Karen Ariely Rocha Arruda

Phenotypic dento-osseous characterization of a Brazilian family with Familial Adenomatous Polyposis

Brasília
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Caracterização fenotípica das anomalias dento-ósseas de uma família brasileira portadora de Polipose Adenomatosa Familial

Trabalho de Conclusão de Curso apresentado ao Departamento de Odontologia da Faculdade de Ciências da Saúde da Universidade de Brasília, como requisito parcial para a conclusão do curso de Graduação em Odontologia.

Orientador: Prof.ª Dra. Eliete Neves Silva Guerra

Coorientador:Prof.ª Dra. Fabiana Tolentino Almeida

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Aos pacientes portadores de Polipose Adenomatosa Familial.

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“Confie no Senhor de todo o seu coração e não se apoie na sua própria inteligência. Lembre-se de Deus em tudo o que fizer, e ele lhe mostrará o caminho certo”

Provérbios 3:5-6

RESUMO

Arruda, Karen. Caracterização fenotípica das anomalias dento-ósseas de uma família brasileira portadora de Polipose Adenomatosa Familial. 2021. Trabalho de Conclusão de Curso (Graduação em Odontologia) – Departamento de Odontologia da Faculdade de Ciências da Saúde da Universidade de Brasília.

Objetivo: realizar uma caracterização fenotípica das anomalias dento-ósseas em uma família brasileira com Polipose Adenomatosa Familial (FAP) e investigar a variante causal no gene Adenomatous Polyposis coli (*APC*). **Métodos**: O estudo incluiu uma família com 14 indivíduos (Grupo A: afetados; Grupo B: Familiares sem diagnóstico). A frequência dos achados radiográficos em ambos os grupos foi avaliada de acordo com o método de diagnóstico Dental Panoramic Radiograph Score (DPRS). A precisão e reprodutibilidade do DPRS foram testadas. O DNA genômico foi isolado da saliva do paciente índice e submetido a sequenciamento total do Exoma e Sanger. **Resultados**: DPRS≥7 foi observado em 80% do Grupo A, mas em 0% do Grupo B. Os achados mais comuns no Grupo A foram ilhas de condensação óssea (60%), esclerose difusa (40%), osteomas (40%) e dentes supranumerários (20%). O DPRS mostrou ser um método confiável quando DPRS≥5 e DPRS≥7 foram considerados positivos para FAP, e reprodutível visto que os avaliadores identificaram corretamente os pacientes afetados (concordância Kappa> 0,8, p=0,002). Foi detectada uma mutação heterozigótica nonsense no gene *APC* (c.1370C> G; p.Ser457 *) do caso índice. **Conclusão**: Pacientes com FAP apresentam maior frequência de anomalias dento-ósseas (p=0,005). As anomalias ósseas foram mais prevalentes do que as anomalias dentárias (p=0,001). Assim, os pacientes com FAP devem ser encaminhados para exame odontológico e aconselhamento genético para realizar o diagnóstico precoce das anomalias dento-ósseas e avaliar as implicações dos achados moleculares em cada família em particular.

ABSTRACT

Arruda, Karen. Phenotypic dento-osseous characterization of a Brazilian family with Familial Adenomatous Polyposis. 2021. Undergraduate Course Final Monograph (Undergraduate Course in Dentistry) – Department of Dentistry, School of Health Sciences, University of Brasília.

Objective: To perform a phenotypic characterization of the dento-osseous anomalies in a Brazilian family with Familial Adenomatous Polyposis (FAP) and to investigate the adenomatous polyposis coli (*APC*) causative variant. **Design:** The study included a family of 14 individuals (Group A: affected; Group B: non-affected). The frequency of radiographic findings in both groups was evaluated according to the Dental Panoramic Radiograph Score (DPRS) diagnostic method. The accuracy and reproducibility of DPRS were tested. The DNA was isolated from the index patient's saliva and submitted to whole-exome and Sanger sequencing approach. **Results**: DPRS≥7 was observed in 80% of Group A but in none of Group B. The most common findings in Group A were dense bone islands (60%), hazy sclerosis (40%), osteomas (40%), and supernumerary tooth (20%). DPRS has proved to be a reliable method while DPRS≥5 and DPRS≥7 were taken as positive for FAP, and reproducible diagnosis test considering that the evaluators correctly identified the affected patients (Kappa agreement>0.8, p=0.002). A nonsense heterozygous mutation in the *APC* gene (c.1370C>G; p.Ser457*) of the index case was detected. **Conclusion**: FAP patients have a higher frequency of dento-osseous anomalies (p=0.005). Bone abnormalities were more prevalent than dental anomalies (p=0.001). Thus, FAP patients should be referred for dental examination and genetic counseling to perform early diagnosis of dento-osseous anomalies and evaluate the implications of the molecular findings in each particular family.

