Third molar agenesis in modern humans with and without agenesis of other teeth

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Background. The number of teeth in the human dentition is of interest both from developmental and evolutionary aspects. The present case-control study focused on the formation of third molars in modern humans aiming to shed more light on the most variable tooth class in the dentition.

Materials and Methods. For this reason, we investigated third molar formation in a sample of 303 individuals with agenesis of teeth other than third molars (agenesis group) and compared it to a sex and age matched control group of 303 individuals without agenesis of teeth other than third molars.

Results. The prevalence of third molar agenesis in the agenesis group was 50.8%, which is significantly higher than the 20.5% in the control group (p < 0.001). The chance of a missing third molar in the agenesis group was increased by 38.3% (p < 0.001), after controlling for the agenesis in other teeth factor. When considering the amount of missing third molars per individual, a clear tendency towards more missing third molars was evident in the agenesis group compared to the control group. The frequency of bilaterally missing third molars in the agenesis group was 29% in the maxilla, as well as in the mandible, which is about three times higher than the frequency of unilaterally missing third molars (p < 0.001). In the control group, bilaterally missing third molars occurred in 8.6% in the maxilla and 8.9% in the mandible.

Conclusion. The present results indicate that genetic factors involved in tooth agenesis affect also the dentition as a whole. Furthermore, the third molars are more vulnerable to factors involved in agenesis of other teeth and they are more often affected as a whole. These findings seem to be associated with the evolutionary trend in humans towards reduced molar number.

1 **Third molar agenesis in modern humans with and without agenesis of other**

2 **teeth**

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14 **ABSTRACT**

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25 third molar in the agenesis group was increased by 38.3% (p < 0.001), after controlling for the
26 agenesis in other teeth factor. When considering the amount of missing third molars per
27 individual, a clear tendency towards more missing third molars was evident in the agenesis group
28 compared to the control group. The frequency of bilaterally missing third molars in the agenesis
29 group was 29% in the maxilla, as well as in the mandible, which is about three times higher than
30 the frequency of unilaterally missing third molars (p < 0.001). In the control group, bilaterally
31 missing third molars occurred in 8.6% in the maxilla and 8.9% in the mandible.

32 **Conclusion.** The present results indicate that genetic factors involved in tooth agenesis affect
33 also the dentition as a whole. Furthermore, the third molars are more vulnerable to factors
34 involved in agenesis of other teeth and they are more often affected as a whole. These findings
35 seem to be associated with the evolutionary trend in humans towards reduced molar number.

INTRODUCTION

Tooth agenesis is the congenital absence of one or more teeth. In the primary dentition, the prevalence ranges between 0.1% and 0.2%. However, in the permanent dentition tooth agenesis is prevalent in 6.4% of the overall population, with similar occurrence in the two jaws (Khalaf et al. 2014). There is a large variation between different population groups and studies (Khalaf et al. 2014).

Tooth agenesis studies generally exclude third molars, due to the high frequency of their absence (Khalaf et al. 2014). Agenesis of third molars is more or less considered a physiologic finding or an evolutionary adaptation of the dentition rather than a developmental disturbance (Koussoulakou et al. 2009). The third molar is the last tooth to develop in the dentition and is characterized by the variability in time of formation and by its diversity in presence or absence (Banks 1934; Celikoglu et al. 2010). The worldwide average of third molar agenesis is 22.6%, with Asian populations showing the highest rate of 29.7% (Carter & Worthington 2015).

A wide range of studies shows that the agenesis of third molars correlates with the number of other teeth in the dentition. According to Garn et al. (Garn et al. 1962), the chance of another tooth to be missing is raised thirteen-fold if at least one-third molar is missing. More recent studies point in the same direction, though with much reduced effect sizes (Bredy et al. 1991; Celikoglu et al. 2011; Endo et al. 2015). Endo et al. (Endo et al. 2013) reported a significant association between missing third molars and bilateral agenesis of other teeth. Other researchers focused on the agenesis of specific teeth and third molar agenesis (Abe et al. 2010; Garib et al. 2010; Garib et al. 2009).

So far, various studies have investigated the association between missing third molars and agenesis of other teeth, but on limited tooth agenesis samples. Furthermore, most relevant studies tested Asian populations. Thus, we performed a study in a large sample of European subjects, aiming to investigate third molar formation in individuals with and without agenesis of other teeth. To obtain a robust sample, we selected a large number of individuals with agenesis of teeth other than third molars and compared it to a matched group without agenesis of teeth other than third molars. The current approach offers the opportunity to assess previously tested, but also novel questions, relevant to the study hypothesis, with adequate sample sizes. The primary null hypothesis was that there is no difference in third molar agenesis patterns between individuals who have agenesis in teeth other than third molars, and those who do not.

MATERIALS AND METHODS

In this case-control study, we followed the STROBE guidelines for reporting observational studies (von Elm et al. 2008).

The ethical approval was provided by the Ethics Commission of the Canton of Bern, Switzerland (Project-ID: 2018-01340) and the Research Committee of the School of Dentistry, National and

72 Kapodistrian University of Athens, Greece (Project-ID: 281, 2/9/2016). The need for informed
73 consent was waived for part of the sample and was obtained for the rest.

