79}. However, it cannot be decisive without analytical assessments, since many studies on orthodontic/dental patients reported higher missing prevalence in the mandibular second premolars^{3,5,16,18,22,63,65,66,68,71,72,75,81,86}, mandibular laterals^{15,88}, mandibular centrals⁷⁶, or even maxillary premolars⁸². Besides, some studies on epidemiologic samples such as school students or defense force recruits found the mandibular second premolar as the most common absent tooth^{11,57,84}. The differences might root in the minimum age¹¹ and the ethnicity of the included subjects^{3,5,12,18}. Despite the numerous controversial results, the current analyses confirmed this suggestion for orthodontic patients, but not for non-orthodontic dental patients. When it came to the percentage of commonly missing teeth (regardless of their missing ranks), all the mean percentages (for the four assessed teeth) were affected by sample types. Only the maxillary laterals tended to be absent more in dental/orthodontic patients. The other teeth were more likely to be absent in epidemiologic studies, but less missing in samples of dental/orthodontic patients. It is much better to enroll random subjects; however, X-ray exposure merely for research purposes might be unethical^{1,12,28,89}.

Are some teeth more likely to be reported missing in larger samples?

An interesting finding was that in larger samples, there might be a greater chance of reporting more missing second premolars. The

factor sample size was demonstrated previously to be able to bias the CMT prevalence²⁸. However, the mechanism is unknown.

Are some teeth more missing in a given gender?

Only mandibular premolar showed a significant correlation with sex (higher missing rates in boys). When the effect of sample type (epidemiological vs. dental/orthodontic) was controlled for, the correlation turned nonsignificant. Therefore, it might be related to potential greater esthetical concerns perceived by female dental patients. On the other hand, Küchler et al.⁷² observed that in orthodontic patients, incisor missing was more common in males while premolar missing was more frequent in females. This biasing factor is as well inconclusive and needs future research.

Does the minimum age of pooled subjects matter?

This factor seemed to affect mostly the maxillary laterals and to a lesser degree the mandibular second. Studies that had enrolled subjects with higher minimum ages were more likely to report more missing laterals and fewer missing premolars. The differences reached the level of significance at around the age of 10 years for the premolar and the ages 8 to 10 for the lateral. Regarding the lateral, an increase in the reported prevalence by enrolling older subjects might be anticipated and reasonable, since at older ages, delayed laterals are more likely erupted. Thus delayed laterals are not confused with missing laterals^{12,23,28,59,62}. A similar finding was anticipated to happen for premolars around the ages 10 to 12 years (when delayed teeth are likely erupted)²⁸. However, instead of an increase, a decline was observed in the results of studies with older subjects. This warrants future research.

CONCLUSIONS

The most affected teeth seem to be mandibular second premolars and maxillary laterals. The discrepancy between the two can vary based on the subsample. However, in any situation, L5 missing surpasses U2 missing in the number of the reports, marking it as the first most commonly missing tooth. Regarding the share of missing of each tooth, L5 and mandibular incisors are more likely to be absent, followed by U5 and U2. The agenesis of different teeth can be affected by the ethnicity, sample types (random vs. non-random), sample sizes (only for bimaxillary second premolars), and the minimum age of pooled subjects (only for U2 and L5). Older children should be sampled to ensure more accurate results. The year of publication seems not affecting the findings.

Table 4. The results of Welch t-test, comparing the mean percentage of missing of four tooth types, according to various minimum ages (as cut-offs).

Tooth	Cutoff age (year)	< Cutoff age		≥ Cutoff age		Difference	P
		N	Mean (%)	N	Mean (%)		
L5	9	20	36.8	14	29.1	-7.8	0.073
	10	20	36.8	11	38.5	15.3	0.003
U2	8	14	23.8	17	32.5	8.7	0.050
	9	17	22.7	14	35.7	13.0	0.005
	11	24	25.9	7	37.9	12.0	0.100

N, number of studies; L5, mandibular second premolar; U2, maxillary lateral.

Only comparisons with P values ≤ 0.1 are demonstrated. Significant P values in bold font.

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