SUMMARY

Scientific Article

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Title Page

Caracterização fenotípica das anomalias dento-ósseas de uma família brasileira portadora de Polipose Adenomatosa Familial

Phenotypic dento-osseous characterization of a Brazilian family with Familial Adenomatous Polyposis

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Resumo

Caracterização fenotípica das anomalias dento-ósseas de uma família brasileira portadora de Polipose Adenomatosa Familial

Objetivo: realizar uma caracterização fenotípica das anomalias dento-ósseas em uma família brasileira com Polipose Adenomatosa Familial (FAP) e investigar a variante causal no gene Adenomatous Polyposis coli (*APC*). **Métodos**: O estudo incluiu uma família com 14 indivíduos (Grupo A: afetados; Grupo B: Familiares sem diagnóstico). A frequência dos achados radiográficos em ambos os grupos foi avaliada de acordo com o método de diagnóstico Dental Panoramic Radiograph Score (DPRS). A precisão e reprodutibilidade do DPRS foram testadas. O DNA genômico foi isolado da saliva do paciente índice e submetido a sequenciamento total do Exoma e Sanger. **Resultados**: DPRS≥7 foi observado em 80% do Grupo A, mas em 0% do Grupo B. Os achados mais comuns no Grupo A foram ilhas de condensação óssea (60%), esclerose difusa (40%), osteomas (40%) e dentes supranumerários (20%). O DPRS mostrou ser um método confiável quando DPRS≥5 e DPRS≥7 foram considerados positivos para FAP, e reprodutível visto que os avaliadores identificaram corretamente os pacientes afetados (concordância Kappa> 0,8, p=0,002). Foi detectada uma mutação heterozigótica nonsense no gene *APC* (c.1370C> G; p.Ser457 *) do caso índice. **Conclusão**: Pacientes com FAP apresentam maior frequência de anomalias dento-ósseas (p=0,005). As anomalias ósseas foram mais prevalentes do que as anomalias dentárias (p=0,001). Assim, os pacientes com FAP devem ser encaminhados para exame odontológico e aconselhamento genético para realizar o diagnóstico precoce das anomalias dento-ósseas e avaliar as implicações dos achados moleculares em cada família em particular.

Palavras-chave

Polipose adenomatosa do colo; anormalidades dentárias; radiografia panorâmica; síndrome de Gardner; neoplasias colorretais.

Clinical Relevance

The early diagnosis of FAP patients is essential in preventing the development of colorectal cancer and consequent improvement in the prognosis of the disease in affected individuals. For this, it is necessary to know about the clinical manifestations of this condition.

Abstract

Phenotypic dento-osseous characterization of a Brazilian family with Familial Adenomatous Polyposis

Objective: To perform a phenotypic characterization of the dento-osseous anomalies in a Brazilian family with Familial Adenomatous Polyposis (FAP) and to investigate the adenomatous polyposis coli (*APC*) causative variant. **Design:** The study included a family of 14 individuals (Group A: affected; Group B: non-affected). The frequency of radiographic findings in both groups was evaluated according to the Dental Panoramic Radiograph Score (DPRS) diagnostic method. The accuracy and reproducibility of DPRS were tested. The DNA was isolated from the index patient's saliva and submitted to whole-exome and Sanger sequencing approach. **Results**: DPRS≥7 was observed in 80% of Group A but in none of Group B. The most common findings in Group A were dense bone islands (60%), hazy sclerosis (40%), osteomas (40%), and supernumerary tooth (20%). DPRS has proved to be a reliable method while DPRS≥5 and DPRS≥7 were taken as positive for FAP, and reproducible diagnosis test considering that the evaluators correctly identified the affected patients (Kappa agreement>0.8, p=0.002). A nonsense heterozygous mutation in the *APC* gene (c.1370C>G; p.Ser457*) of the index case was detected. **Conclusion**: FAP patients have a higher frequency of dento-osseous anomalies (p=0.005). Bone abnormalities were more prevalent than dental anomalies (p=0.001). Thus, FAP patients should be referred for dental examination and genetic counseling to perform early diagnosis of dento-osseous anomalies and evaluate the implications of the molecular findings in each particular family.