Study sample

75 Consecutive orthodontic records of various time periods within a 12-year period (2006 - 2018,
76 depending on the place of sample collection) were searched for eligible subjects at the following
77 clinics: A) University of Bern, Switzerland b) University of Athens, Greece, c) two private
78 practices in Athens and two in Thessaloniki, Greece, and d) one private practice in Biel,
79 Switzerland. Sample collection was performed at the place of data generation by colleagues who
80 were blinded to the aim of this study.

81 The sample was collected based on the following inclusion criteria:

82 • Individuals with an age between 12.5 and 40 years

83 • Individuals with and without agenesis of teeth other than third molars for the agenesis and the
84 control group, respectively

85 • European ancestry

86 • No syndromes, systemic diseases or other defects that affect the craniofacial complex
87 development, as reported in the subjects' medical records

88 • Adequate quality panoramic radiographs for identification of missing teeth (Figure S1)

89 • No individuals where the cause of missing teeth was unclear

90 • No individuals where the presence or absence of teeth could not be confirmed

91 The minimum age limit of 12.5 years was determined according to previous studies that
92 evaluated the correlation between chronological age and the degree of third molar mineralization
93 (Caldas et al. 2011; de Oliveira et al. 2012; Karataş et al. 2013; Soares et al. 2015; Zandi et al.
94 2015). They showed that in 95% of cases, Demirjian's stage A was observed at the age of 12.5 or
95 younger, which means that the mineralization of third molar crowns has already started and is
96 clearly visible on the panoramic radiographs.

97 Finally, the panoramic radiographs of 303 individuals with agenesis of teeth other than third
98 molars (agenesis group) were selected from a large orthodontic sample of approximately 10.000
99 individuals, based on availability. A control group of 303 individuals without agenesis of teeth
100 other than third molars, matched for age (within 6 months) and sex was formed from the same
101 archives. All other inclusion criteria for the control group, were the same as mentioned above for
102 the agenesis group.

Data extraction

104 After reviewing the orthodontic files (medical and dental history, intraoral and extraoral photos,
105 radiographs) at the place of sample collection, the relevant data were recorded in an Excel sheet
106 (Microsoft Excel, Microsoft Corporation, Redmond WA, USA) in a standardized manner. To

107 identify tooth agenesis, the panoramic radiographs were digitized and viewed on screen. A single
108 researcher (M.S.) performed the data extraction procedure of the entire sample in terms of
109 missing teeth, and repeated it for 40 randomly selected subjects (https://www.random.org/)
110 following a 1-month washout period. In case of disagreement, the radiographs were controlled
111 by all authors and a consensus was reached.

112 To record tooth agenesis patterns, the TAC (Tooth Agenesis Code) system was used (van Wijk
113 & Tan 2006). This system assigns a binary value to each tooth providing a unique numeric value
114 for each pattern. Each dental quadrant is analyzed separately, and thus, the combined values
115 assigned to each of the quadrants (q1, q2, q3, and q4) represent a unique tooth agenesis pattern
116 (van Wijk & Tan 2006).

117 **Statistical analysis**

118 All statistical analyses were conducted with SPSS software (IBM SPSS Statistics for Windows,
119 Version 23.0. Armonk, NY: IBM Corp). Descriptive statistics were also calculated through the
120 Tooth Agenesis Code Data Analysis Tool (http://www.toothagenesiscode.com/, last accessed 15
121 May 2019). Intra-rater agreement was evaluated through the percentage of different patterns
122 identified in the two repeated assessments. The two-tailed Pearson's Chi square test was used to
123 assess differences in the frequencies observed in the control and the agenesis samples. The
124 Spearman's correlation coefficient was used to investigate the relation of the number of agenesis
125 of teeth other than third molars to the number of third molar agenesis, overall, as well as within
126 quadrants.

127 **RESULTS**

128 **Method error**

129 The Intra-rater agreement between repeated tooth agenesis pattern identification was 97.5%.

130 **Agenesis group without considering third molars**

131 In the 303 individuals (170 females, 133 males) of the agenesis sample, in total 799 teeth, other
132 than third molars, were congenitally missing. In 38.6% of the sample one tooth, in 33.3% two,
133 and in 7.9% three teeth were missing (Table S1). The incidence for missing teeth in the maxilla
134 was 57.1%, compared to 68.6% in the mandible (p = 0.079). The most common missing tooth
135 was the mandibular second premolar (29.3%), followed by the maxillary lateral incisor (21.0%),
136 and the maxillary second premolar (14.0%; Table 1).

137 Regarding tooth symmetry, the teeth that were most often symmetrically missing in the maxilla
138 were the lateral incisors (19.5%) followed by the second premolars (14.2%). In the mandible, the
139 second premolars were missing bilaterally in 25.4%, followed by the central incisors in 7.9%
140 (Table 2).

141 The most common agenesis patterns in the maxilla were bilaterally missing lateral incisors
142 (23.1%), followed by bilaterally missing second premolars (12.7%). In the mandible, the most

143   common patterns were bilateral agenesis of second premolars in 27.9%, followed by unilateral
144   agenesis of the right second premolar (17.3%). In the whole dentition, bilateral agenesis of
145   maxillary lateral incisors occurred most often (11.2%), followed by bilateral agenesis of
146   mandibular second premolars (10.2%; Table 3).

