Universidad Europea

GRADUATION PROJECT

Degree in Dentistry

A POPULATION-BASED STUDY OF DENTAL ANATOMICAL VARIATIONS AND THEIR POTENTIAL CLINICAL SIGNIFICANCE

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SUMMARY

Introduction: Dental morphological traits vary across human populations and may reflect underlying genetic differences. Non-metric crown traits, such as the Carabelli cusp and molar cusp number, have been identified as potential population markers in dental anthropology; **Objectives**: This study aimed to assess the prevalence and distribution of non-metric dental morphological traits and to determine whether geographic origin significantly influences their expression in a diverse population sample; **Material and Methods:** A cross-sectional observational study was conducted at the European University of Madrid. Participants underwent a standardized visual intraoral examination, and multi-generational genetic background data were collected via questionnaire. Statistical associations were evaluated using Pearson's Chi-square test and Fisher's exact test; **Results:** Significant associations were found between geographic origin and several maxillary traits, particularly the Carabelli cusp and molar cusp number. Central European participants displayed greater morphological complexity than Mediterranean participants. No significant variation was identified for mandibular teeth; **Conclusions**: Genetic background, assessed through a multi-generational approach, influences maxillary dental crown morphology. These findings support the clinical relevance of population-specific dental traits in preventive and forensic dentistry contexts. Further research with larger and more balanced samples is needed to confirm these results.

KEYWORDS

Dentistry, Dental anatomy, non-metric traits, genetic background, geographic origin.

RESUMEN

Introducción: Los rasgos morfológicos dentales varían entre las distintas poblaciones humanas y pueden reflejar diferencias genéticas subyacentes. Los rasgos no métricos de la corona, como la cúspide de Carabelli y el número de cúspides molares, se han identificado como posibles marcadores poblacionales en la antropología dental; **Objetivos**: El objetivo de este estudio fue evaluar la prevalencia y la distribución de los rasgos morfológicos dentales no métricos y determinar si el origen geográfico influyen de manera significativa en su expresión en una muestra poblacional diversa; **Material y métodos:** Se llevó a cabo un estudio observacional transversal en la Universidad Europea de Madrid. Los participantes se sometieron a un examen intraoral visual estandarizado y se recopilaron datos sobre los antecedentes genéticos multigeneracionales mediante un cuestionario. Las asociaciones estadísticas se evaluaron utilizando la prueba de chi-cuadrado de Pearson y la prueba exacta de Fisher; **Resultados:** se encontraron asociaciones significativas entre el origen geográfico y varios rasgos maxilares, en particular la cúspide de Carabelli y el número de cúspides molares. Los participantes de Europa Central mostraron una mayor complejidad morfológica que los participantes mediterráneos. No se identificó ninguna variación significativa en los dientes mandibulares; **Conclusiones:** El bagaje genético, evaluado mediante un enfoque multigeneracional, influye en la morfología de la corona dental maxilar. Estos hallazgos respaldan la relevancia clínica de los rasgos dentales específicos de cada población en los contextos de la odontología preventiva y forense. Se necesitan más investigaciones con muestras más amplias y equilibradas para confirmar estos resultados.

PALABRAS CLAVE

Odontología, Anatomía dental, rasgos no métricos, bagaje genético, origen geográfico.

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1. INTRODUCTION

1.1. Dental anatomical variations

Every individual is unique — in skin tone, hair color, height, facial features, and morphology. Yet, beyond these visible differences, there are also subtle variations hidden in our mouths.
Although humans generally share the same dental formula — with incisors, canines, premolars, and molars — dental anatomy itself can vary from one person to another.(1)

Could changes on the genetic background influence prevalence of these dental traits, and if so, what are the implications for clinical practice in Spain or other countries?

In this study, we will focus specifically on variations in the **crown morphology** of teeth so-called non-metric anatomical dental variations.
Eight main dental non-metric anatomical variations will be examined: **the cusp of Carabelli, conoid teeth** (particularly in lateral incisors), **prominent cingulum**, a**genesis, dens invaginatus** (also known as *dens in dente*), **Microdontia Unitary** and the **number of cusps in molars** (Maxillary and Mandibular) and **secondary premolars** (Mandibular). All of them have in common that they are easily observable without any intra-oral manipulation or procedure which could involve a particular risk to the person or discomfort. The analyzed dental variations are listed below: (1)(2–4)

- **Cusp of Carabelli** (also called the tubercle of Carabelli or fifth cusp) is more frequently observed on maxillary first molars and is located on the mesiolingual surface of the mesiolingual cusp. The cusp ridge of the fifth cusp is situated approximately 2 mm cervically to the cusp ridge of the mesiolingual cusp. Its presence is variable and depends on the individual. If the fifth cusp is present, its cusp angle will be sharper than the mesiolingual cusp.

  Moreover, the cusp of Carabelli, due to its morphology, can create additional grooves that are difficult to clean and can lead to caries development.(5)(6)
- **Conoid Teeth** represent a morphological anomaly in which the tooth—most commonly the maxillary lateral incisor—is smaller and shorter, with a pointed, conical shape. This can present both aesthetic and functional issues associated with occlusion defects or biting pattern.

- **Prominent cingulum** refers to a noticeable bulge on the lingual or palatal surface of a tooth, particularly in anterior teeth. The presence of a cingulum is normal and common, although in some cases it may be abnormally pronounced leading to gingival attachment problems.
- **Dental agenesis** is defined as the congenital absence of one or more permanent tooth (or teeth). This condition can occur in isolation or in association with syndromes such as cleft lip and palate or Down syndrome and may result from genetic or environmental factors. In general, it has been described that can have some aesthetic or occlusion problems. The most common agenesis is linked to the third molars.
- **Dens Invaginatus** (dens in dente) is a development anomaly of the enamel, characterized by an invagination of the enamel. The depth and morphology of the invagination can vary considerably, which makes its diagnosis challenging. It starts at the crown and can extend to the root.
- **Microdontia unitary** is where a single tooth is affected with microdontia. The tooth is smaller than normal. It most commonly affects maxillary lateral incisors and mandibular 3rd molars. It has been described that can affect to aesthetic and occlusion issues.
- **Number of cusps in molars** (maxillary and mandibular), In normal dental anatomy, the maxillary first molar typically presents four to five cusps (with the fifth cusp corresponding to the cusp of Carabelli), while the maxillary second molar usually has three to four cusps. In mandibular molars, the first molar exhibits five cusps, whereas the second molar presents four. In the cases of changes in cusp numbers does not really have a major clinical relevance but due to the size reduction of some cusp or the presence of extra ones can be associated with occlusion issues or increment on caries risk due to the presence of extra grooves.
- **Number of cusps in secondary premolars** (mandibular), this tooth has two common forms: the three-cusp type (the most common) (1) which present an angular occlusal aspect and the two-cusp type who is more rounded.

1.2. Genetic Background

Having established the dental anatomical variations examined in this study, it is essential to consider the larger biological and population-based factors that may influence their expression. Among these factors, genetic background has been widely discussed in the literature as a potential determinant and influencing factor of morphologic dental traits.(5)

Genetic and geographic variations in dental anatomic traits have been extensively documented in recent literature. For example, the cusp of Carabelli remains one of the most studied traits, with a prevalence of 87% in the Bengaluru (India) population, predominantly bilateral. In contrast, its frequency drops to 23% in Lahore (Pakistan) and 57.6% in Saudi Arabia, where it is often expressed as a groove rather than a fully developed cusp. (7)(8)(9)

Conoid lateral maxillary incisors, often associated with microdontia, show a strong genetic link, particularly with mutations in MSX1, PAX9, DLX1 and DLX2. These anomalies are frequently reported in Asian and Middle Eastern populations, where conoid lateral incisors are a common feature.(10,11).

Dental agenesis (excluding third molars) affects 1.6-9.6% of individuals globally, with higher rates in Europe (males 4.6%, females 6.3%) and Australia (males 5.5%, females 7.6%) compared to North America. In Spain, recent data confirm a prevalence of 6.757%, with the mandibular second premolar being the most frequently missing tooth.(12)

The number of cusps in molars varies significantly: in Saudi Arabia, the mandibular first molar most commonly presents a 5 cusps pattern (85%), while in India, the maxillary first molars exhibit 5 cusps in 32.6% of cases and 4 cusps in 67.08%. The mandibular second molar often has 4 cusps, with a “Y” or “+” groove pattern, reflecting evolutionary and genetic diversity. (13,14)

Within the context of this study, the genetic background is not treated as a static or simplistic category. To recognize/define/research the complexity of modern identities, this study goes beyond self-reported physical related characteristics/phenotype by collecting detailed information on the genetic background of participants, as well as those of their parents and grandparents. This multi-generational and geographic approach allows for more nuanced and precise understanding of genetic inheritance patterns and helps mitigate the limitations of self-identification, which can be influenced by cultural or social factors. (6)

Some countries characterized by a rich history of cultural and genetic exchange makes them a particularly interesting region for studying dental morphological diversity. The cultural and demographic exchanges in these regions have become particularly significant in recent decades, driven by increased migration from diverse countries. Spain serves as a notable example of this phenomenon.(15)(16)

By examining a sample of individuals with diverse genetic backgrounds, this study aims to identify whether specific dental traits frequency change or become more prevalent on the population due to the change on the genetic background within Spain or other analyzed countries.

Spain is a prime example of a country that as recently incorporated to its population a considerable number of habitants which different genetic background who came from different countries/continents. This demographic shift makes Spain a particularly relevant region for studying the impact of genetic diversity on dental morphology.

This focus on genetic influence is not merely descriptive but has direct clinical implications. Identifying genetic-specific or population-specific trends in dental anatomy could guide personalized preventive strategies, orthodontic planning, and restoratives approaches, particularly in Spain's increasingly multicultural population.

1.3. Potential clinical significance

While this study is based on a limited sample and mainly composed of individuals linked to the European University of Madrid, the findings could provide valuable insights into the prevalence of specific dental anatomical variations among the Spanish population. Understanding these variations will have direct implications for clinical practice, preventive care and public health strategies in dentistry.

For instance, if the cusp of Carabelli is found to be more prevalent in this sample, it could suggest a higher susceptibility to caries in the first maxillary molars among Spaniards. The cusp of Carabelli, due to its morphology, can create additional grooves that are difficult to clean and can lead to caries development.(17) Dentists in Spain could use this information to emphasize targeted preventive measures, such as sealants or more rigorous oral hygiene instructions for these specific teeth and patients.

Similarly, a higher prevalence of conoid teeth or microdontia could indicate a need for increased awareness among practicians. These anomalies often require interdisciplinary management, including aesthetic restorations or orthodontic interventions, to address both functional and cosmetic concerns.(18)

Furthermore, identifying trends in dental agenesis or dens invaginatus could inform early diagnostic protocols. Dens invaginatus, if left undetected can lead up to pulp necrosis and periapical lesions. Early detection through routine x-rays could prevent complications and reduce the need for more invasive treatments. (19)

Finally, variations in the number of cusps in molars and premolars might influence occlusal patterns and the long-term stability of dental restorations.(20)

In summary, by recognizing the unique anatomical traits of the population, practitioners can tailor their approaches to prevention, diagnosis and treatment, ultimately improving oral health outcome.

2. HYPOTHESIS AND OBJECTIVE

2.1. Null hypothesis

Genetic background does not influence the dental anatomy of people. The country of origin of the participant and its ascendant relatives until the second order has no significant effect on the prevalence of the eight dental anatomical variations studied in this population.

2.2. Alternative hypothesis

Genetic background does influence the dental anatomy of people. The country of origin of the participant and its ascendant relatives until the second order significantly affects the prevalence of one or more of the eight dental anatomical variations studied in this population.

2.3. General Objective

The objective of this study is to explore demographic patterns in the distribution of dental anatomical variations and to assess their relationship with genetic background within a population-based sample.

To determine whether the prevalence, frequency and distribution of specific dental anatomical variations differ among patients with different genetic backgrounds.

PICO question:
Among the general population (P), does the analysis of dental anatomical variations (I), show us different variations in other different country origins groups within the studied population (C), reveal significant differences in their prevalence, and how will it shape the dental demographics in Spain or other countries (O)?

3. MATERIAL AND METHODS

This final research project was designed as a cross-sectional observational analysis dealing with dental anatomical variations in the general population.

The present study has the approval of the Pre-Clinical Department of the Faculty of Biomedical and Health Sciences under code OD.028/2526.

The Research Ethic Committee (CEIm) of the Hospital of Getafe approved this study with minor changes. (ANNEX)

Data collection was carried out through visual examination and two surveys (ANNEX 1) administered via Microsoft Forms, which were displayed in three languages (English, Spanish and French) to ensure participant comprehension.

The first survey (ANNEX) was used as a recruitment and scheduling tool. Teachers and Students attending the University were invited to participate voluntarily in the study and selected a preferred appointment date for the intraoral visual examination. The visual analysis was conducted during the second semester. Examinations were scheduled every Monday and Wednesday, with 20-minutes intervals between participants. The examination was conducted in a room allowed by the Dental Laboratory of the University with the approval of the department.

The second survey (ANNEX) was given on the day of the visual examination and constituted the primary data collection instrument. It collected demographic information, such as age, sex and detailed country of origin or genetic background. To allow a more precise classification of participant's genetic background, information regarding the genetic origin of the participant himself, of both parents and all four grandparents was obtained to ensure a correct and complete information. Including a self-identification with specific human phenotype usually associated with country/region/areas.

To ensure precise linkage between questionnaire responses and visual findings while maintaining confidentiality, each patient was assigned a unique identification code which included one letter and a number, to constitute a letter-number code.

Following completion of the second survey, a standardized visual intraoral examination was performed to determine the presence or absence of specific dental anatomical variations. The

examination was conducted using single-use examination gloves and a flashlight. The analysis was limited to visible anatomical characteristics while avoiding direct manipulation of oral tissues. Although visual examination limits the detection of certain internal abnormalities (e.g., deep dens invaginatus), this method was chosen for its non-invasiveness and feasibility in an academic setting.

Clinical findings collected manually on a table prepared “ad hoc” including the unique identification code and dental data. Results were then entered into a Microsoft Excel Spreadsheet at the end of each data collection day for organization and future statistical analysis.

Prior to the visual examination, participants were provided with an information sheet and a consent form, and verbal consent was obtained. (ANNEX)

4. RESULTS

4.1. Description of the study population

78 patients were contacted through our surveys. Two of them did not give their consent, and 15 gave their consent but were unable to come to the University for visual examination. Six patients gave their consent and took an appointment but did not attend. Finally, 55 patients were included and underwent a visual examination in the dental laboratory.

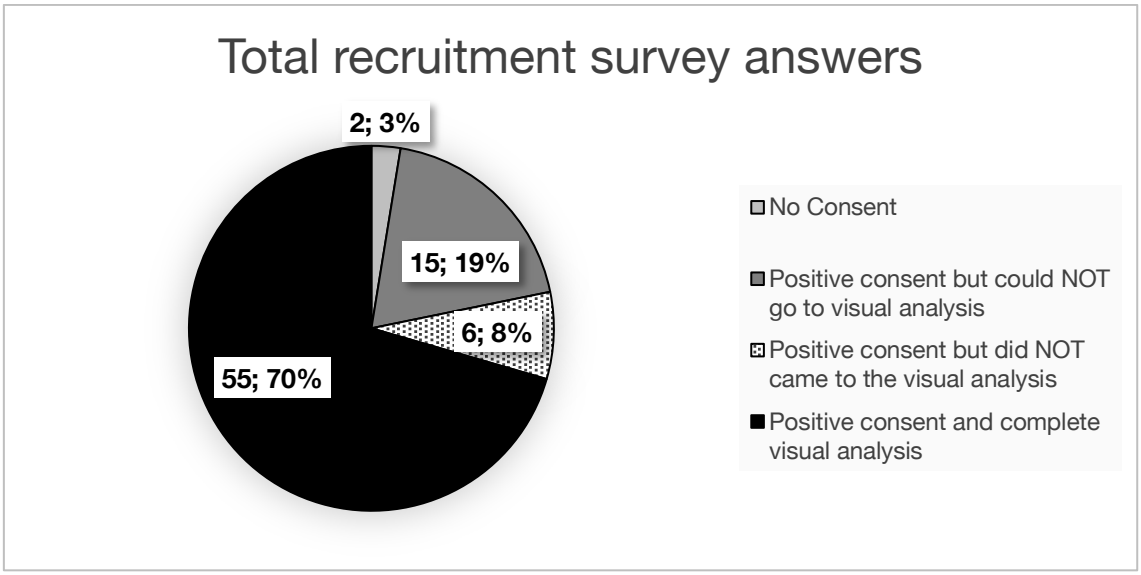


Figure 1 Total recruitments surveys answers

On the examined population (n=55), 36 were females (65%) and 19 were males (35%).