Key Words

Familial Adenomatous Polyposis; Dento-osseous Anomalies; Adenomatous Polyposis Coli Gene; Gardner Syndrome; Colorectal Cancer.

Introduction

Familial Adenomatous Polyposis (FAP- MIM175100) is an autosomal dominant inherited syndrome predisposing to colorectal cancer. FAP is caused by variants in the Adenomatous Polyposis Coli (*APC*) tumor suppressor gene, mapped to the long arm of chromosome 5 (5q21) band q21 (MIM 611731). Pathogenic variants in the *APC* gene can cause two distinct phenotypes: the classic FAP and the Attenuated Familial Adenomatous Polyposis (Knudsen et al., 2010). The encoded apc protein is a nucleus-cytoplasmic shuttling protein, known to antagonize the Wnt signaling pathway by the formation of a cytoplasmic complex that targets β-catenin for degradation (Zeineldin et al., 2014); therefore, the loss of apc function contributes to the increase of cytoplasmic β-catenin levels. The β-catenin protein is related to the process of cell adhesion and gene transcription (Amado et al., 2012). In high concentrations, β-catenin is translocated to the nucleus, where it binds to transcription factors of target genes. The dysregulated signaling pathway induces cell proliferation and the formation of intestinal adenomas, resulting in malignant transformation of normal cells (Fujii et al., 2019; Nusse and Clevers, 2017; Yang et al., 2014;).

When pathogenic variants in the *APC* gene are not identified in FAP patients, a second gene must be investigated, the *MUTYH* gene (chromosome 1p34.1; MIM 604933). *MUTYH* pathogenic variants cause an attenuated phenotype similar to Attenuated Familial Adenomatous Polyposis that is called MAP, *MUTYH*-Associated Polyposis, and has an autosomal mode of inheritance (Torrezan et al.2013; Nielsen et al., 2009).

In general, FAP patients develop multiple intestinal polyps around the second decade of life. Approximately 50% of patients with classic FAP have these colorectal adenomas by the age of 15 years, and this percentage increases to 95% by the age of 35 years (Half et al., 2009). The attenuated form of the disease called Attenuated Familial Adenomatous Polyposis is clinically characterized by the development of less than 100 colorectal adenomatous and by a delay in the polyps' onset, on average of up to 20-30 years (Hernegger et al., 2002). In addition to the development of adenomatous polyps in the colon and rectum, classic FAP patients may present extracolonic manifestations,

including gastric and duodenal adenomas, congenital hypertrophy of the retinal pigment epithelium desmoid tumors, fibromas, and dento-osseous anomalies (odontomas, osteomas, dense bone islands, and supernumerary tooth) (Gardner, 1962; Campos et al., 2015). Although commonly noted in FAP, extraintestinal manifestations are rare in Attenuated Familial Adenomatous Polyposis patients, and affected individuals are likely to have a reduced lifetime risk of colorectal cancer, less than in 100% of cases as in FAP (Jasperson et al., 2010). The diagnosis and follow-up of FAP are achieved by periodic colonoscopy examination and, if indicated, prophylactic or elective colectomy (Syngal et al., 2015).

The presence of both intestinal polyps and dental anomalies in patients with classic FAP were first described by Gardner (Gardner, 1951). Gardner Syndrome is a term which has been used to refer to this phenotypic characterization of FAP. Bone alterations in the jaw and dental anomalies have been reported in 65% and 30% of patients respectively and may precede the development of intestinal polyps and the colorectal cancer (Almeida et al., 2016; Septer et al., 2018; Almeida et al., 2020).