147   Third molar agenesis in the agenesis and the control group

148   The prevalence of third molar agenesis in the agenesis group was 50.8%, which is significantly
149   larger than the 20.5% in the control group (p < 0.001). 418 third molars were congenitally
150   missing in the agenesis group (n = 303) compared to 144 in the control group (n = 303). If the
151   probability of third molar agenesis in the agenesis group was equal to that of teeth other than
152   third molars, this would increase the value of 144 missing third molars, observed in the control
153   group, by 114. Consequently, 258 missing third molars would have been expected in the
154   agenesis group. This value is significantly lower than the actual value observed (418; p < 0.001).
155   Thus, the chance of a missing third molar in the agenesis group is increased by 38.3%, compared
156   to controls.

157   In the agenesis group, there was a significant, though weak correlation, of the total number of
158   other missing teeth to the total number of missing third molars (rho = 0.31, p < 0.001). Similarly,
159   very weak correlations were identified when third molar agenesis was correlated to the number
160   of other tooth agenesis within quadrants (Q1: rho = 0.16, p = 0.006; Q2: rho = 0.14, p = 0.015;
161   Q3: rho = 0.20, p = 0.001; Q4: rho = 0.29, p = 0.001).

162   The frequency of bilaterally missing third molars in the agenesis group was 29% in the maxilla,
163   as well as in the mandible. This is about three times higher than the frequency of unilaterally
164   missing third molars (maxilla: 9.9%, p < 0.001, mandible: 11.9%, p < 0.001; Table 2). The ratio
165   of bilateral to unilateral third molar agenesis was significantly higher in the agenesis group
166   compared to the control group (maxilla: 2.93 vs. 1.53, respectively, p < 0.001; mandible: 2.44 vs.
167   1.29, respectively, p < 0.001; Table 2).

168   In the tooth agenesis group, symmetrical third molar agenesis occurred in a similar manner
169   within jaws (29% within each jaw), between jaws (right side: 24%, left side: 24%), or crossed
170   quadrant (q1 vs. q3: 22.1%; q2 vs q4: 24.4%) (p > 0.05; Table 4). The same was true for the
171   control groups (p > 0.05; Table 4), though the prevalence of all respective symmetrical patterns
172   was much lower (range: 6.6 - 8.9%, p < 0.001).

173   In both groups, there was no statistically significant difference between the number of missing
174   third molars in the different quadrants (Chi square test, p > 0.05; Table 1). The agenesis group
175   differed significantly from the control group in the distribution of the number of missing third
176   molars (p < 0.001). There is a clear tendency towards more missing third molars in the agenesis
177   group compared to the controls. The agenesis group has 1.55, 2.14, 3.80, and 3.48 times higher
178   possibility of having one, two, three, or four missing third molars respectively, when compared
179   to the control group (Figure 1).

180   Table S2 shows the most common patterns of tooth agenesis in the agenesis group, including
181   third molars. In the maxilla, the lateral incisors were most commonly missing in 14.1%, followed
182   by bilaterally missing third molars in 13.6% of the sample. In the mandible, the second
183   premolars were most commonly missing bilaterally in 12.8% of the sample, followed by
184   unilateral second premolar agenesis.

185   Table S3 shows the most common patterns of third molar agenesis in control subjects where
186   agenesis was observed. In the maxilla, as well as in the mandible, bilateral third molar agenesis
187   was the most common pattern (60.5% and 56.3%, respectively). In the entire dentition, the most
188   common pattern was the four missing third molars (17.4%), followed by bilateral third molar
189   agenesis in the mandible, in 14.5%. Table S4 shows the most common patterns of third molar
190   agenesis in the agenesis group, where third molar agenesis was observed. In this group also,
191   bilateral third molar agenesis was the most common pattern within jaws (74.6% and 71.0%, in
192   the maxilla and the mandible, respectively). Furthermore, in the entire dentition, the most
193   common pattern was also in this group the four missing third molars (38.3%), followed by
194   bilateral third molar agenesis in the mandible (12.3%).

195   **DISCUSSION**

196   The purpose of this study was, to explore the patterns of third molar agenesis in a large sample of
197   modern European subjects with and without agenesis of other teeth. The prevalence of third
198   molar agenesis in the agenesis group was 50.8%, which is about 2.5 times higher than in the
199   control group. In the agenesis group, there was a weak correlation of the number of agenesis of
200   other teeth with the number of third molar agenesis within individuals, as well as very weak
201   correlations of third molar agenesis to the number of agenesis of other teeth within quadrants.
202   When considering the percentages of the amount of missing third molars per individual in the
203   control and the agenesis group, there was a tendency towards more missing third molars in the
204   agenesis group. The frequency for bilaterally missing third molars in the agenesis group was
205   about three times higher than the frequency of unilateral absence. The ratio of bilateral to
206   unilateral third molar agenesis was also significantly higher in the agenesis group, compared to
207   the control group.

208   It could also be useful to note, that based on our findings, in young patients with severe tooth
209   agenesis, the clinician should expect that probably the third molars will also be missing. This
210   should be considered in the treatment planning of severe tooth agenesis cases, which is usually
211   complex and requires a multidisciplinary approach.