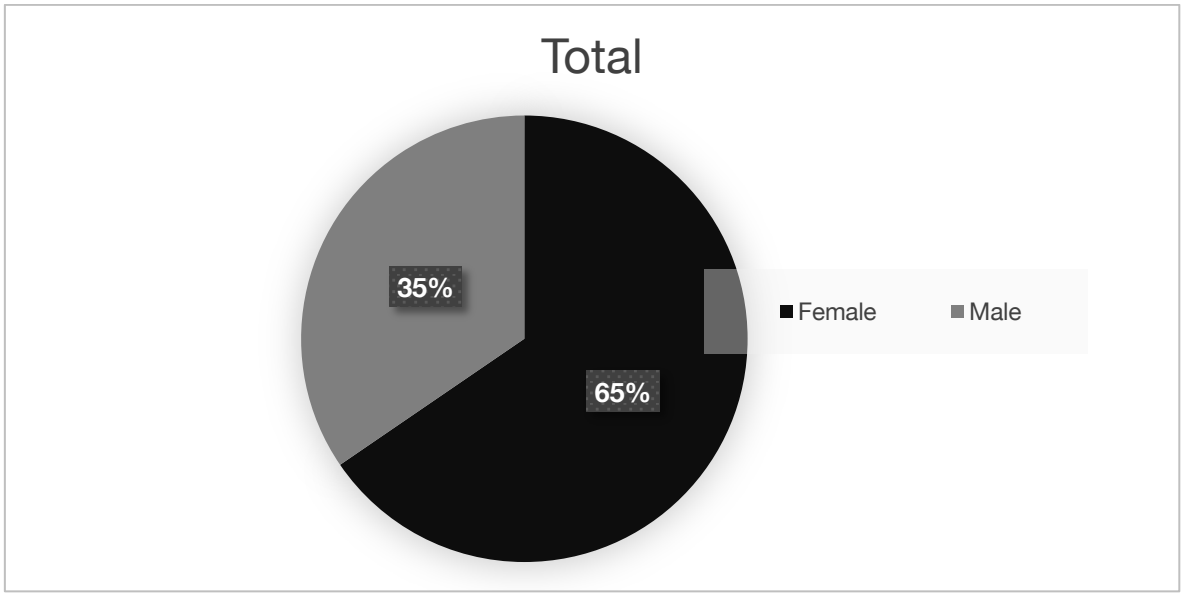


Figure 2 Proportion of males and females in our study

Figure 3 showed that participants were mainly aged between 18 and 34 years (65.4%), with smaller proportions in older age groups.

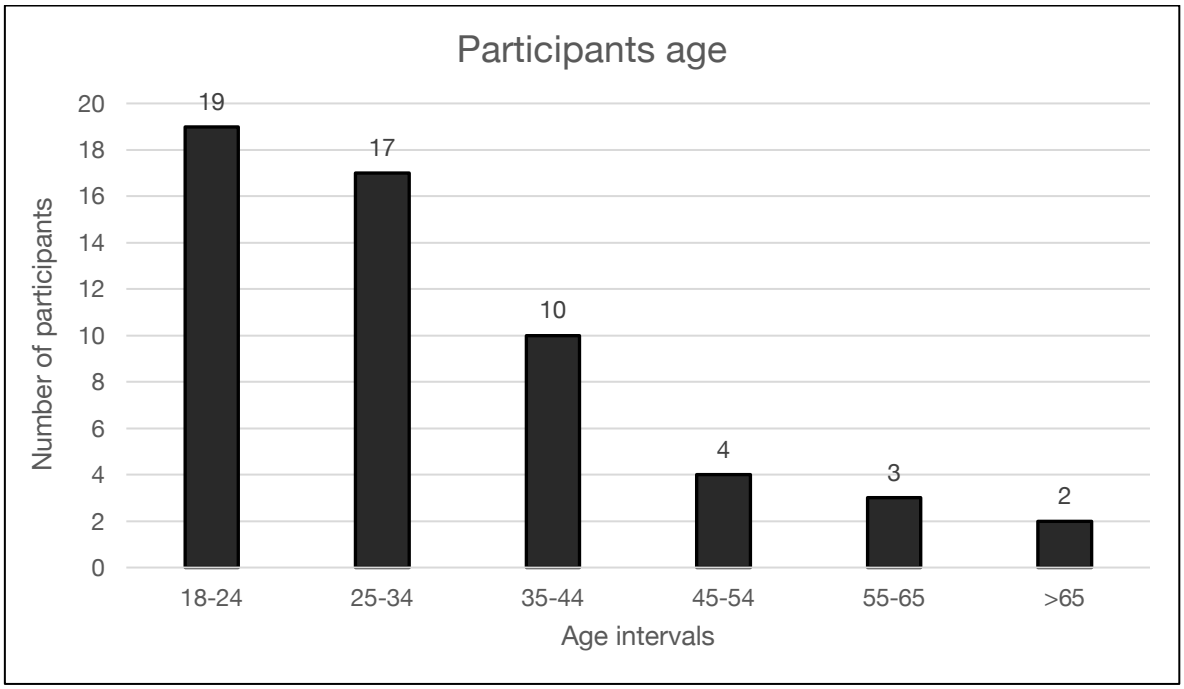


Figure 3 Age of our participants

Figures 4 and 5 showed that most participants originated from France (51%) and Spain (24%), while other countries were represented by small sample sizes.

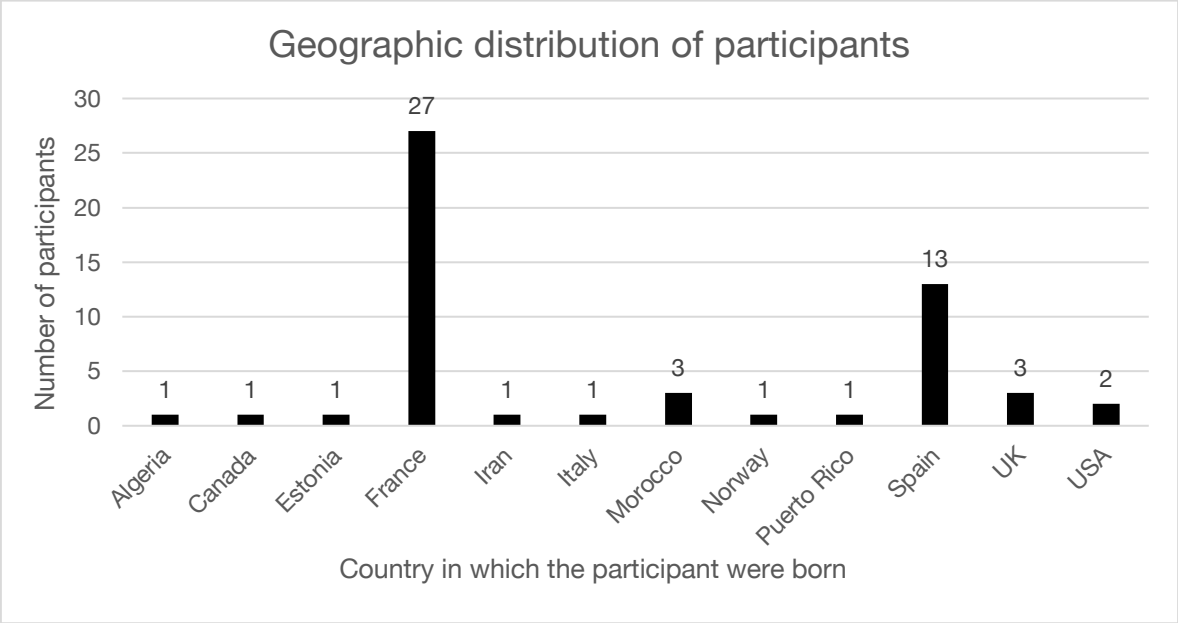


Figure 4 Geographic Distribution of our participants

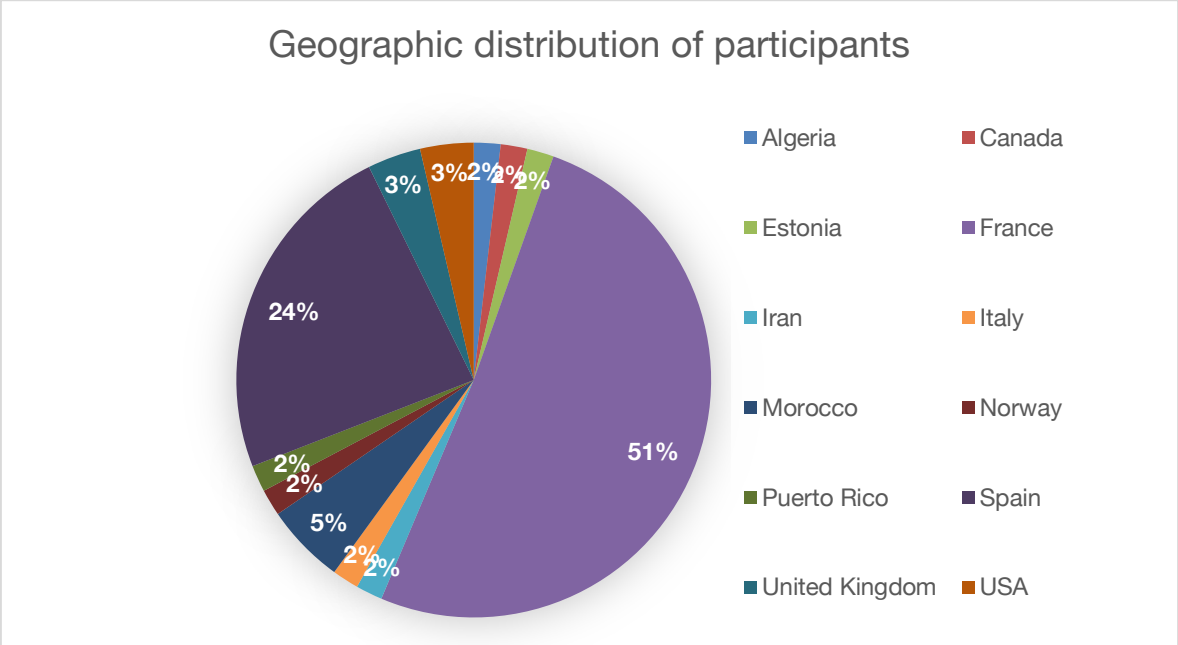


Figure 5 Geographic Origins of our participants

Figure 6 showed that the majority self-identified as Caucasian-European (n = 33), followed by North African/Mediterranean groups (n = 11). Other phenotypes were marginal or mixed.

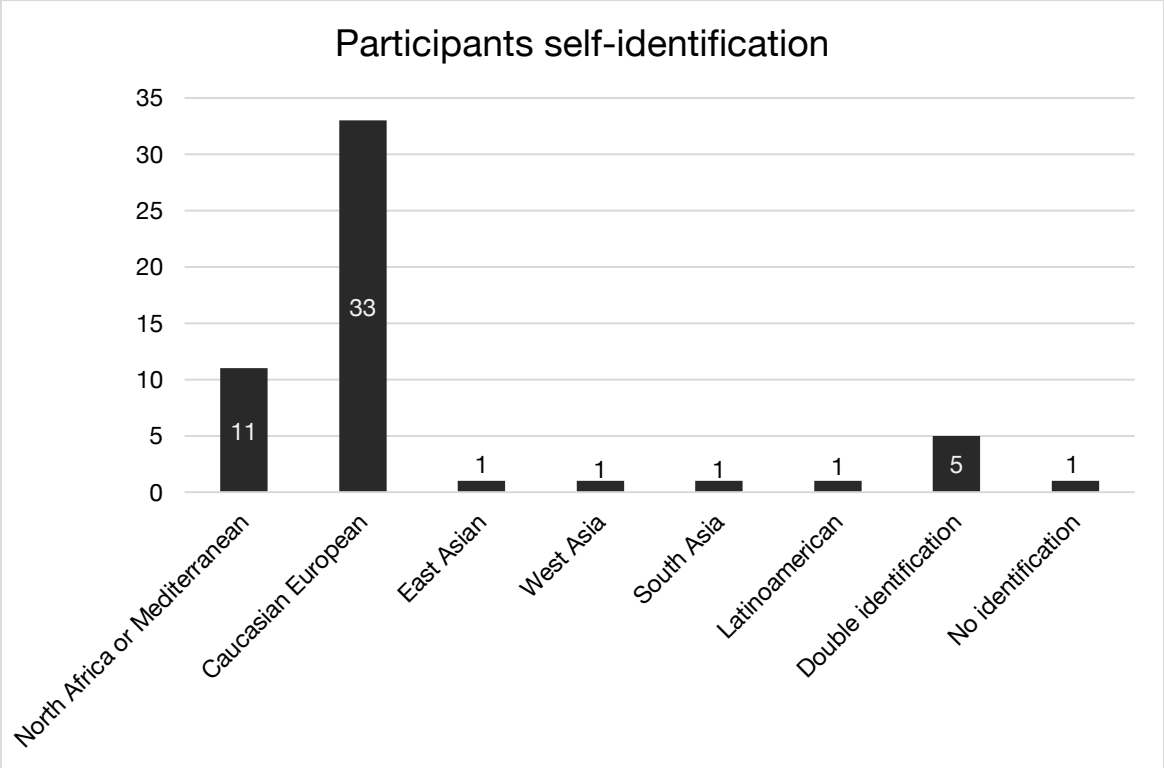
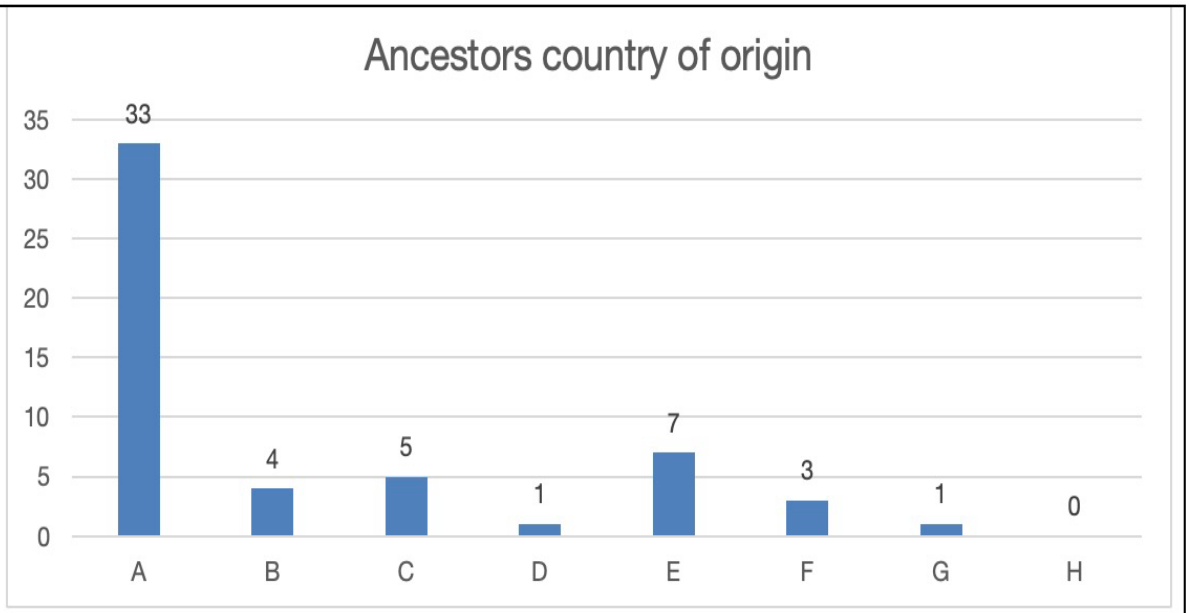


Figure 6 Participants self-identification

The figure 7 showed that most participants (n = 33) and their ancestors originated from the same country (Group A), while smaller groups showed increasing geographic diversity across generations.



A: Participant and ancestors' same country all (n=33)
B: Participant and ancestors' different country but all ancestors' same origin (n=4)
C: Participant and first ancestors' same country, different on grandparents but similar region (n=5)
D: Participant and at least one of the first ancestor's different country with similar geographic area (n=1)
E: Participant and at least one of the first ancestor's different country with similar geographic area (n=7)
F: Participant and first ancestors' same country, different country and region on grandparents (n=3)
G: Participant with unknown parents' origin (n=1)

Figure 7 Ancestors country of origin

Regarding genetic background, Figures 8a and 8b depicted that most participants originated from Central Europe (39%) and Mediterranean regions (36%), with other regions minimally represented.