Thakker et al. (1995) developed a diagnostic method using panoramic radiographs to identify high-risk FAP patients called Dental Panoramic Radiograph Score (DPRS). This method has demonstrated a significant association between the presence of substantial changes and the presence of FAP, allowing the early diagnosis of the disease (Aggarwal et al., 2003; Thakker et al., 1995). Although the literature presents several studies showing the phenotypic characterization of FAP, studies still lack on showing the genotypic correlation with the clinical data of patients with FAP. Thus, the present study aimed to perform a phenotype characterization of a family with FAP in order to detail the dento-osseous anomalies using the DPRS diagnostic method to determine its diagnostic accuracy. In addition, genetic analyses using whole-exome sequencing and Sanger sequencing validation were performed.

Methods

This project was approved by the Research Ethics Committee of the Health Sciences Faculty, University of Brasília with a Certificate of Presentation for Ethical Appreciation number 12696913.0.0000.0030 and it is in accordance with the Declaration of Helsinki.

The study includes one Brazilian family with FAP consisting of 14 individuals divided into 2 groups, Group A comprised of FAP-confirmed patients (n=5), and Group B with family members without FAP diagnosis (n=9). The diagnosis of FAP was based on confirmation in medical records, and the evaluated criteria were the number of polyps and the age at diagnosis of FAP. This cross-sectional study performed a phenotype characterization of a family with FAP syndrome by the description of radiographic findings using the Dental Panoramic Radiograph Score (DPRS) diagnostic method. Also, we evaluated the proportion of dento-osseous anomalies in FAP patients (Group A) comparing to non-FAP family members (Group B). Medical and dental records were reviewed for contributory history. Furthermore, we carried out a genetic analysis to investigate the causative variant in the family.

All patients were submitted to the following procedures: signature of informed consent; in cases of underage patients, the consent form was signed by the legal responsible; clinical examination with anamnesis, including extra and intraoral physical examination; and panoramic radiography for diagnosis. Cone-beam Computed Tomography (CBCT) was performed exclusively in patients with bone and dental alterations that needed further investigation. For genetic analysis, DNA was isolated from the index patient's saliva.

Phenotype characterization

All patients from a family with classical FAP were evaluated at the School of Dentistry and Pharmacy Center of the University Hospital of Brasília (Brasília, Brazil). Since the index patient referred other affected patients in the family, all family members were contacted and invited to participate in the study.

Familial adenomatous polyposis is a clinical diagnosis that is typically based on the presence of more than 100

colorectal adenomas, age at diagnosis of FAP and occurrence of colorectal cancer (Friedl and Arentz, 2005). Thereby, anamnesis included an investigation for colorectal cancer and other malignancies as well as family history. The analysis of medical records available at the University Hospital of Brasília confirmed all information such as the presence of FAP and colorectal cancer. In order to confirm the autosomal dominant inheritance, a heredogram was performed using GenoPro2018 - version 3.0.1.4 (Brazil, 2019).

The extraoral physical examination evaluated facial asymmetry, skin, scalp, and lymph nodes. The intraoral examination thoroughly assessed the oral cavity (oral mucosa and teeth). A panoramic radiograph was performed on all patients, using the Kodak 8.000C Digital Panoramic and Cephalometric System (Trophy, France), to diagnose and/or monitor the dento-osseous anomalies. CBCT was performed on an I-CAT Platinum device (Imaging Sciences International, United States) with the following technical parameters: 120kVp, 8mA, and voxel size of 0.2mm. Advanced imaging was acquired to assist in the diagnosis and extension of the lesions.

For the assessment of dento-osseous anomalies, panoramic radiographs were blindly analyzed by two Oral and Maxillofacial Radiologists (OMR) evaluators, following the validated DPRS criterion by Thakker et al. (1995). This method considers the nature, extent, frequency, and location of dento-osseous anomalies noted on panoramic radiographs of FAP patients compared to unaffected individuals (Thakker et al. 1995). The DPRS considers four possible outcomes: 1- normal changes, 2- minimal changes, 3- ambiguous changes, and 4- significant changes. Each outcome is associated with a final score, which was obtained by adding the individual scores for each dento osseous anomaly (Appendix I and II). The size and quantity of these anomalies are taken into consideration to reflect the clinical level of significance if this anomaly occurs in isolation.