212   Our methodology differs from all previous studies, in terms that we investigated the patterns of
213   third molar agenesis in a large sample with agenesis of other teeth. To our knowledge, all the
214   existing studies divided their groups according to third molar agenesis. Through the latter
215   approach, only a small percentage of the subsequent subsamples had agenesis in teeth other than
216   third molars, and thus, these groups did not have adequate or comparable size to the control
217   groups. Our study tested a large agenesis sample of 303 agenesis individuals, as well as 303

218 controls, selected out of a total of around 10000 records. This allowed for findings that are
219 presented for the first time in the literature, such as those related to symmetry or to occurrences
220 within quadrants. Furthermore, the groups were matched for sex and age, accounting for any
221 confounding effects of these factors. For younger individuals, these might be related to the
222 differences in dental maturity between sexes of the same chronological age or to the etiology of
223 tooth absence in older individuals. Furthermore, studies on tooth agenesis (Khalaf et al. 2014), as
224 well as on third molar agenesis (Carter & Worthington 2015), agree in the higher prevalence of
225 agenesis in females than in males. Differences between sexes were not investigated here, since
226 this was beyond the scope of the present study. A potential effect of the sex factor on the
227 outcomes is not expected because the sample was matched for sex.

228 The age range that we considered was limited from 12.5 to 40 years old. The minimum limit was
229 defined according to various longitudinal studies that showed the correlation between
230 chronological age and the degree of third molar mineralization using Demirjian's developmental
231 stages. This classification has been widely used and tested to facilitate age estimation. Therefore,
232 the choice of this age limit is considered to be appropriate for our purpose (Caldas et al. 2011; de
233 Oliveira et al. 2012; Karataş et al. 2013; Soares et al. 2015; Zandi et al. 2015). The upper age
234 limit of 40 years was chosen to avoid false positive results due to extraction or tooth loss due to
235 other reasons that could have been registered as agenesis.

236 We found a prevalence of 50.8% for third molar agenesis in the agenesis group compared to
237 20.5% in the control group. According to a recent meta-analysis (Carter & Worthington 2015),
238 the worldwide average of third molar agenesis is 22.6% (21.6% for Europeans), confirming the
239 validity of our control group. Our results clearly demonstrated that in individuals with agenesis
240 of other teeth, the prevalence of third molar agenesis is higher. This points in the same direction
241 with previous studies that showed an increased prevalence of agenesis of other teeth in
242 individuals with third molar agenesis (Bredy et al. 1991; Celikoglu et al. 2011; Endo et al. 2015).

243 In our control group, the sequence of the number of missing third molars was similar to that of
244 Carter and Worthington (Carter & Worthington 2015) that showed the highest prevalence for one
245 missing third molar, followed by two, and four missing third molars. However, the most
246 common amount of missing third molars in the agenesis group was four, followed by two and
247 one third molar. This inconsistency is attributed to the different sample composition. The
248 aforementioned meta-analysis tested third molar agenesis in the general population, meaning that
249 individuals with agenesis of other teeth would be limited. The above findings clearly show that
250 the presence of agenesis, in teeth other than third molars, has a considerable effect on third molar
251 agenesis patterns. Especially, the probability to have four missing third molars increases. This
252 suggests that the third molars might be more vulnerable to genetic factors involved in tooth
253 agenesis, as compared to other tooth types. Indeed, this is also supported by the increased
254 number of missing third molars in the agenesis sample compared to that expected by chance. A
255 recent study analysing data from 172 monozygotic and 112 dizygotic twins concluded that third
256 molar formation is strongly controlled by additive genetic factors, providing further support to

257 our statements (Trakinienė et al. 2018). This concept is in line with the evolutionary trend in
258 humans towards less teeth, and more specifically, less molars (Kavanagh et al. 2007). Facial size
259 has also been reduced during evolution (Bastir et al. 2010). Recent evidence showed that the
260 number of teeth that are formed in a dentition is associated with facial size in modern humans.
261 This indicates that a biological mechanism of tooth number reduction that has evolved during
262 time might still be active and continue to regulate the number of teeth and facial size in a
263 coordinated manner (Oeschger et al. 2020). The findings of the present study, along with the
264 high prevalence of third molar agenesis in the population (Carter & Worthington 2015) suggest
265 that the third molars might be affected to a higher degree from such mechanisms, compared to
266 other teeth in the dentition.

267 Furthermore, in terms of developmental timing, the third molar is the last tooth in the molar
268 series and also the last tooth to develop in the dentition. Evidence supports that the last tooth in
269 each tooth series shows more often developmental disturbances, including agenesis, thus being
270 more vulnerable to genetic or environmental factors that might be present during development
271 (Townsend et al. 2009; Gkantidis et al. 2017). This might be another contributing factor relevant
272 to the present results. It has also been shown that overall dental development is delayed in
273 patients with tooth agenesis, compared to controls, with a weak correlation between dental
274 developmental stage and number of missing teeth (Lebbe et al. 2017).

275 In the agenesis group, the prevalence for bilaterally missing third molars was more than three
276 times higher than in the control group, in the maxilla as well as in the mandible. The ratio of
277 bilateral to unilateral third molar agenesis was significantly higher in the agenesis group
278 compared to the control group. The same was true for all types of symmetry. Furthermore, in the
279 agenesis and the control group, the most common third molar agenesis pattern was four missing
280 third molars, followed by bilateral third molar agenesis in the mandible. This is in line with our
281 previous statement that third molars are more susceptible to genetic or epigenetic factors that
282 cause tooth agenesis, and might more possibly be affected as a whole. The above claim is also
283 supported by the increasing possibility for more missing third molars in the agenesis group than
284 in the controls. Furthermore, very weak correlations were identified between other missing teeth
285 and third molar agenesis within quadrants, suggesting that there are no significant genetic effects
286 limited within quadrants.