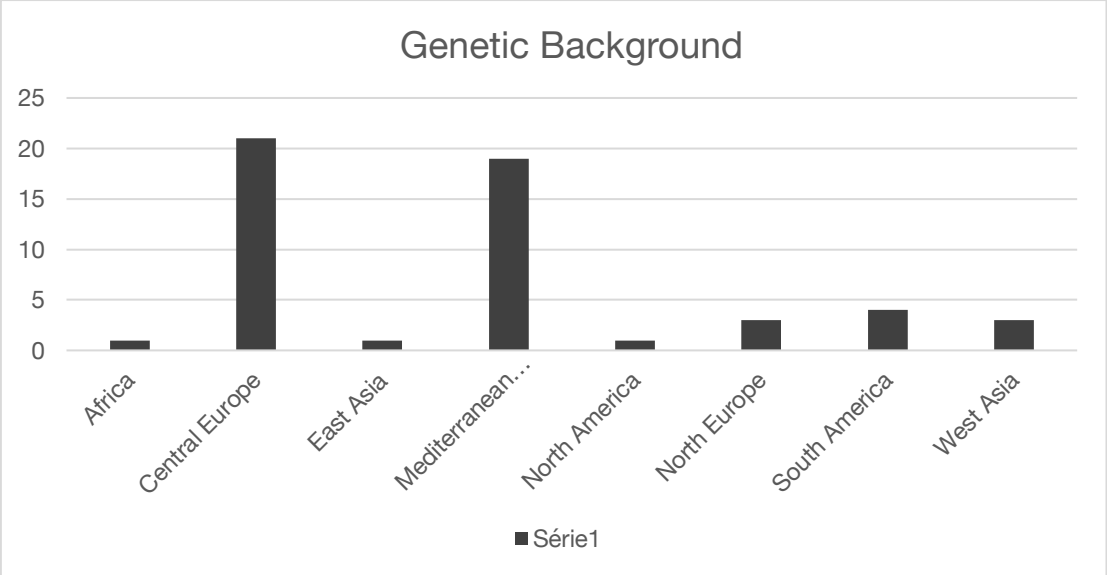


Figure 8a Genetic Background of participants

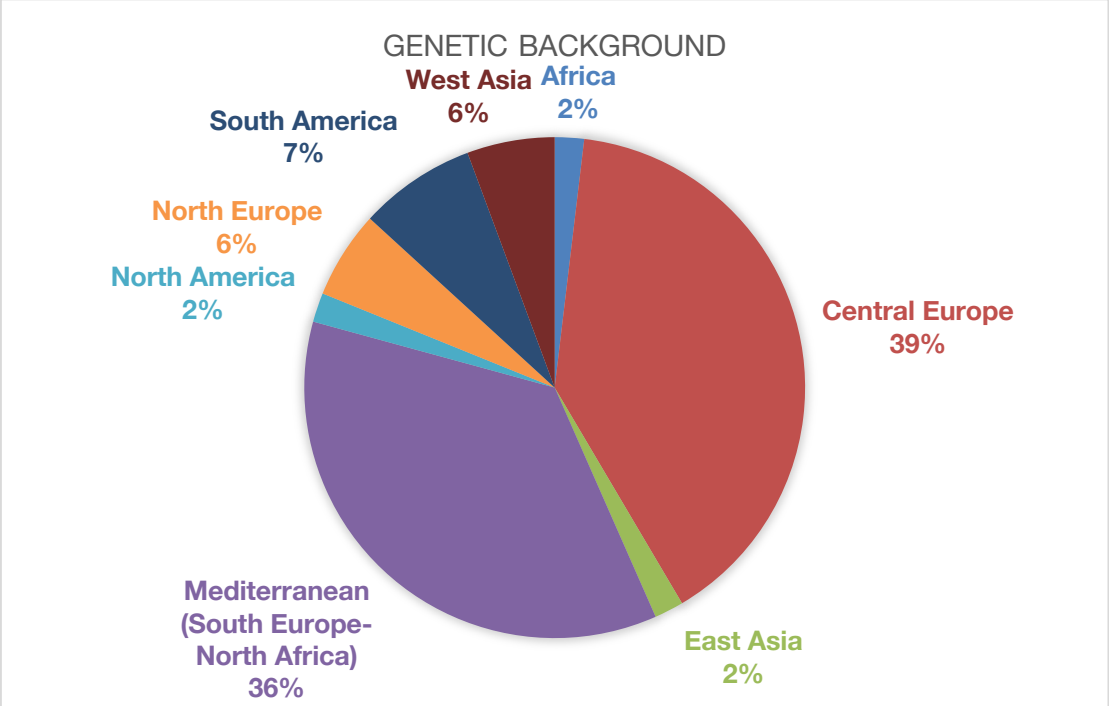


Figure 7b Genetic Background in percentages

4.2. Overall prevalence of dental traits

The most relevant dental traits results are represented below. More detailed analysis between the two most represented countries and sexual dimorphism is presented in the annexes.

A) Cusp of Carabelli

Out of 104 examined maxillary first molars, 36 presented a cusp of Carabelli (also referred to as fifth cusp). A total of 110 maxillary first molars were expected; however, 6 could not be assessed due to either prosthetic crowns or tooth absence, resulting in 104 molars available for analysis. Therefore, among the 104 examined molars, 35% showed the presence of the cusp of Carabelli.

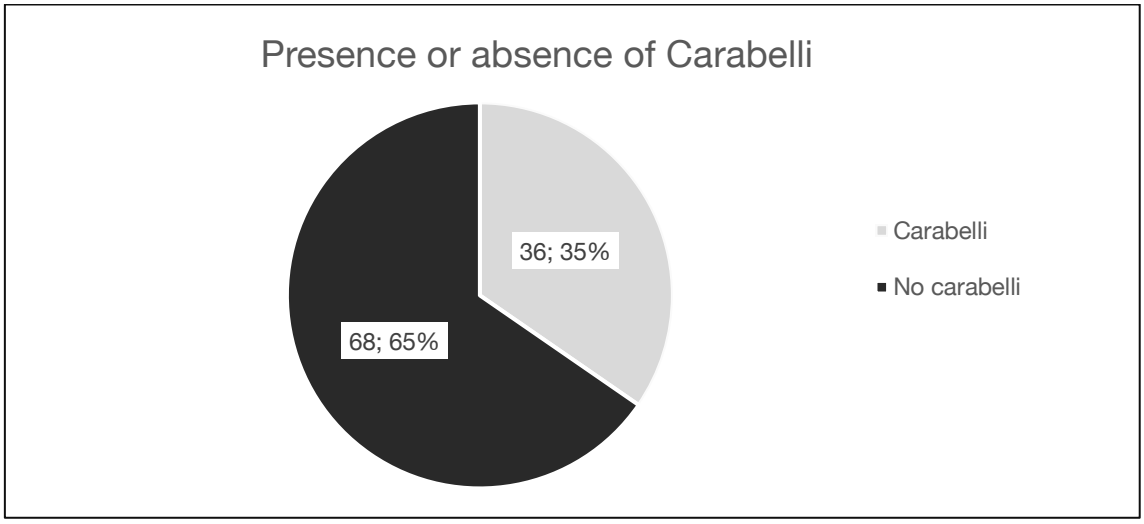


Figure 8 Presence or Absence of Cusp of Carabelli on maxillary first molars

B) Number of cusps on maxillary first molars

Figure 10 showed that most molars had 4 cusps (n = 96), with few presenting 3 (n = 2) or 5 cusps (n = 6).

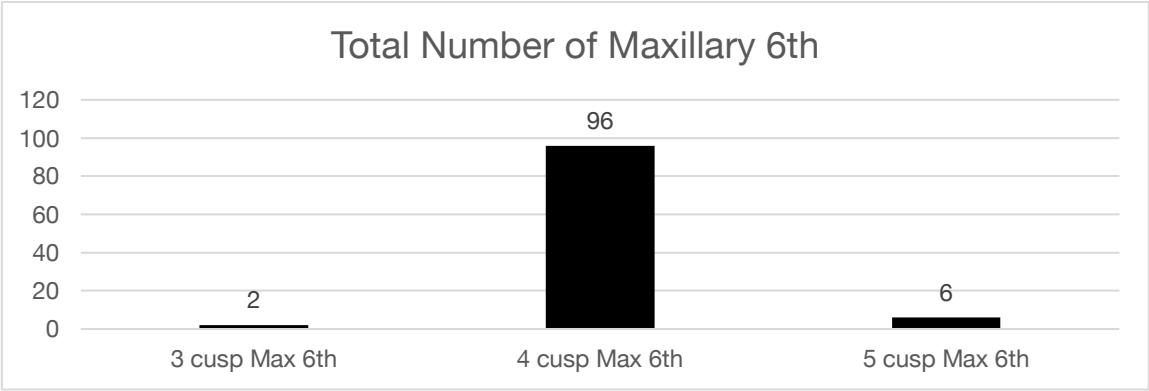


Figure 9 Total number of first maxillary molars

C) Number of cusps on maxillary second molars

In Figure 11, cusp distribution was almost evenly split between 3 and 4 cusps (n = 53 each), with rare 5- cusp cases (n = 4).

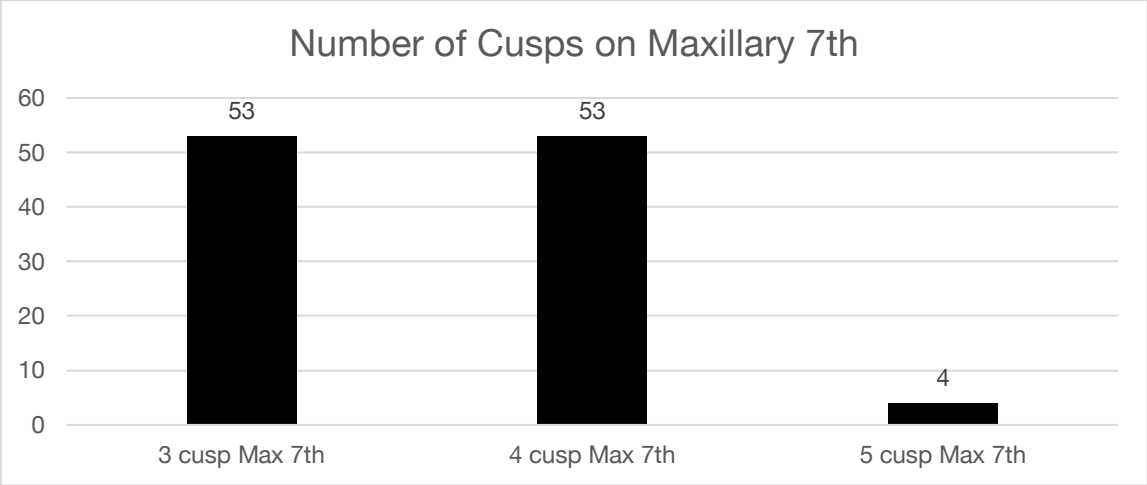


Figure 10 Total number of second maxillary molars

D) Number of cusps on mandibular first molars

In Figure 12, the majority presented 5 cusps (n = 76), followed by 4 cusps (n = 33); one case showed 6 cusps.

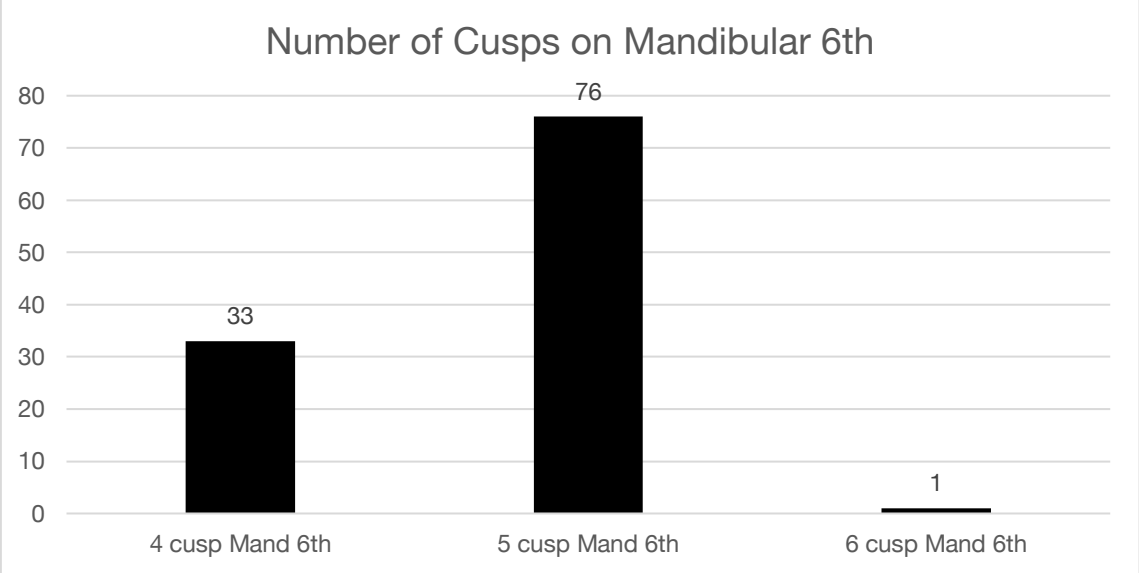


Figure 11 Total number of first mandibular molars

E) Number of cusps on mandibular second molars

In Figure 13, most molars had 4 cusps (n = 92), with fewer showing 5 cusps (n = 18).

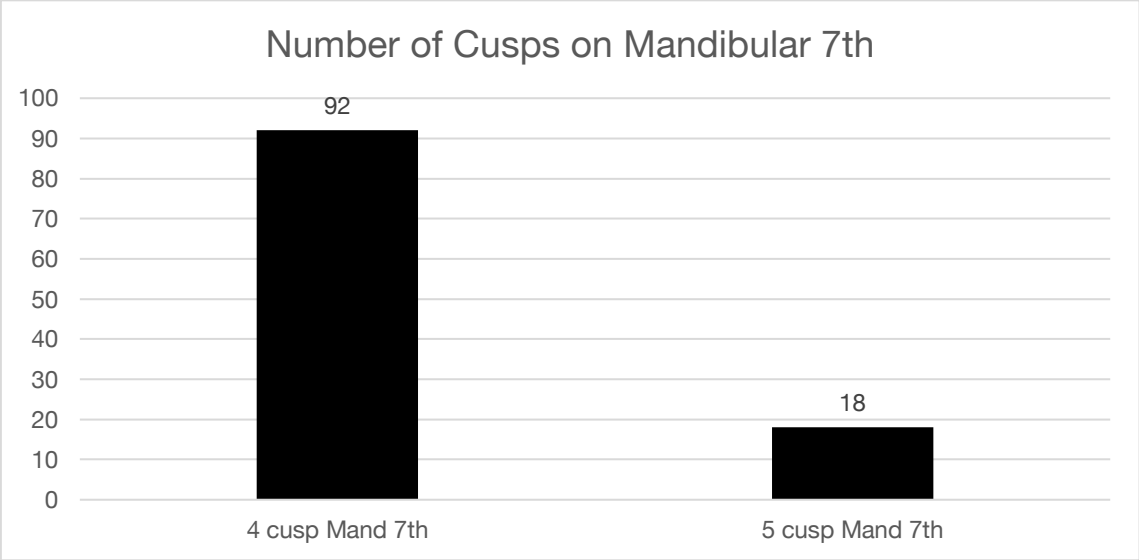


Figure 12 Total number of second mandibular molars

F) Number of cusps on mandibular second premolars

A nearly equal distribution was observed between 2-cusp (n = 60) and 3-cusp types (n = 50).

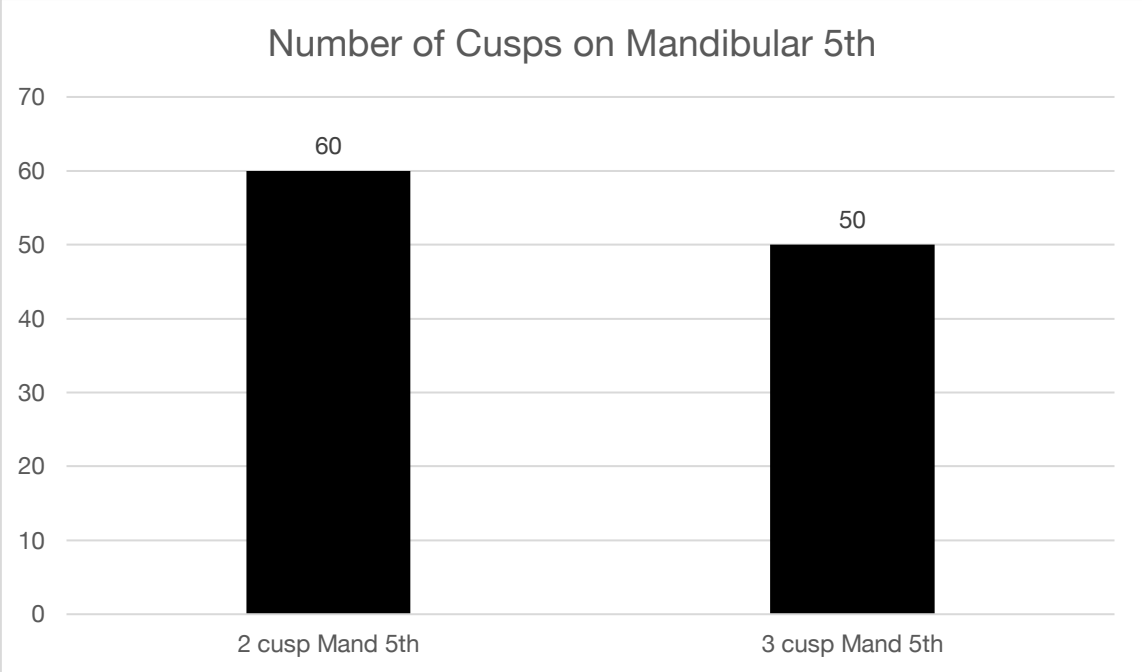


Figure 13 Total number of Mandibular second premolars

G) Participants presenting agenesia

Nine participants out of the 55 presented agenesis.