To determine the diagnostic accuracy of this tool, several parameters were established (specificity, sensitivity, false positive and negative rate, positive and negative predictive accuracy, and precision) from the final DPRS values for Groups A and B.

Aiming to measure the agreement between the evaluators and the reproducibility of the DPRS, the reliability test was carried

out twice by the same OMR, using the same images within a 1-month interval.

Genetic analysis

Genomic DNA from the index patient was isolated from buccal mucosa cells in saliva as previously described (Aidar and Line, 2007; Almeida et al., 2020).

Whole-exome sequencing was performed in the sequencing facility Macrogen (Seoul, Korea) using Sure select V6 for captures and sequenced in Illumina platform. Candidate variants were filtered against exome data in publicly available databases, including the 1000 Genomes Project, ExAC, UCSC common Single Nucleotide Polymorphisms database, ClinVar, and results of “in silico” algorithms (softwares Polyphen, SIFT, MutationTaster, CADD, Human Splicing Finder). Variants were filtered out if they had low quality, insertions/deletions in regions of homopolymers, and allelic imbalance greater than 80:20. Furthermore, the pathogenicity of variants was determined based on the latest American College of Medical Genetics guidelines (Richards et al, 2015)

To validate the heterozygous nonsense variant detected by the whole-exome sequencing, the Polymerase chain reaction and Sanger sequencing of *APC* exon 11 was carried out according to standard protocols. Primers were designed using ExonPrimer software (http://ihg.gsf.de/ihg/ExonPrimer.html).

Statistical analysis

Fisher's exact test was applied to investigate the association between patients with FAP / non-FAP and the presence of dento-osseous anomalies through the DPRS results. The Cohen’s Kappa Coefficient (k), a quantitative measure of the magnitude of agreement, was applied to analyze the intra and inter reliability between the OMR while evaluating the images. The parameters specificity, sensitivity, false positive (FP) and negative rate (FN), positive predictive value (PPV) and negative predictive value (NPV) accuracy, and precision were determined for the diagnostic study. Analysis of the data was conducted using The Statistical Package for the Social Sciences (version 22;

SPSS, IBM, Armonk, NY). P-values below 0.05 indicate statistical significance.

Results

A total of five affected and nine unaffected family members (Figure 1A) were included in the study showing the autosomal dominant inheritance usually present in the classic FAP form. Two of the five patients in Group A (II.09 and III.30), developed colorectal cancer in the third decade of life, and the patient III.30 progressed to liver and lung metastasis. However, these individuals were already diagnosed at a late stage with adenocarcinoma. Patient III.03 was diagnosed with FAP in 2011 at 32 years old, and five years later developed gastric adenomas with low-grade dysplasia. Patient IV.02 was diagnosed in childhood (11 years old) and although had less than 100 intestinal adenomas at that date, five years later the intestinal polyps counted more than 100. Patient II.17 presented tubular adenomas with moderate rectal dysplasia nine years after the initial diagnosis of FAP. The treatment performed was total proctocolectomy with ileal pouch and protective ileostomy. The demographics of the affected individuals are summarized in Table 1.

Genetic findings

Whole-exome sequencing was performed in the index patient (II.09). The overall targeted nucleotide coverage was 99.8% at ≥ × 20. An heterozygous nonsense variant was identified at exon 11 (NM_000038.5: c.1370C>G, p.Ser457*) and confirmed by Sanger sequencing (Figure 1B). This variant was predicted to be deleterious and disease causing by SIFT, Polyphen2, and Mutation Taster with a CADD score of 38 and predicted pathogenic according to ACMG guidelines.

Clinical and radiographic findings

Of all the individuals analyzed by the DPRS method, 80% of Group A (n = 5) was classified with significant changes, DPRS ≥ 7, and 20% was classified with normal changes, DPRS 0-2. In Group B (n = 9), only one patient was classified with minimal changes (Score 3-4) and all the other eight patients were

classified with normal changes (Score 0-2). There was an association between DPRS 0-2, and absence of FAP (p=0.005), and DPRS≥ 7 was related to group A (p=0.005).