287 A limitation of the study could be that the sample was selected from orthodontic practices,
288 meaning that it may not be representative of the general population. For example, it might be
289 evident that the percentage of severe tooth agenesis occurrences is higher in our sample, since it
290 derived from orthodontic patients, including two university centres. However, the study aimed to
291 test the association of third molar formation with the formation of other teeth using a case-
292 control study design. Thus, the study did not aim to represent the general population, but it aimed
293 to test the association of the severity of agenesis of other teeth with third molar formation.
294 Therefore, this is not considered a limitation of the study. On the contrary, it led to adequate
295 number of cases representing the occurrence of severe agenesis (n = 27 cases with 6 or more

296 teeth missing, 8.9% of the agenesis sample). The fact that the sample derived from orthodontic
297 patients, which are thoroughly documented and followed over time, reduces the possibility that
298 certain severe cases might represent non-diagnosed syndromes. Even if limited misdiagnosed
299 cases were present in the sample, our analysis showed very weak correlations of the total number
300 of other missing teeth to the total number of missing third molars. Thus, these cases could have
301 not confounded our findings considerably. Without considering third molars, the present tooth
302 agenesis patterns are comparable to those of other studies presented in the literature (Gkantidis et
303 al. 2017; Khalaf et al. 2014), concerning the most common missing teeth and other tooth
304 agenesis patterns. Regarding other characteristics of an orthodontic population, when considering
305 that malocclusion is endemic in recent years, it is not expected that our sample would highly
306 differ from the general population. Another limitation could be the inclusion of patients up to 40
307 years old, which might increase the chance to miss information on causes of tooth loss, such as
308 due to extractions. To control for this confounding, according to our inclusion criteria, a case was
309 excluded when the treating doctors judged that the cause for a missing tooth was unclear.
310 Through this approach, misdiagnosis might not be fully excluded, but it was limited
311 considerably, in order not to critically affect the outcomes. Finally, the present results are based
312 only on subjects of the European population, and thus, they have to be confirmed on other
313 ancestries. However, the study sample originated from places where the white European
314 background is highly represented. Thus, we decided to include only white subjects of European
315 ancestry to avoid confounding.

316 **CONCLUSION**

317 The present study showed that individuals with non-syndromic tooth agenesis in teeth other than
318 third molars show a higher prevalence of third molar agenesis compared to matched control
319 individuals without agenesis of other teeth. There was also a clear tendency towards more
320 missing third molars in the agenesis group. Furthermore, in the agenesis group, the prevalence
321 for bilaterally missing third molars was more than three times higher than in the control group.
322 The ratio of bilateral to unilateral third molar agenesis was also significantly higher. The above
323 findings indicate that the third molars might be more vulnerable to genetic or epigenetic factors
324 involved in agenesis of other teeth and they are often affected as a whole. These findings seem to
325 be associated with the evolutionary trend in humans towards reduced number of teeth.