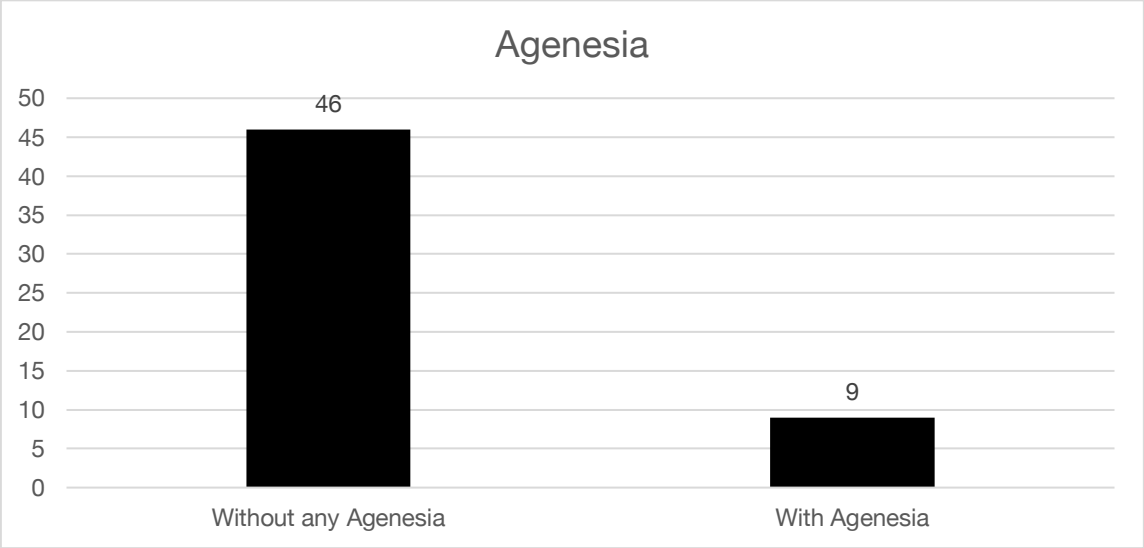


Figure 14 Number of participants presenting dental agenesia

In Figure 16, multiple agenesis (>3 teeth) was the most frequent pattern (n = 4), followed by single (n = 3) and two-teeth agenesis (n = 2). Four third molar agenesis was observed in 3 cases.

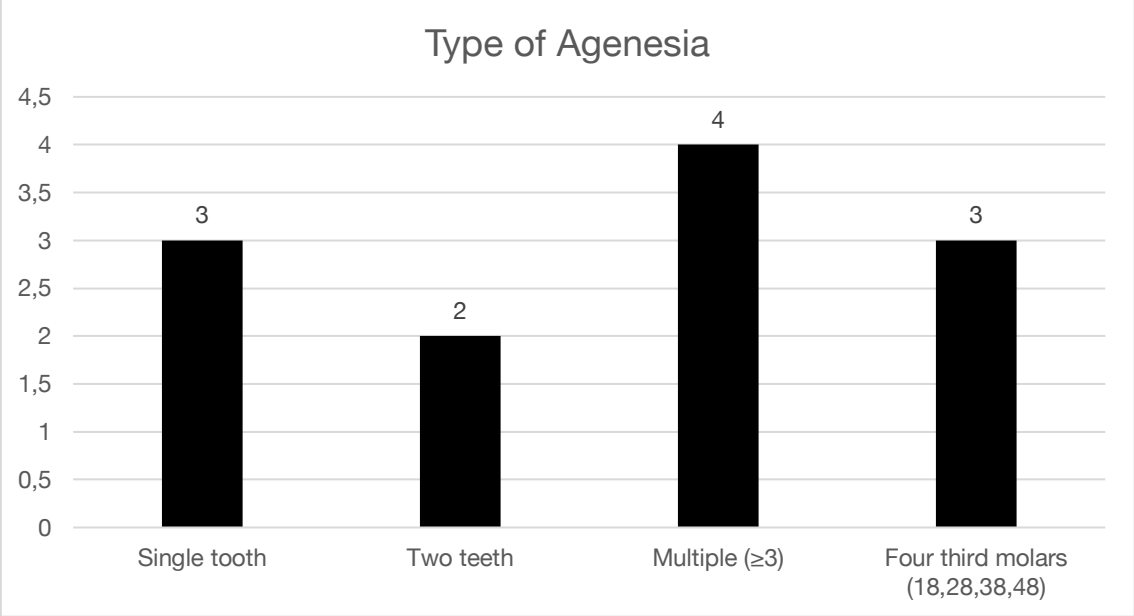


Figure 15 Type of Agenesia among our participants

4.3. Distribution according to geographic origin/genetic background

A) Cusp of Carabelli

In Figure 17, 18 and 19, the presence of the Carabelli cusp varied across countries and regions, with higher frequencies observed in France and Mediterranean populations. Central Europe showed a more balanced distribution between presence and absence.

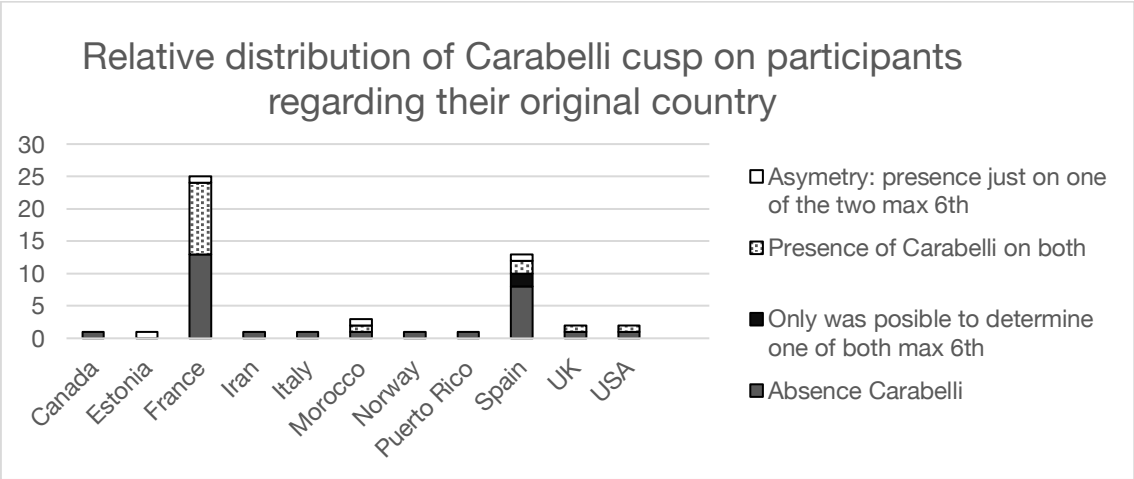


Figure 16 Distribution of the Carabelli cusp among countries of origin

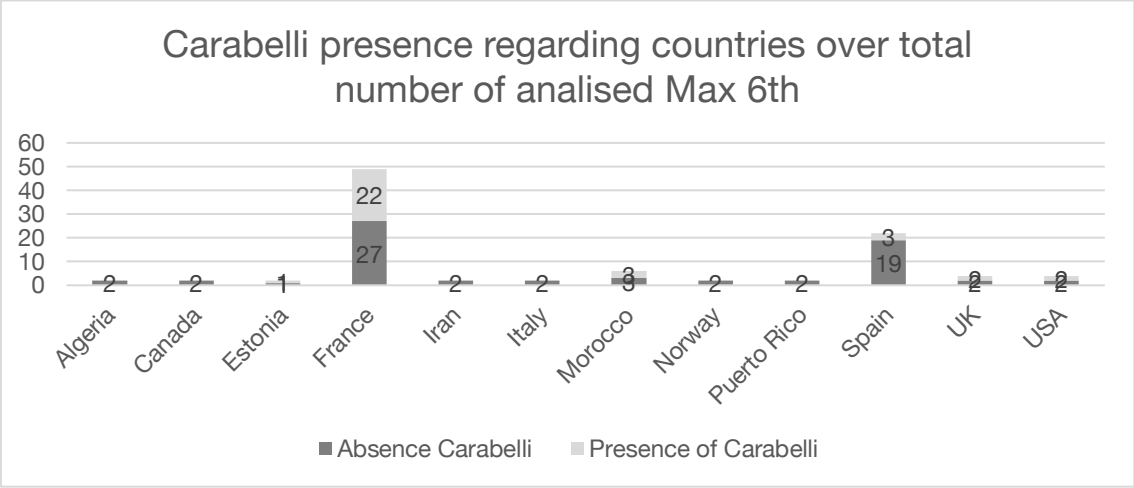


Figure 17 Distribution of Carabelli Cusp depending on the country of Origin

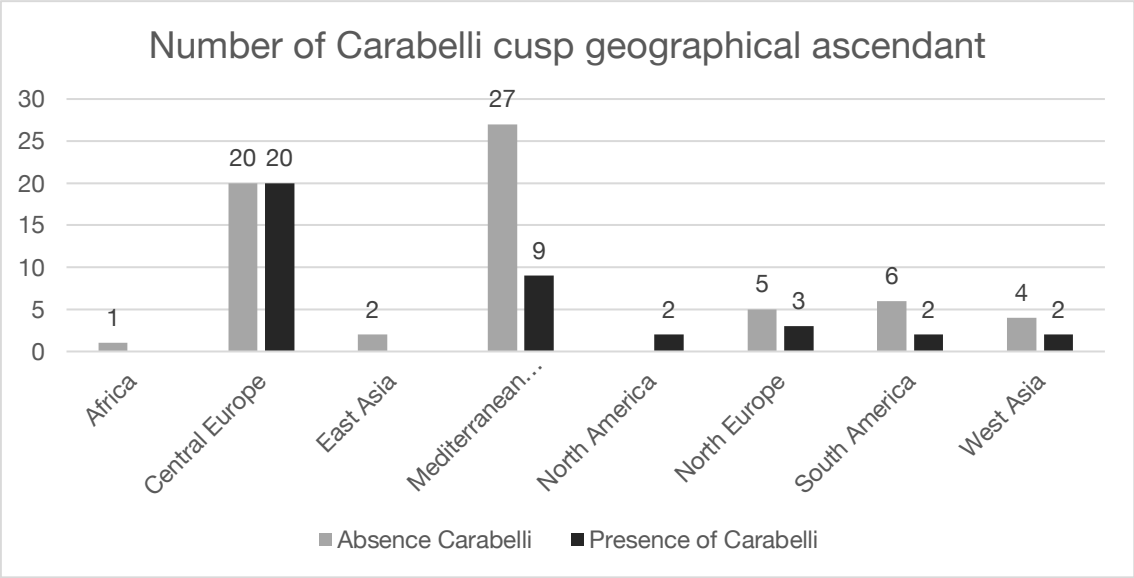


Figure 18 Number of Carabelli cusp according to geographic ascendant

B) Number of cusps on maxillary first molars

In Figure 20, according to country, France and Spain accounted for the most analyzed teeth, with a predominance of the 4-cusp pattern. The 5-cusp configuration remained rare, and other countries were minimally represented.

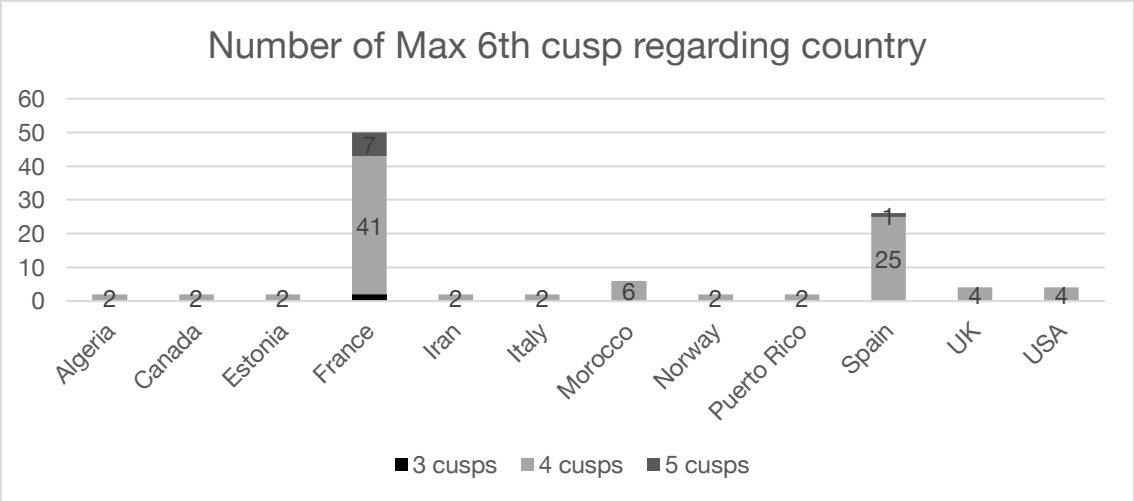


Figure 19 Number of maxillary first molars according to Countries

In Figure 21, according to geographical ascendant, the 4-cusp pattern predominated across all regions, particularly the Mediterranean (n = 51) and Central Europe (n = 31). The 3-cusp configuration was rare, while the 5-cusp pattern was mainly observed in Central Europe.

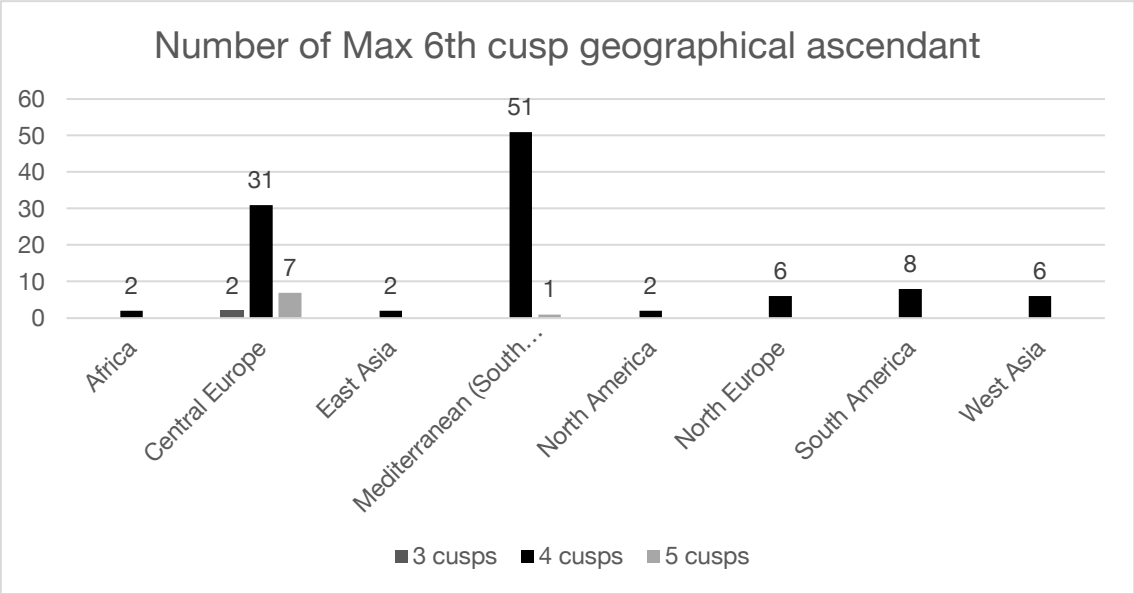


Figure 20 Number of Maxillary first molars according to geographic ascendant

C) Number of cusps on maxillary second molars

In Figure 22, according to country, France and Spain showed the highest number of analyzed teeth, mainly presenting 4 cusps, with some 5-cusps cases.

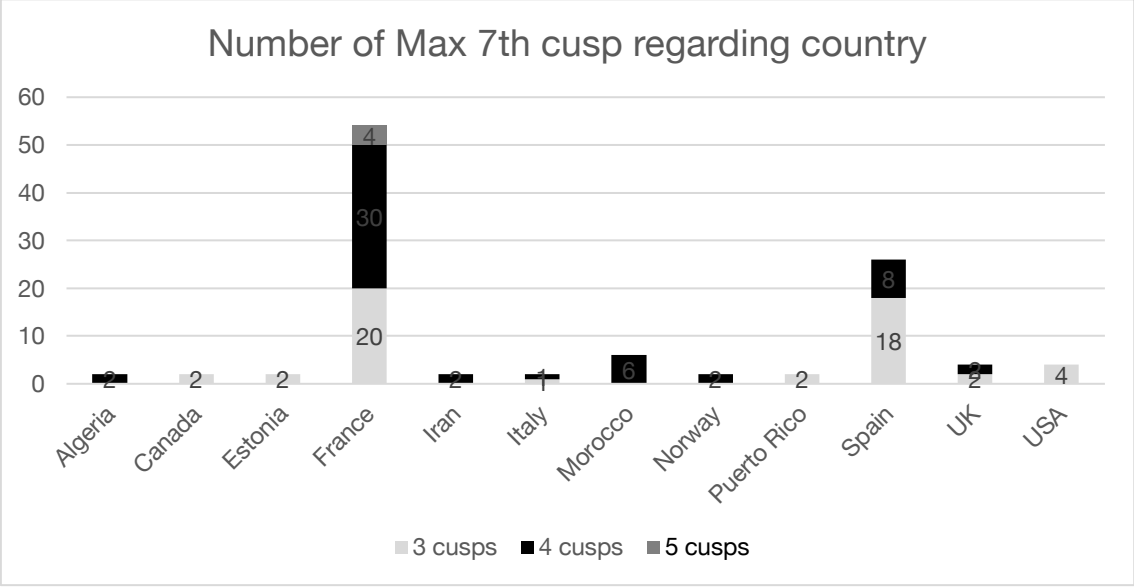


Figure 21 Number of maxillary second molars according to Countries

In Figure 23, according to geographical ascendant, a balanced distribution between 3 and 4 cusps was observed, especially in Central Europe and Mediterranean regions, while other regions had limited representations.