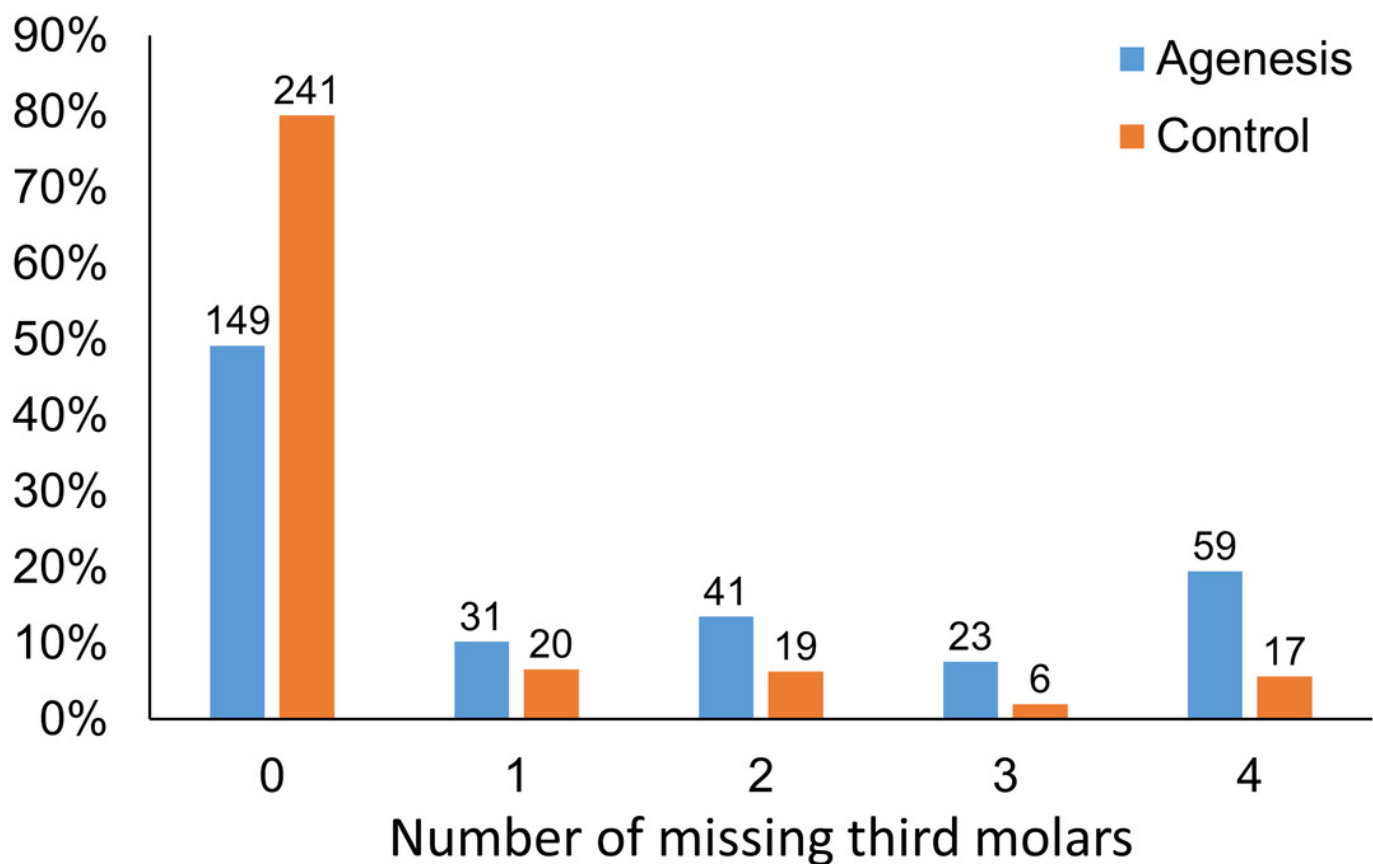
326 **REFERENCES**

327 Abe R, Endo T, and Shimooka S. 2010. Maxillary first molar agenesis and other dental
328   anomalies. *Angle Orthod* 80:1002-1009. 10.2319/020210-69.1
329 Banks HV. 1934. Incidence of Third Molar Development. *The Angle Orthodontist* 4:223-233.
330   10.1043/0003-3219(1934)004<0223:Iotmd>2.0.Co;2
331 Bastir M, Rosas A, Stringer C, Cuetara JM, Kruszynski R, Weber GW, Ross CF, and Ravosa
332   MJ. 2010. Effects of brain and facial size on basicranial form in human and primate
333   evolution. *J Hum Evol* 58:424-431. 10.1016/j.jhevol.2010.03.001

334   Bredy E, Erbring C, and Hubenthal B. 1991. [The incidence of hypodontia with the presence and
335   absence of wisdom teeth]. *Dtsch Zahn Mund Kieferheilkd Zentralbl* 79:357-363.
336   Caldas IM, Julio P, Simoes RJ, Matos E, Afonso A, and Magalhaes T. 2011. Chronological age
337   estimation based on third molar development in a Portuguese population. *Int J Legal Med*
338   125:235-243. 10.1007/s00414-010-0531-8
339   Carter K, and Worthington S. 2015. Morphologic and Demographic Predictors of Third Molar
340   Agenesis: A Systematic Review and Meta-analysis. *J Dent Res* 94:886-894.
341   10.1177/0022034515581644
342   Celikoglu M, Bayram M, and Nur M. 2011. Patterns of third-molar agenesis and associated
343   dental anomalies in an orthodontic population. *Am J Orthod Dentofacial Orthop* 140:856-
344   860. 10.1016/j.ajodo.2011.05.021
345   Celikoglu M, Kazanci F, Miloglu O, Oztek O, Kamak H, and Ceylan I. 2010. Frequency and
346   characteristics of tooth agenesis among an orthodontic patient population. *Med Oral*
347   *Patol Oral Cir Bucal* 15:e797-801. 10.4317/medoral.15.e797
348   de Oliveira FT, Capelozza AL, Lauris JR, and de Bullen IR. 2012. Mineralization of mandibular
349   third molars can estimate chronological age--Brazilian indices. *Forensic Sci Int* 219:147-
350   150. 10.1016/j.forsciint.2011.12.013
351   Endo S, Sanpei S, Ishida R, Sanpei S, Abe R, and Endo T. 2015. Association between third
352   molar agenesis patterns and agenesis of other teeth in a Japanese orthodontic population.
353   *Odontology* 103:89-96. 10.1007/s10266-013-0134-1
354   Endo T, Sanpei S, Komatsuzaki A, Endo S, Takakuwa A, and Oka K. 2013. Patterns of tooth
355   agenesis in Japanese subjects with bilateral agenesis of mandibular second premolars.
356   *Odontology* 101:216-221. 10.1007/s10266-012-0080-3
357   Garib DG, Alencar BM, Lauris JR, and Baccetti T. 2010. Agenesis of maxillary lateral incisors
358   and associated dental anomalies. *Am J Orthod Dentofacial Orthop* 137:732.e731-736;
359   discussion 732-733. 10.1016/j.ajodo.2009.12.024
360   Garib DG, Peck S, and Gomes SC. 2009. Increased occurrence of dental anomalies associated
361   with second-premolar agenesis. *Angle Orthod* 79:436-441. 10.2319/021308-87.1
362   Garn SM, Lewis AB, and Vicinus JH. 1962. Third molar agenesis and reduction in the number of
363   other teeth. *J Dent Res* 41:717. 10.1177/00220345620410033001
364   Gkantidis N, Katib H, Oeschger E, Karamolegkou M, Topouzelis N, and Kanavakis G. 2017.
365   Patterns of non-syndromic permanent tooth agenesis in a large orthodontic population.
366   *Arch Oral Biol* 79:42-47. 10.1016/j.archoralbio.2017.02.020
367   Karataş OH, Öztürk F, Dedeoğlu N, Çolak C, and Altun O. 2013. Radiographic evaluation of
368   third-molar development in relation to the chronological age of Turkish children in the
369   southwest Eastern Anatolia region. *Forensic Sci Int* 232:238.e231-235.
370   10.1016/j.forsciint.2013.07.023
371   Kavanagh KD, Evans AR, and Jernvall J. 2007. Predicting evolutionary patterns of mammalian
372   teeth from development. *Nature* 449:427-432. 10.1038/nature06153
373   Khalaf K, Miskelly J, Voge E, and Macfarlane TV. 2014. Prevalence of hypodontia and
374   associated factors: a systematic review and meta-analysis. *J Orthod* 41:299-316.
375   10.1179/1465313314Y.0000000116
376   Koussoulakou DS, Margaritis LH, and Koussoulakos SL. 2009. A curriculum vitae of teeth:
377   evolution, generation, regeneration. *Int J Biol Sci* 5:226-243. 10.7150/ijbs.5.226

378 Lebbe A, Cadenas de Llano-Pérula M, Thevissen P, Verdonck A, Fieuws S, and Willems G.
379   2017. Dental development in patients with agenesis. *Int J Legal Med* 131:537-546.
380   10.1007/s00414-016-1450-0
381 Oeschger ES, Kanavakis G, Halazonetis DJ, and Gkantidis N. 2020. Number of teeth is
382   associated with facial size in humans. *Sci Rep* 10:1820. 10.1038/s41598-020-58565-8
383 Soares CB, Figueiroa JN, Dantas RM, Kurita LM, Pontual Ados A, Ramos-Perez FM, Perez DE,
384   and Pontual ML. 2015. Evaluation of third molar development in the estimation of
385   chronological age. *Forensic Sci Int* 254:13-17. 10.1016/j.forsciint.2015.06.022
386 Townsend G, Harris EF, Lesot H, Clauss F, and Brook A. 2009. Morphogenetic fields within the
387   human dentition: A new: Clinically relevant synthesis of and old concept. *Archives of*
388   *Oral Biology* 54: S34-S44.
389 Trakinienė et al. 2018. Impact of genetics on third molar agenesis. *Sci Rep* 8:8307.
390   10.1038/s41598-018-26740-7
391 van Wijk AJ, and Tan SP. 2006. A numeric code for identifying patterns of human tooth
392   agenesis: a new approach. *Eur J Oral Sci* 114:97-101. 10.1111/j.1600-0722.2006.00340.x
393 von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, and Vandenbroucke JP. 2008. The
394   Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)
395   statement: guidelines for reporting observational studies. *J Clin Epidemiol* 61:344-349.
396   10.1016/j.jclinepi.2007.11.008
397 Zandi M, Shokri A, Malekzadeh H, Amini P, and Shafiey P. 2015. Evaluation of third molar
398   development and its relation to chronological age: a panoramic radiographic study. *Oral*
399   *Maxillofac Surg* 19:183-189. 10.1007/s10006-014-0475-0

Figure 1

Distribution of individuals with different number of missing third molars (x-axis) in the agenesis and the control group.

Distribution of missing teeth across quadrant and tooth number.

1 **Table 1** Distribution of missing teeth across quadrant and tooth number.

Tooth number*	Upper right (%)	Upper left (%)	Lower right (%)	Lower left (%)	Total (%)
Agenesis group					
1	3 (0.25)	2 (0.2)	29 (2.4)	30 (2.5)	64 (5.3)
2	85 (7)	83 (6.8)	15 (1.2)	18 (1.5)	201 (16.5)
3	14 (1.2)	13 (1.1)	5 (0.4)	4 (0.3)	36 (3)
4	20 (1.6)	21 (1.7)	15 (1.2)	15 (1.2)	71 (5.8)
5	60 (4.9)	52 (4.3)	117 (9.6)	117 (9.6)	346 (28.4)
6	5 (0.4)	4 (0.3)	9 (0.7)	7 (0.6)	25 (2.1)
7	13 (1.1)	14 (1.2)	15 (1.2)	14 (1.2)	56 (4.6)
8	101 (8.3)	105 (8.6)	104 (8.5)	108 (8.9)	418 (34.3)
Total	301 (24.7)	294 (24.2)	309 (25.4)	313 (25.7)	1217 (100)
Control group					
8	37 (25.7)	32 (22.2)	39 (27.1)	36 (25)	144 (100)

2 *Tooth number 1 stands for all central incisors (11, 21, 31, 41) and so on. For example, Upper right 1 is
3 tooth 11 in the FDI system.