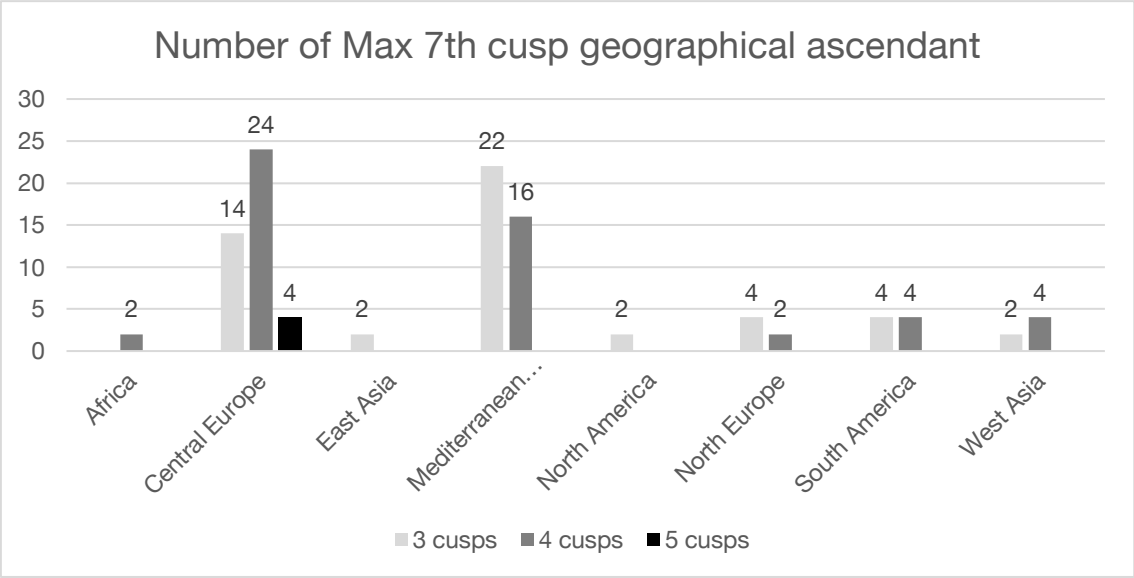


Figure 22 Number of Maxillary second molars according to geographic ascendant

D) Number of cusps on mandibular first molars

In Figure 24, according to country, France and Spain contributed most of the data, with a predominance of the 5-cusp pattern.

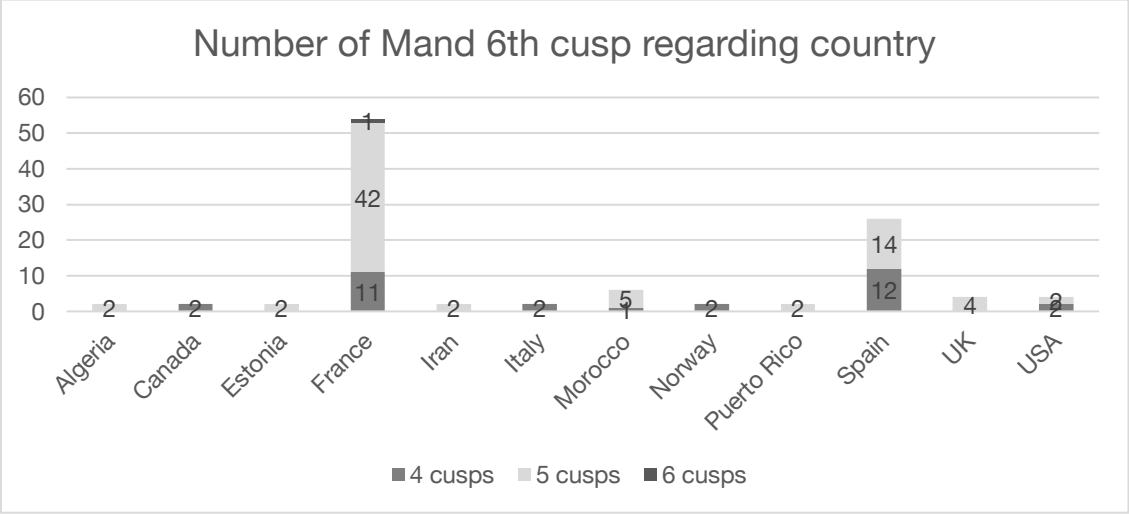


Figure 23 Number of Mandibular first molars according to Countries

In Figure 25, according to geographical ascendant, the 5-cusp configuration was dominant across all regions, particularly in Central Europe and the Mediterranean regions (South Europe-North Africa).

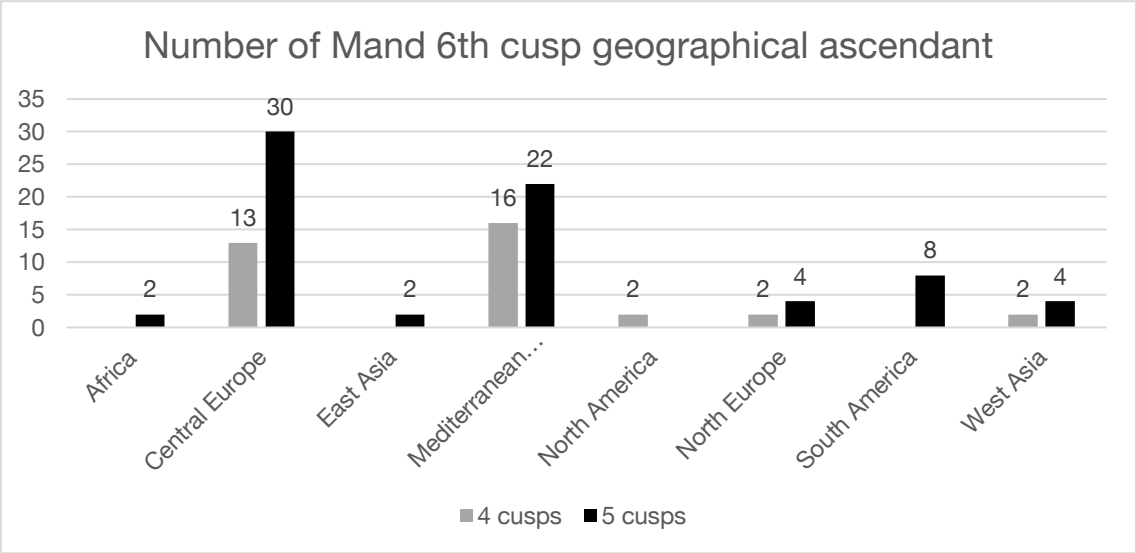


Figure 24 Number of Mandibular first molars according to geographic ascendant

E) Number of cusps on mandibular second molars

In Figure 26, according to country, most molars exhibited 4 cusps, especially in France and Spain, with fewer 5-cusps cases.

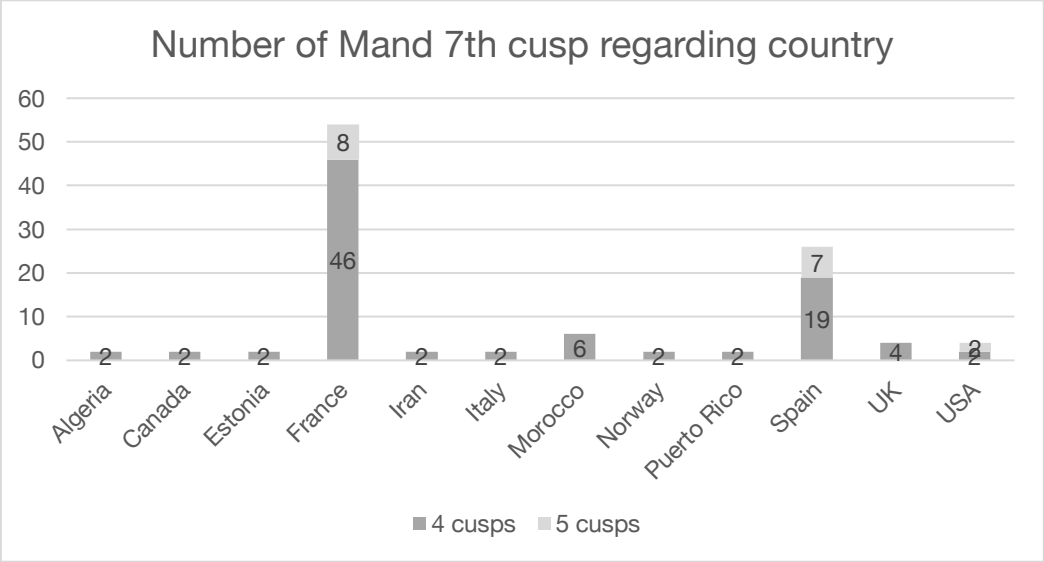


Figure 25 Number of Mandibular second molars according to Countries

In Figure 27, according to geographical ascendant, the 4-cusp pattern predominated in all regions, with limited variability.

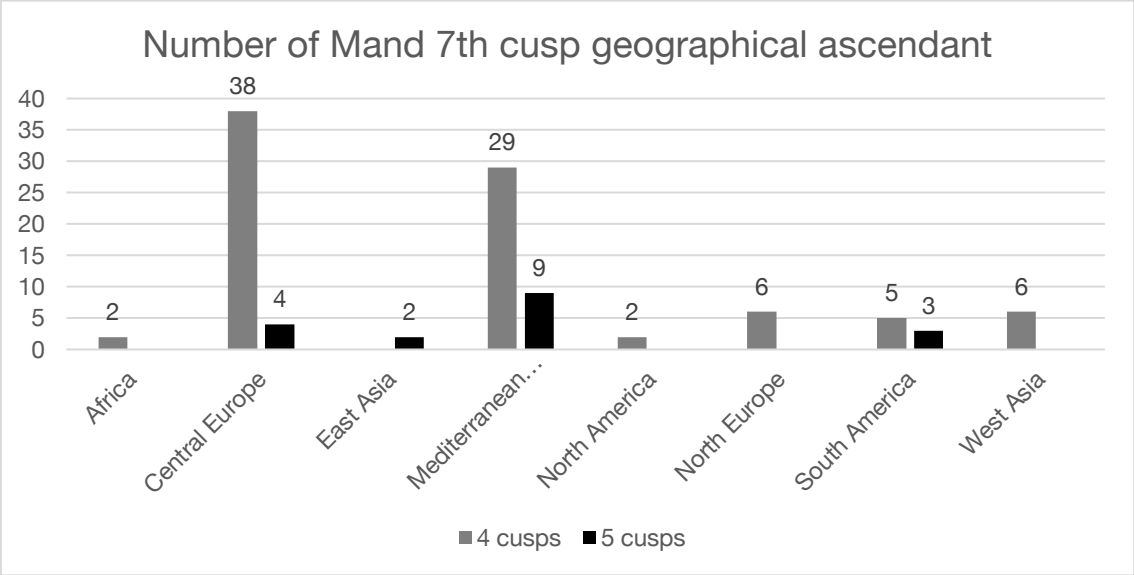


Figure 26 Number of Mandibular second molars according to geographic ascendant

F) Number of cusps on mandibular second premolars

In Figure 28, according to country, France and Spain showed the highest number of analyzed teeth, with both 2- and 3-cusp patterns represented.

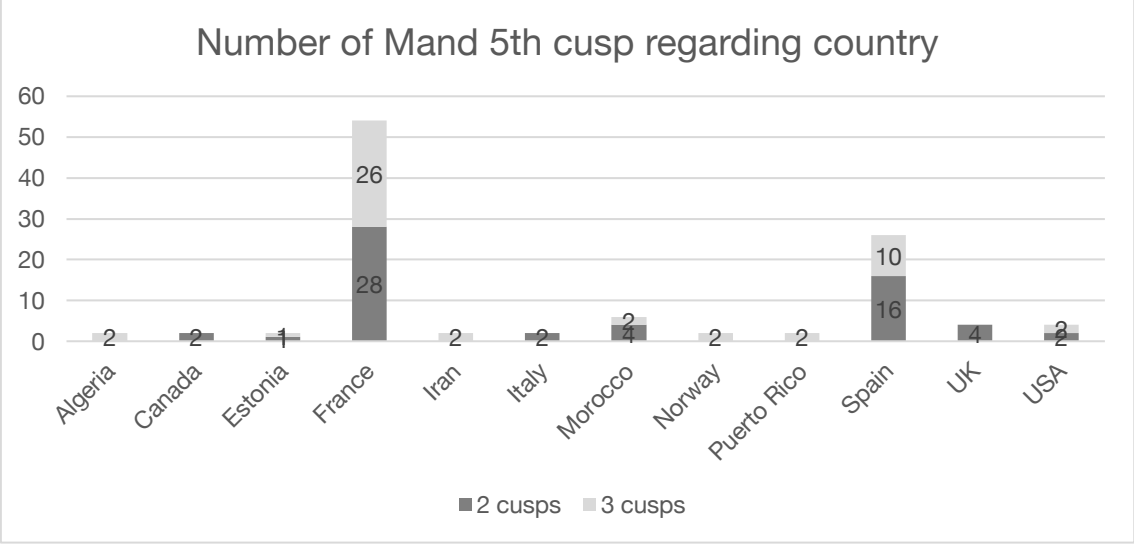


Figure 27 Number of Mandibular second premolars according to Countries

In Figure 29, according to geographical ascendant, both configurations were observed across all regions without a clear predominance.

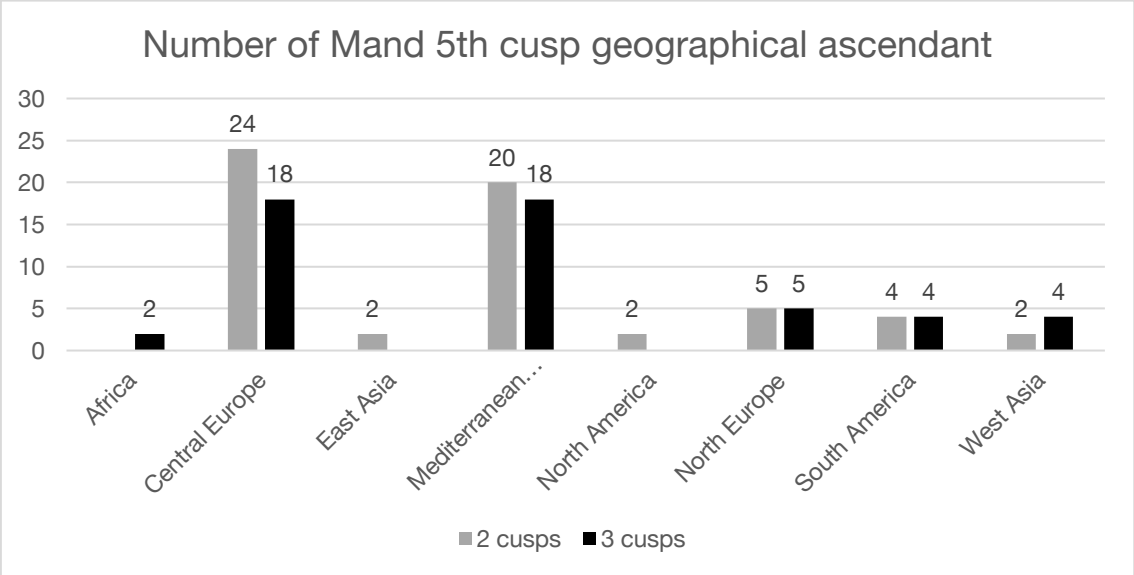


Figure 28 Number of Mandibular second molars according to geographic ascendant

G) Participants presenting dental agenesia

In Figure 30, agenesis was mainly observed in France (n = 7), followed by Italy (n = 2) and Iran (n = 1).

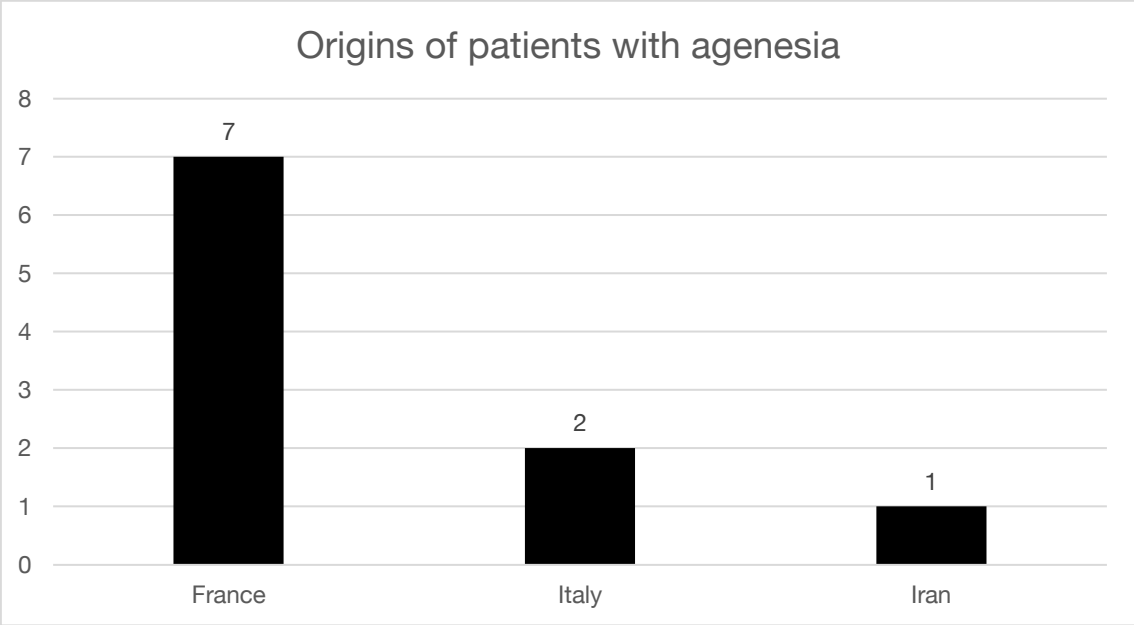


Figure 29 Origins of participants with agenesia

4.4. Statistical analysis

A) Methods

Pearson's Chi-square test of independence (χ^2) was used to evaluate associations between dental morphological traits and geographic origin and biological sex ($\alpha = 0.05$). Participants were grouped into three regions based on ancestral background: Mediterranean (Southern Europe – North Africa), Central Europe, and Others. Supplementary pairwise comparisons between France and Spain, and between Central Europe and the Mediterranean, were also performed. Fisher's exact test was applied where expected cell frequencies fell below 5.

B) Results

Regarding **geographic origin**, three traits showed statistically significant associations. The **Carabelli Cusp** was significantly associated with geographic origin across all comparison levels ($p = 0.019$-0.045), with the Mediterranean region, presenting a lower frequency than Central Europe, and France showing a higher prevalence than Spain. The number of cusps on **maxillary first molars** differed significantly between Central Europe and the Mediterranean ($\chi^2 = 9.98$, $p = 0.007$), with Central Europe displaying greater morphological variability and a higher proportion of 5-cusp pattern.

Similarly, **maxillary second molar** cusp distribution differed significantly between these two regions ($\chi^2 = 7.20$, $p = 0.027$) and between France and Spain ($\chi^2 = 8.03$, $p = 0.018$), with France showing more complex morphologies. A significant difference was also found for **mandibular first molars** at the three-region level ($\chi^2 = 6.28$, $p = 0.043$), though this did not hold in the focused Central Europe vs. Mediterranean comparison ($p = 0.348$). No significant geographic association was identified for mandibular second molars or mandibular second premolars ($p > 0.15$ across all comparisons).

Regarding **biological sex,** no statistically significant difference was observed for any of the tested traits (χ^2 range: 0.00-1.69; p range: 0.430-1.000), indicating an absence of sex-related dimorphism in cusp number and accessory cusp expression in this sample.

C) Summary table

Dental Trait	χ^2	df	p-value	Significant?	Note
Carabelli cusp — Geo. origin (3 regions)	7.94	2	**0.019**	**Yes**	
Carabelli cusp — C. Europe vs Mediterranean	4.01	1	**0.045**	**Yes**	
Carabelli cusp — France vs Spain	5.21	1	**0.023**	**Yes**	
Max 1st molar cusps — Geo. origin	9.72	4	**0.045**	**Yes**	Min exp. < 5
Max 1st molar cusps — C. Europe vs Mediterranean	9.98	2	**0.007**	**Yes**	
Max 2nd molar cusps — C. Europe vs Mediterranean	7.20	2	**0.027**	**Yes**	
Max 2nd molar cusps — France vs Spain	8.03	2	**0.018**	**Yes**	
Mand 1st molar cusps — Geo. origin	6.28	2	**0.043**	**Yes**	
Mand 1st molar cusps — France vs Spain	5.98	2	0.050	Border	P = 0.050
Mand 2nd molar cusps — Geo. origin	2.93	2	0.232	No	Min exp. < 5
Mand 2nd premolar cusps — Geo. origin	0.19	2	0.910	No	
All traits — Sex (M/F)	0.00-1.69	1-2	0.42-1.00	No	All traits

Table 1 Chi-square test results for all dental traits and comparison levels.

5. DISCUSSION

This study examined the distribution of non-metric dental morphological traits in a sample of 55 participants and explored their relationship with geographic origin and biological sex. The results partially support the alternative hypothesis: genetic background does appear to influence the prevalence of certain dental traits, particularly those of the maxillary dentition, while mandibular posterior teeth and biological sex showed no significant associations.

Carabelli Cusp. The overall prevalence of the cusp of Carabelli in our sample (35% of maxillary first molars) falls within the lower range of values reported for European populations. This is consistent with the mixed ancestral composition of our cohort, which combined Central European and Mediterranean participants alongside smaller groups of diverse origins. The significant geographic variation identified across all comparison levels ($p = 0.019$-0.045) aligns with the well-established role of this trait as a population marker. The Carabelli cusp is widely regarded as a polygenic, highly heritable trait (5), and its higher frequency in Central Europe compared to the Mediterranean region is coherent with the broader anthropological literature (7–9). The significant difference between France and Spain ($p = 0.023$) likely reflects their distinct underlying compositions rather than a strict national effect, further reinforcing the value of using multi-generational ancestral origin as a classification variable, as applied in this study.

Maxillary molar cusp number. The significant differences observed in cusp number on maxillary first and second molars between Central European and Mediterranean participants ($p = 0.007$ and $p= 0.027$, respectively) are consistent with the established view that cusp number frequencies differ across populations and can serve as useful anthropological discriminators (13).

Central Europe showed greater morphological complexity, with a higher proportion of 5-cusp configurations on maxillary first molars, while the Mediterranean group tended toward simpler forms. These patterns are consistent with the known evolutionary gradient of dental complexity across European and circum-Mediterranean populations (15,16). It should be noted, however, that the three-region geographic comparison for maxillary first molars ($p = 0.045$) must be interpreted with caution due to minimum expected cell frequencies below 5 in several cells, which may limit the robustness of this specific result.

Mandibular teeth. In contrast, no significant geographic variation was found for mandibular second molars or mandibular second premolars ($p > 0.15$ across all comparisons), and

mandibular first molars only reached significance at the broad three-region level (p = 0.043). This pattern is consistent with the well-documented evolutionary conservatism of mandibular posterior teeth: the reduction of the distal cusp over time, transitioning from 5- to 4-cusp patterns, is considered an evolutionarily derived pattern that appears to have progressed relatively uniformly across European and Mediterranean groups (14). The mandibular dentition therefore appears to be a weaker discriminator of geographic origin in our sample than the maxillary dentition, a finding in line with studies showing greater inter-population variability in upper molar morphology (5,15)

Absence of sexual dimorphism. The complete absence of sex-related differences across all traits (p = 0.430-1.000) is consistent with current evidence. Non-metric crown morphological traits, including accessory cusps and cusp number, are not considered significantly sex-dimorphic in modern human populations, which distinguishes them from metric dental traits where a degree of sexual dimorphism has been documented (17,20)

Limitations. Several methodological constraints should be considered. First, the sample size was relatively small (n = 55), and the geographic distribution was uneven with France and Spain accounting for 75% of the participants. Which substantially limits the statistical power of comparisons involving smaller groups and increases the risk of errors.

Second, dental examinations were conducted through direct visual inspection without the use of standardized scoring scales such as the Arizona State University Dental Anthropology System (ASUDAS) ,which limits the precision of trait recording and the comparability of our results with the existing literature (5,21)

Third, while the multi-generational approach to genetic background classification is a notable strength of this study, it relied entirely on self-reported data, which may introduce recall bias, particularly regarding grandparent's origins.

Fourth, recruitment within a university setting may have introduced selection bias, limiting the generalizability of the findings.

Finally, several traits described in the introduction – including conoid teeth, prominent cingulum, dens invaginatus, and microdontia – were not observed in the study sample and could therefore not be subjected to statistical analysis, which limits the scope of the conclusions.

Clinical and research implication. Despite these limitations, the findings have relevant clinical implications. The greater morphological complexity of maxillary molars observed in Central

European individuals – including additional cusps and more intricate fissures patterns – may be associated with an increased susceptibility to dental caries in this group (5,17).

In an increasingly multicultural clinical setting such as contemporary Spain, awareness of population-specific dental morphological patterns may help guide more targeted preventive strategies, including systematic fissure sealants.

Furthermore, these results support existing evidence that non-metric dental traits are valuable tools in forensic identification and the reconstruction of population history (15).

Future research should focus on larger and more geographically balanced samples, the use of standardized scoring systems, and the inclusion of additional traits not observed in this study, to provide a more comprehensive understanding of dental morphological diversity within Spain's evolving population.

6. CONCLUSIONS

This study investigated the prevalence and distribution of non-metric dental morphological traits in a sample of 55 participants with diverse genetic backgrounds, with the aim of exploring whether geographic origin influences their expression.

The results partially support the alternative hypothesis. Statistically significant associations were identified between the geographic origin and three maxillary traits: the presence of the Cusp of Carabelli and the number of cusps on both maxillary first and second molars. In each case, Central European participants exhibited greater morphological complexity than their Mediterranean neighbors, a pattern consistent with established findings in dental anthropology. In contrast, mandibular posterior teeth showed no significant geographic variation, reflecting their relative evolutionary conservatism. No sex-related dimorphism was observed for any of the traits examined.

These findings suggest that genetic background, assessed through a multi-generational ancestral approach, does influence certain aspects of dental crown morphology, particularly in the maxillary dentition. From a clinical perspective, awareness of these population-specific patterns may help inform more targeted preventive and restorative strategies in increasingly diverse patient populations, such as those encountered in contemporary Spain.

However, the limitations of this study – including the modest sample size, uneven geographic distribution – require caution when interpreting and generalizing these findings. They should therefore be considered preliminary, providing a foundation for further research.

Future studies should focus on larger and more geographically balanced samples, the use of standardized trait-recording systems (ASUDAS), and the inclusion of a broader range of non-metric traits, to develop a more comprehensive understanding of dental morphological diversity within Spain's multicultural population.

7. SUSTAINABILITY

This study on dental anatomical variations and their potential clinical significance contributes to **Sustainable Development Goal (SDG 3): Good Health and Well-Being** by promoting sustainable approaches in dentistry and public health. By identifying population-specific dental traits such as the Cusp of Carabelli and dental agenesis, the research supports targeted preventive strategies, including early interventions like sealants and tailored oral hygiene education, aligning with SDG Targets 3.4 (reduce premature mortality from non-communicable diseases) and 3.8 (achieve universal health coverage). It also encourages innovation in cost-effective diagnosis tools and public health policies, fostering SDG Targets 3.b (research and development of vaccines and medicines) and 3.9 (reduces deaths and illnesses from hazardous chemicals and pollution).

Additionally, the study emphasizes the importance of education and international collaboration, enhancing health workforce training and cross-regional comparisons, thus supporting SDG Targets 3.c, 3.3, and 3.7.

Overall, this proactive and inclusive approach contributes to equitable oral health and the long-term viability of health systems, in line with the broader objectives of SDG 3.

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9. ANNEXES

9.1. Surveys

Survey N°1

1. **INFORMED CONSENT** This questionnaire is part of the Final Degree Project in Dentistry at the European University of Madrid entitled 'Population study of dental anatomical variations and their potential clinical significance' and directed by Tutor. The purpose of this study is to establish the relationship between the most frequent anatomical variations and population origin, and the information will be collected through a brief survey and a simple visual analysis to be carried out on the campus of the European University. Your participation in this study is voluntary, and you may request to be excluded from it without prior justification or prejudice to yourself. The information collected will be confidential and will not be used for any purpose other than this research and its dissemination. The data collected will be completely anonymous. No personally identifiable information will be requested. The data collected in the survey and visual analysis will be processed in accordance with the provisions of Organic Law 3/2018, of 5 December, on the Protection of Personal Data and the guarantee of digital rights. For the purposes of the provisions of the regulations of Organic Law 3/2018, of 5 December, on the Protection of Personal Data and Guarantee of Digital Rights, you are hereby informed and expressly aware of the use of the data provided in the survey and visual analysis for the purposes indicated above. This consent is granted without prejudice to all your rights in relation to the forementioned regulations, and you have the possibility to access, rectify, cancel and oppose the information provided at any time you wish. To do so, you must write to the tutor. *You give your consent to participate in the survey and visual analysis as a volunteer so that the results of the study can be used in the Final Degree Project* 'A Population-Based Study of Dental Anatomical Variations and Their Potential Clinical Significance'.

- Yes
- No

2. Could you go to the European University campus in Villaviciosa de Odón to have the visual analysis done?

- Yes
- No

3. If you answer yes, choose the day you will be able to visit the European University Campus in Villaviciosa de Odón:

On the day you choose to attend the European University Campus in Villaviciosa de Odón, you must wait at the door of the Dental Laboratory on campus at the assigned time to undergo the visual analysis.

4. Are you currently also a patient at the university clinic?

- Yes
- No

Survey N°2

1. **INFORMED CONSENT** This questionnaire is part of the Final Degree Project in Dentistry at the European University of Madrid entitled 'Population study of dental anatomical variations and their potential clinical significance' and directed by Tutor. The purpose of this study is to establish the relationship between the most frequent anatomical variations and population origin, and the information will be collected through a brief survey and a simple visual analysis to be carried out on the campus of the European University. Your participation in this study is voluntary, and you may request to be excluded from it without prior justification or prejudice to yourself. The information collected will be confidential and will not be used for any purpose other than this research and its dissemination. The data collected will be completely anonymous. No personally identifiable information will be requested. The data collected in the survey and visual analysis will be processed in accordance with the provisions of Organic Law 3/2018, of 5 December, on the Protection of Personal Data and the guarantee of digital rights. For the purposes of the provisions of the regulations of Organic Law 3/2018, of 5 December, on the Protection of Personal Data and Guarantee of Digital Rights, you are hereby informed and expressly aware of the use of the data provided in the survey and visual analysis for the purposes indicated above. This consent is granted without prejudice to all your rights in relation to the forementioned regulations, and you have the possibility to access, rectify, cancel and oppose the information provided at any time you wish. To do so, you must write to the tutor.

You give your consent to participate in the survey and visual analysis as a volunteer so that the results of the study can be used in the Final Degree Project 'A Population-Based Study of Dental Anatomical Variations and Their Potential Clinical Significance'.

- Yes
- No

2. Which code was assigned to you during the examination?

3. Sex Assigned at Birth

- Female
- Male

4. Age

5. What is your country of birth?

- United States of America
- United Kingdom
- France
- Spain
- Italy
- Portugal
- Germany
- Norway
- Sweden
- Turkey
- Algeria
- Morocco
- Tunisia
- Senegal
- Mali
- Mauritania
- China
- Vietnam
- Pakistan
- Brazil

- Colombia
- Venezuela
- Ecuador
- Argentina
- Peru
- Romania
- Ukraine
- Bulgaria
- Honduras
- Paraguay
- I don’t know
- It’s not in the list

6. If your country of birth is not listed, please indicate it here.

7. What is your mother's country of birth?

8. If your mother's country of birth is not listed, please indicate it here.

9. What is your father's country of birth?

10. If your father's country of birth is not listed, please indicate it here.

11. What is your maternal grandmother's country of birth?

12. If the country of birth of your maternal grandmother is not listed, please indicate it here.

13. What is your maternal grandfather's country of birth?

14. If the country of birth of your maternal grandfather is not listed, please indicate it here.

15. What is your paternal grandmother's country of birth?

16. If the country of birth of your paternal grandmother is not listed, please indicate it here.

17. What is your paternal grandfather's country of birth?

18. If the country of birth of your paternal grandfather is not listed, please indicate it here.

19. Do you identify with any of the CHARACTERISTIC phenotypic groups in the following areas? (The term phenotype refers to the set of observable characteristics or traits of an organism and can be equivalent to ethnicity.)

- Caucasian European
- Mediterranean European
- Native American
- Native Alaskan
- Native Oceanian or another Pacific Islander
- Southeast Asian
- East Asian
- South Asian
- West Asian
- North or Mediterranean African
- Native African
- I do not identify with any of the above groups
- I don't know/I won't answer
- Other options

20. Other (specify):

21. Have you ever experienced any of the following conditions?

- Bruxism (teeth grinding or clenching)
- Tooth extractions
- Orthodontic treatment (braces, aligners)
- None

22. If you selected any of the previous answers, please specify whether you wear a splint or not, which tooth was extracted or what orthodontic treatment you had and at what age.