Frequency tables that show single tooth agenesis and the prevalence of right sided, left sided or bilateral agenesis in the whole sample (n = 606).

1 **Table 2** Frequency tables that show single tooth agenesis and the prevalence of right sided, left
2 sided or bilateral agenesis in the whole sample (n = 606).

Maxilla

Tooth number*	Present bilaterally (%)	Missing right side (q1) (%)	Missing left side (q2) (%)	Missing unilaterally (%)	Missing bilaterally (%)
Agenesis group					
1	300 (99.0)	1 (0.3)	0 (0.0)	1 (0.3)	2 (0.7)
2	194 (64.0)	26 (8.6)	24 (7.9)	50 (16.5)	59 (19.5)
3	286 (94.4)	4 (1.5)	3 (1.0)	7 (2.5)	10 (3.3)
4	277 (91.4)	5 (1.7)	6 (2.0)	11 (3.7)	15 (5.0)
5	234 (77.2)	17 (5.6)	9 (3.0)	26 (8.6)	43 (14.2)
6	297 (98.0)	2 (0.7)	1 (0.3)	3 (1.0)	3 (1.0)
7	287 (94.7)	2 (0.7)	3 (1.0)	5 (1.7)	11 (3.6)
8	185 (61.1)	13 (4.3)	17 (5.6)	30 (9.9)	88 (29.0)
Control group					
8	260 (85.8)	11 (3.6)	6 (2.0)	17 (5.6)	26 (8.6)

Mandible

Tooth number	Present bilaterally (%)	Missing right side (q4) (%)	Missing left side (q3) (%)	Missing unilaterally (%)	Missing bilaterally (%)
Agenesis group					
1	268 (88.4)	5 (1.7)	6 (2.0)	11 (3.7)	24 (7.9)
2	281 (92.7)	4 (1.3)	7 (2.3)	11 (3.6)	11 (3.6)
3	297 (98.0)	2 (0.7)	1 (0.3)	3 (1.0)	3 (1.0)
4	284 (93.7)	4 (1.3)	4 (1.3)	8 (2.6)	11 (3.6)
5	146 (48.2)	40 (13.2)	40 (13.2)	80 (26.4)	77 (25.4)
6	292 (96.4)	4 (1.3)	2 (0.7)	6 (2.0)	5 (1.7)
7	283 (93.4)	6 (2.0)	5 (1.7)	11 (3.7)	9 (3.0)
8	179 (59.1)	16 (5.3)	20 (6.6)	36 (11.9)	88 (29.0)
Control group					
8	255 (84.2)	12 (4.0)	9 (3.0)	21 (7.0)	27 (8.9)

3 *Tooth number 1 stands for all central incisors (11, 21, 31, 41) and so on. For example, Upper right 1 is
4 tooth 11 in the FDI system.

Most common tooth agenesis patterns in the agenesis group excluding third molars.

1 **Table 3** Most common tooth agenesis patterns in the agenesis group excluding third molars.

	Frequency (%)	Missing teeth		Frequency (%)	Missing teeth
Maxilla			**Mandible**		
1	40/173 (23.1)	12, 22	1	58/208 (27.9)	35, 45
2	22/173 (12.7)	15, 25	2	36/208 (17.3)	45
3	21/173 (12.1)	12	3	34/208 (16.3)	35
4	20/173 (11.6)	22	4	10/208 (4.8)	31, 41
5	11/173 (6.4)	15	5	5/208 (2.4)	34, 35, 44, 45
					or
					32, 42
Overall	114/173 (65.9)		Overall	143/208 (68.8)	
Whole dentition					
1	34/303 (11.2)			12, 22	
2	31/303 (10.2)			35, 45	
3	29/303 (9.6)			45	
4	27/303 (9.0)			35	
5	18/303 (6.0)			22	
Overall	139/303 (45.9)				

Symmetry of tooth agenesis patterns

1   **Table 4** Symmetry of tooth agenesis patterns.

Comparison			Symmetry I (%)	Symmetry II (%)
Maxilla	Right vs. left side	No 3rd - Agenesis	26.7	46.8
		3rd - Agenesis	29.0	74.6
		3rd - Control	8.6	60.5
Mandible	Right vs. left side	No 3rd - Agenesis	30.0	43.8
		3rd - Agenesis	29.0	71.0
		3rd - Control	8.9	56.3
Left side	Upper left vs. lower left	No 3rd - Agenesis	6.9	8.8
		3rd - Agenesis	24.1	52.1
		3rd - Control	6.6	41.7
Right side	Upper right vs. lower right	No 3rd - Agenesis	7.9	9.8
		3rd - Agenesis	24.4	56.5
		3rd - Control	8.6	52.0
Crossed q1 vs q3	Upper right vs. lower left	No 3rd - Agenesis	6.9	8.71
		3rd - Agenesis	22.1	47.2
		3rd - Control	6.6	37.7
Crossed q2 vs q4	Upper left vs. lower right	No 3rd - Agenesis	7.6	9.5
		3rd - Agenesis	24.4	54.8
		3rd - Control	6.6	39.2

2   Symmetry I: percentage relative to the whole sample ($n = 303$) without considering the patterns of no
3   missing teeth as symmetrical. Symmetry II: percentage relative to subsample of subjects with missing
4   teeth in the respective area (i.e. maxilla, mandible etc.).