9.2. Extra figures regarding sex distribution

A) Cusp of Carabelli

Figure 31 & 32: Among male participants, the Carabelli cusp was absent in 50% of cases, present bilaterally in 39%, determinable on one molar only in 6% and asymmetric in 5%.
Among female participants, absence was observed in 62% of cases, bilateral presence in 26%, unilateral only in 9%, and asymmetry in 3%.

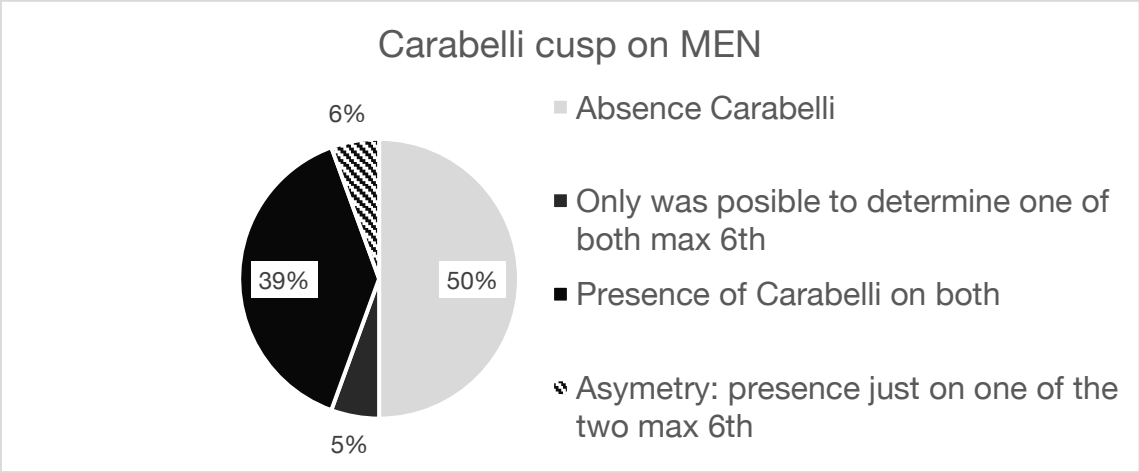


Figure 30 Distribution of the Carabelli Cusp among Men

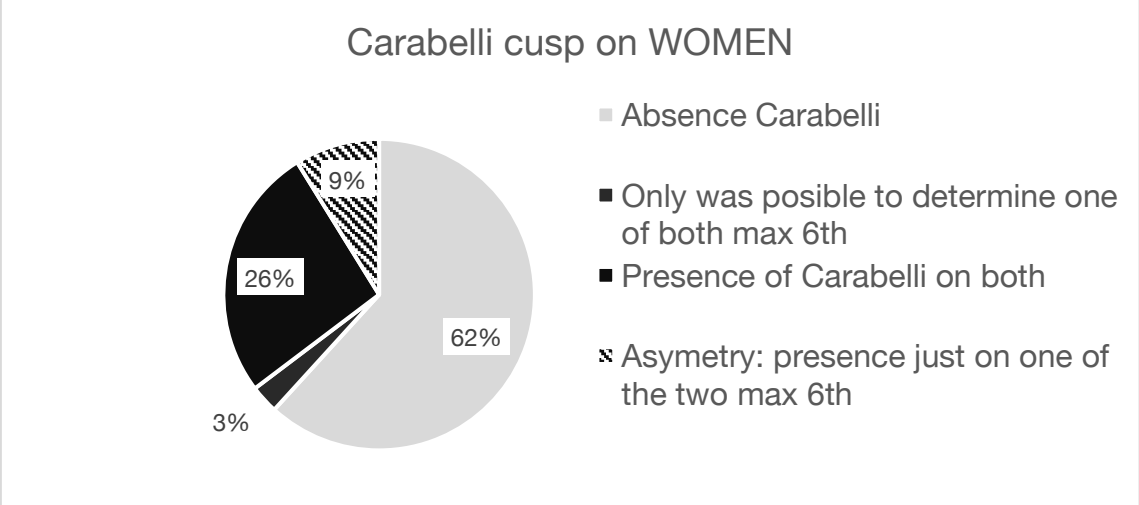


Figure 31 Distribution of the Carabelli Cusp among Women

Figure 33: Comparison of the Carabelli Cusp between Men and Women, it shows that absence of Carabelli Cusp was more frequent in women (n = 21) than in men (n = 9). Bilateral presence is comparable and asymmetric cases were slightly more frequent in women than in men.

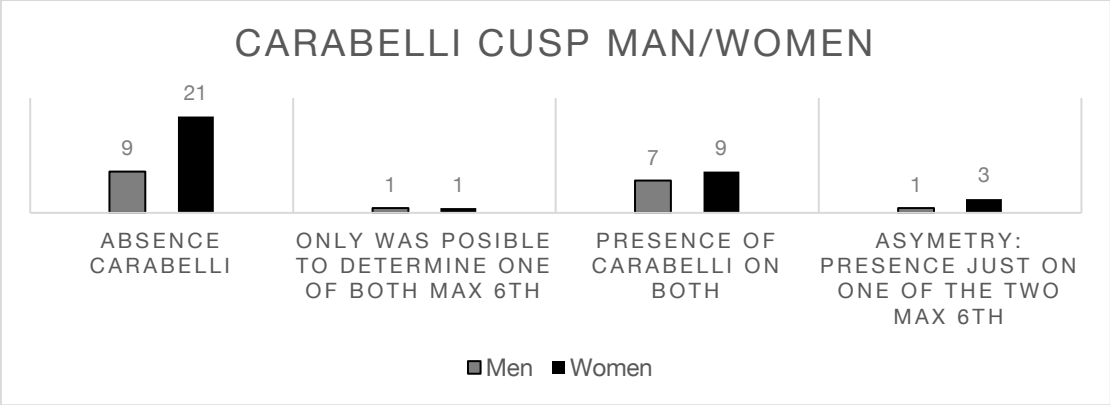


Figure 32 Comparison of the presence of the cusp of Carabelli between Men and Women

B) Number of cusps on maxillary first molars

In Figure 34 both sexes showed a predominant 4-cusp pattern (83% in males, 86% in females). The 5-cusp configuration was observed in 17% of males and 11% of females. The 3-cusp pattern was absent in males and represented in only 3% of females

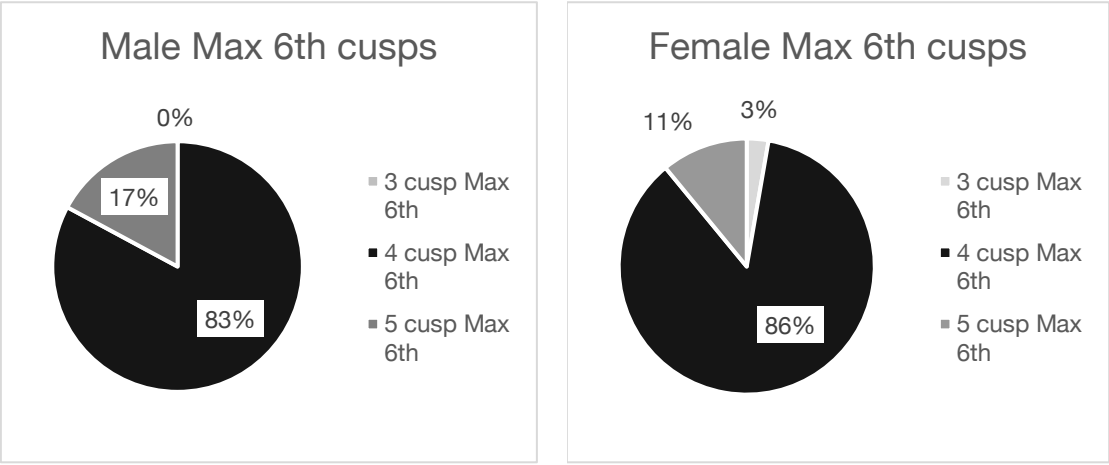


Figure 33 Comparison of the number of cusps of 6th between men and women

C) Number of cusps on maxillary second molars

In Figure 35, both males and females showed a relatively balanced distribution between 3 and 4 cusps. The 4-cup pattern was slightly predominant in males (51%) and equally distributed in females (46% for 3 cusps, 51% for 4 cusps). The 5-cusp configuration remained rare in both sexes.

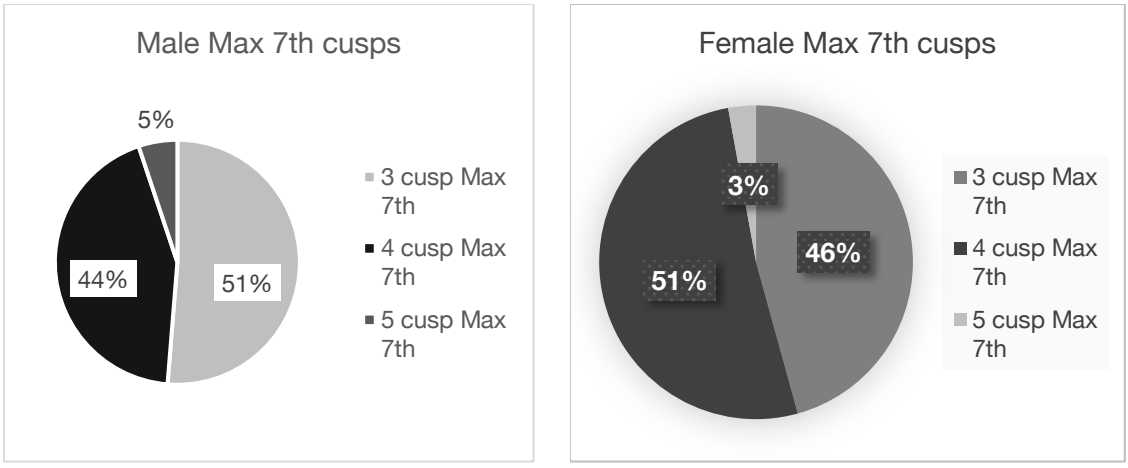


Figure 34 Comparison of the number of cusps in maxillary 7th between men and women

D) Number of cusps on mandibular first molars

The 5-cusp pattern was predominant in both sexes (74% in males, 67% in females), followed by the 4-cusp configuration (26% in males, 32% in females). The 6-cusp pattern was mostly absent.

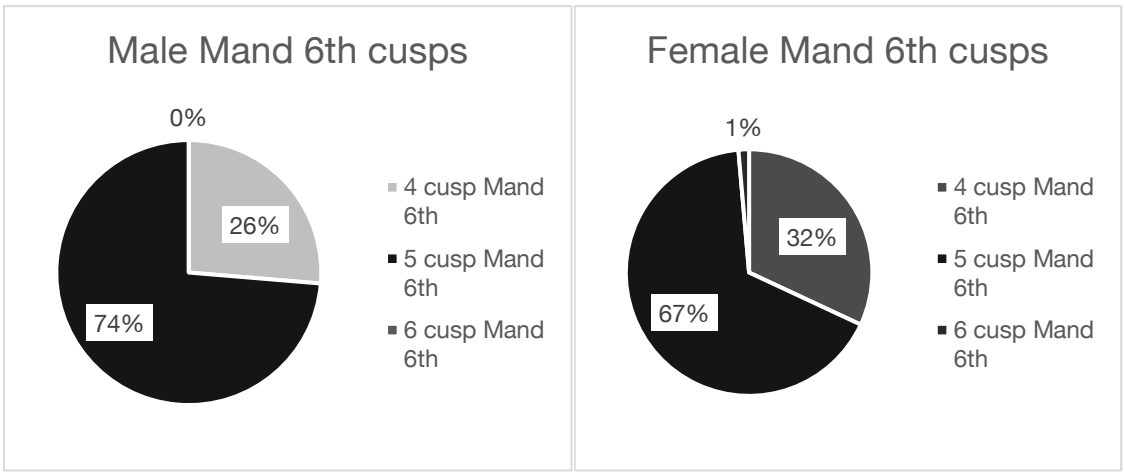


Figure 35 Comparison of the number of cusps in mandibular 6th between men and women

E) Number of cusps on mandibular second molars

The 4-cusp pattern was largely dominant in both groups (84% in males, 83% in females). And the level of 5-cusp pattern is equal.

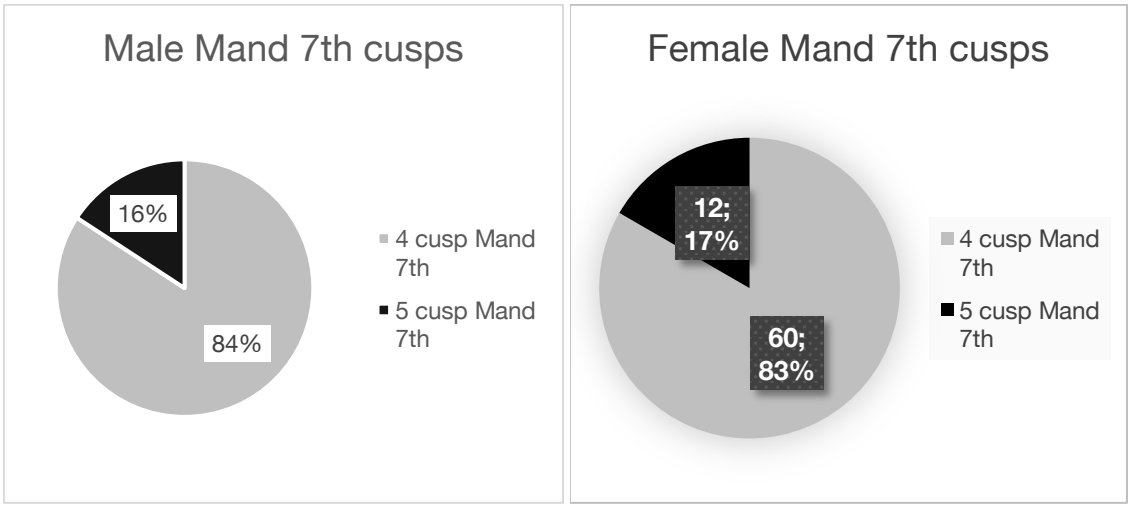


Figure 36 Comparison of the number of cusps in mandibular 7th between men and women

F) Number of cusps on mandibular second premolars

In Figure 38, both sexes showed a near-equal distribution between the 2- and 3- configurations. But in males the 3-cusp was slightly predominant (53%) over the 2-cusp type (47%). A similar trend was observed in females, with the 2-cusp pattern (44%) and the 3-cusp pattern (56%).

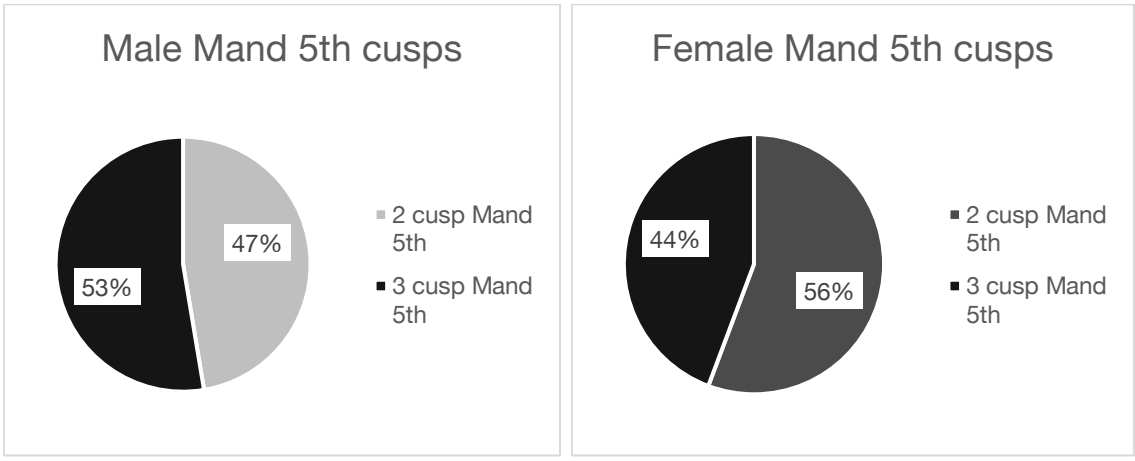


Figure 37 Comparison of the number of cusps in mandibular 5th between men and women

No major sex-related difference was observed, consistent with the absence of statistically significant dimorphism found in the statistical analysis.

9.3. Comparison between the two more represented countries/region

A) Cusp of Carabelli

This chart presents the distribution of the Carabelli cusp in the two most represented countries in our study. Among French participants, 22 teeth exhibited a Carabelli cusp, while 27 did not. In Spanish participants, 3 teeth presented a Carabelli cusp, compared to 19 without it.

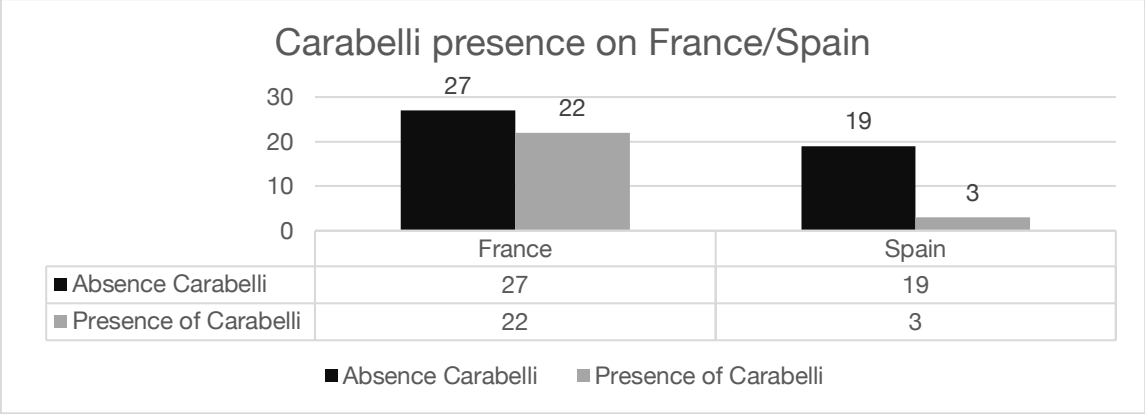


Figure 38 Presence of the Carabelli cusp in the two countries most frequently represented in our study

B) Number of cusps on maxillary first molars

This chart presented the distribution of number of cusps in maxillary first molars in the most represented countries in our study. Among French participants, 2 teeth presented 3 cusps, 41 teeth presented 4 cusps, and 7 teeth presented 5 cusps. Among Spaniards, no one presented first molars with 3 cusps, 25 teeth presented 4 cusps, and 1 tooth had 5 cusps.

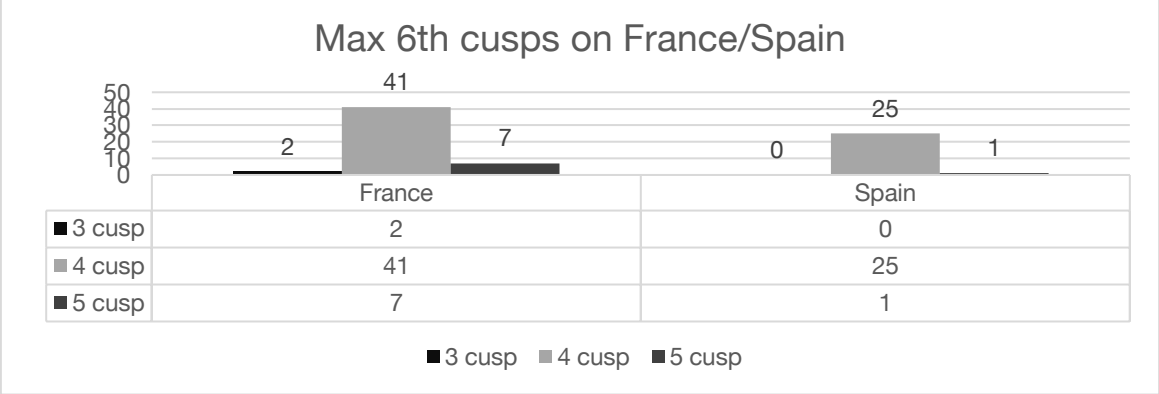


Figure 39 Focus on France and Spain regarding the distribution of Maxillary first Molars

C) Number of cusps on maxillary second molars

This chart presented the distribution of number of cusps in maxillary second molars in the most represented countries in our study. Among French participants, 20 teeth presented 3 cusps, 30 teeth presented 4 cusps, and 4 teeth presented 5 cusps. Among Spaniards, 18 teeth presented 3 cusps, 8 teeth presented 4 cusps, and no one presented teeth with 5 cusps.

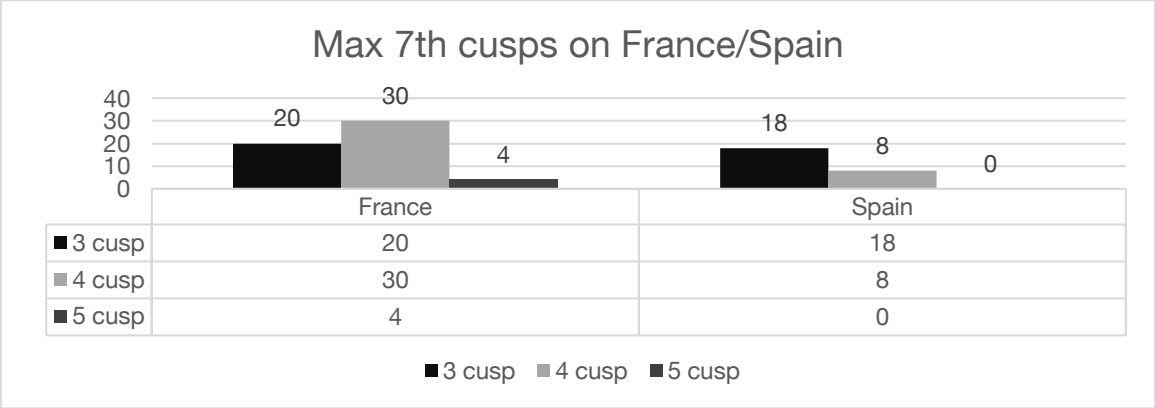


Figure 40 Focus on France and Spain regarding the distribution of Maxillary second Molars

D) Number of cusps on mandibular first molars

This chart presented the distribution of number of cusps in mandibular first molars in the most represented countries in our study. Among French participants, 11 teeth presented 4 cusps, 42 teeth presented 5 cusps, and 1 tooth presented 6 cusps. Among Spaniards, 12 teeth presented 4 cusps, 14 teeth presented 5 cusps, and no one presented teeth with 6 cusps.

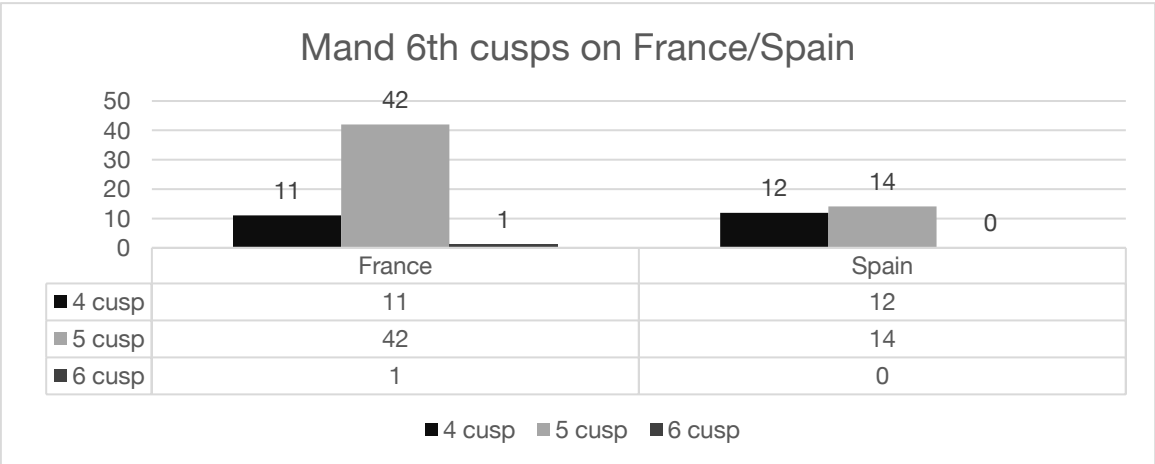


Figure 41 Focus on France and Spain regarding the distribution of Mandibular first Molars

E) Number of cusps on mandibular second molars

This chart presented the distribution of number of cusps in mandibular second molars in the most represented countries in our study. Among French participants, 46 teeth presented 4 cusps, 8 teeth presented 5 cusps. Among Spaniards, 19 teeth presented 4 cusps, 7 teeth presented 5 cusps.

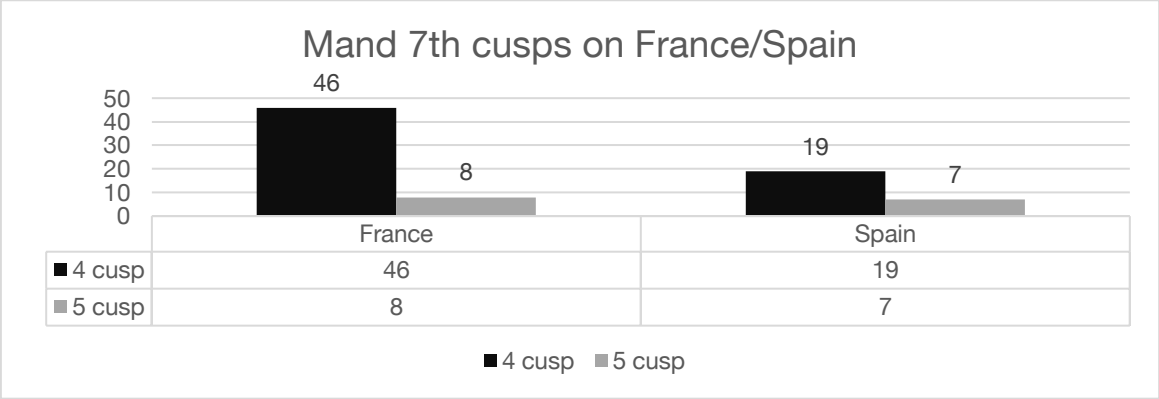


Figure 42 Focus on France and Spain regarding the distribution of Mandibular second Molars

F) Number of cusps on mandibular second premolars

This chart presented the distribution of number of cusps in mandibular second premolars in the most represented countries in our study. Among French participants, 28 teeth presented 2 cusps, 26 teeth presented 3 cusps. Among Spaniards, 16 teeth presented 2 cusps, 10 teeth presented 3 cusps.

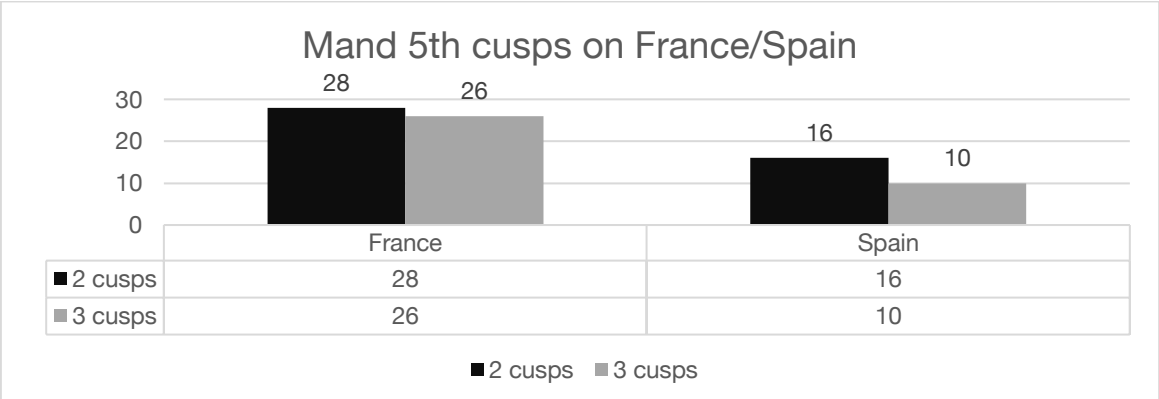


Figure 43 Focus on France and Spain regarding the distribution of Mandibular second Premolars

Hospital Universitario de Getafe

DE: COMITÉ DE ÉTICA DE LA INVESTIGACIÓN CON MEDICAMENTOS (CEIm)

A: [redacted]

El Comité de Ética de la Investigación con Medicamentos (CEIm) del Hospital Universitario de Getafe, en su reunión del día jueves, 26 de marzo de 2026 (A04/26), acordó:

[X] APROBAR CONDICIONADO A LOS SIGUIENTES CAMBIOS:

[...] INFORME NO FAVORABLE POR LOS SIGUIENTES MOTIVOS:

[...] SOLICITAR LAS SIGUIENTES ACLARACIONES:

- Protocolo:
  - El criterio 7 no es criterio de inclusión sino actividad del estudio.
  - Debe definir correctamente cómo reclutaran, qué van a enviar con el QR, ¿qué es eso de la encuesta anónima...?
  - Deben volver a presentar la hoja de de información o únicamente decir que darán consentimiento verbal para sentarse para la visualización y cumplimentar la encuesta de datos geográficos.

- Hoja de Información al Participante y Consentimiento Informado:
  - Se indica que el estudio está aprobado por la AEMPS, debe aclararlo ya que no es un ensayo clínico con medicamentos.
  - Debe eliminar el RD1090/2015, no aplica (no es ensayo clínico con medicamentos).
  - Debe indicar que además de rellenar la encuesta, se le hará una exploración visual.
  - Debe especificar en qué consiste el estudio ...
  - Aspectos que se han de incluir en la hoja de información como beneficio/riesgo, o voluntariedad están en el protocolo y no en la hoja de información.

Del Estudio observacional (Trabajo Fin de Grado) titulado: **""A Population-Based Study of Dental Anatomical Variations and Their Potential Clinical Significance" (Estudio poblacional de variaciones anatómicas dentales y su potencial relevancia clínica)".** *V2, 09/03/2026*

[redacted]

Presidente del CEIm
Hospital Universitario de Getafe

HOJA DE INFORMACIÓN

El presente cuestionario forma parte del Trabajo de Fin de Grado en Odontología de la Universidad Europea de Madrid titulado *"A Population-Based Study of Dental Anatomical Variations and Their Potential Clinical Significance" (Estudio poblacional de variaciones anatómicas dentales y su potencial significancia clínica)*. El estudio ha sido aprobado por el Comité de Ética de la Investigación (CEIm) del Hospital Universitario de Getafe, de acuerdo a la legislación vigente, Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales y Reglamento (UE) 2016/679 del Parlamento Europeo y del Consejo de 27 de abril de 2016 relativo a la protección de las personas físicas en lo que respecta al tratamiento de datos personales y a la libre circulación de estos datos. Este estudio no requiere en ninguno de sus pasos que la persona participante consuma ningún tipo de medicamento, ni se realizará manipulación alguna durante la fase de observación de las piezas dentarias.

Nuestra intención es que usted reciba la información correcta y suficiente para que pueda decidir si acepta o no participar en este estudio. Para ello lea la hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir. Si tiene alguna duda puede dirigirse a la [redacted]

El propósito del presente trabajo es *establecer la relación entre las variaciones anatómicas más frecuentes en dientes y la posible relación con el origen geográfico de la persona*. La información será recogida a través de una **breve encuesta** y un **análisis visual simple** para el cual se requerirá que mantenga la boca abierta durante un corto periodo de tiempo, máximo 5 minutos, necesario para la observación de sus piezas dentarias.

El procedimiento es de **nulo o muy bajo riesgo**, implicando únicamente la observación de sus piezas dentarias con linterna médica y requiriendo la apertura voluntaria de la boca. Entre los riesgos mínimos se pueden incluir la incomodidad temporal debido a la apertura de la boca o sensación de exposición. No se realizarán manipulaciones, procedimientos invasivos ni toma de muestras ni se requerirá el uso de ningún medicamento.

Su participación en este estudio es de carácter **libre y voluntario**, sin beneficio clínico directo ni económico para los participantes, pudiendo solicitar ser excluido del mismo, sin justificación previa ni perjuicio para usted. La información recogida será confidencial y no se usará para ningún otro propósito fuera de esta investigación y derivados de la divulgación investigativa. Los datos recogidos serán completamente anónimos en cumplimiento de la Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales y Reglamento (UE) 2016/679 del Parlamento Europeo y del Consejo de 27 de abril de 2016 relativo a la protección de las personas físicas en lo que respecta al tratamiento de datos personales y a la libre circulación de estos datos. Los datos recogidos para el estudio estarán identificados mediante un código, de manera que no incluya información que pueda identificarle. El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los participantes se ajustarán a lo dispuesto en esta ley.

¿Da su consentimiento de participación como voluntario/a para que los resultados se utilicen en el Trabajo Fin de Grado *"A Population-Based Study of Dental Anatomical Variations and Their Potential Clinical Significance" (Estudio poblacional de variaciones anatómicas dentales y su potencial relevancia clínica)*

Puede contestar:
Si o No

HOJA DE CONSENTIMIENTO INFORMADO

Título del estudio: "*A Population-Based Study of Dental Anatomical Variations and Their Potential Clinical Significance*" (Estudio poblacional de variaciones anatómicas dentales y su potencial relevancia clínica)
Código: 156

Yo, INCLUIR CÓDIGO ASIGNADO EN EL ESTUDIO

☐ He leído la hoja de información que se me ha entregado sobre el estudio.

☐ He podido hacer preguntas sobre el estudio.

☐ He recibido suficiente información sobre el estudio.

☐ He hablado con [redacted], estudiante de odontología en la Universidad Europea de Madrid y/o [redacted] tutora del TFG.

☐ Comprendo que mi participación es voluntaria.
- Sin riesgo o perjuicio para mi salud.
- Sin ningún tipo de beneficio económico ni académico.

☐ Comprendo que puedo retirarme del estudio:
- Cuando quiera.
- Sin tener que dar explicaciones.
- Sin que esto repercuta en mi relación con la universidad.