The radiographs of patients in Group A showed that 20% of them presented supernumerary tooth, 40% showed osteomas; 40% had hazy sclerosis (not so defined increase in bone density) associated with the root of a single tooth; 40% had hazy sclerosis not associated with teeth roots; 60% had dense bone islands (well-defined radiodensities with irregularly shaped margins) (Figure 2 - 4). Group B showed that only 11.1% of the group presented hazy sclerosis not associated with teeth roots and the others did not present any dento-osseous anomalies. Images from the patient index II.09 (Figure 2A-D) showed area of dense bone island in the mandible, and an osteoma located at the right condyle neck (this finding was confirmed on the CBCT). Patient III.03 (Figure 3A-E) presented areas of dense bone island and hazy sclerosis associated with the teeth roots. In addition, an osteoma located in the left maxillary sinus was detected on the CBCT scan. However, the osteoma was not considered in the DPRS evaluation, because it could not be seen in the panoramic radiograph. Patient IV.02 (Figure 4A-B), 14 years old, presented hazy sclerosis associated and non-associated with the teeth roots. Besides, a supernumerary tooth localized between the right lateral and central incisors, as seen in the periapical radiograph (Figure 4B). The supernumerary was not considered in the DPRS evaluation, but even so, the patient presented DPRS score=8 significant changes. Patient III.30, the only female, presented hazy sclerosis and dense bone island bilaterally in the mandible (Appendix III). It is important to reinforce that the DPRS was calculated according to the dento-osseous alterations detected on the panoramic radiograph, therefore alterations identified in other images modalities were not considered for this purpose. The frequency of anomalies in each group is detailed in Figure 5A. Table 2 describes the dento-osseous changes found in all patients in this study and their respective DPRS.

Diagnostic capability of the DPRS

The reliability of the DPRS test evaluated specificity, sensitivity, FP and FN rates, PPV, NPV, and precision for different outcomes. When significant changes (DPRS ≥7) were

counted as positive for the diagnosis of FAP and all other categories were classified as negative, 100% of specificity and PPV, 93% of precision, 90% of NPV, 80% of sensitivity and 20% FN rate were obtained. When the significant and ambiguous changes (DPRS 5-6) were counted as positive for the diagnosis of FAP and the other categories were classified as negative, we obtained the same results as the previous classification and the FP rates remained zero. When the significant, ambiguous, and minimal changes (DPRS 3-4) were counted as positive for the diagnosis of FAP and only normal changes (DPRS 0-2) were excluded, there was a reduction in specificity, NPV, precision, and PPV to 89%, 89%, 86%, and 80% respectively, and an increase in the FP rates to 11% (Figure 5B). Therefore, the results of this analysis suggest that it is reliable to diagnose as FAP the individuals who have DPRS values relative to significant and ambiguous changes and that it is not reliable to take as positive for FAP diagnostics DPRS results equal to or below 3-4, including all the changes (minimal, ambiguous, and significant changes). Concerning all the dependent variables evaluated, the inter, k= 1 (p=0.000), and intrareliability values, k1=0.81 (p=0.002) k2=1 (p=0.000) respectively to evaluators 1 and 2, were excellent.

Discussion

The early diagnosis of individuals affected by FAP is essential in preventing the development of colorectal cancer and consequent improvement in the prognosis of the disease (Dinarvand et al., 2019; Septer et al., 2018; Aggarwal et al., 2003). For this reason, it is necessary to better understand the clinical phenotype of this condition including the extraintestinal manifestations.

In the present study, the difference between the frequency of dento-osseous anomalies observed in the affected group compared to the group of undiagnosed family members was significant (p=0.005). These results demonstrate that dento-osseous changes may be present in groups of people without FAP, but these changes are not significant in this population. These anomalies appear in a higher proportion in affected patients; 4: 5 Group A patients with FAP compared to 1: 9 Group

B. In this study, as described by others (Almeida et al., 2016; Almeida et al.,2020; Aggarwal et al., 2003; Thakker et al., 1995), a higher frequency of bone alterations (osteomas, islands of bone condensation, and diffuse sclerosis) was observed in comparison to the presence of dental anomalies in affected patients (p=0.001).

Numerous studies demonstrated that the presence of osteomas can be an important marker of FAP, highlighting the importance of knowledge about this anomaly and its potential in early diagnosis of FAP (Almeida et al. 2016; Septer et al., 2018; Ida et al., 1981; Bülow et al., 1984; Wolf et al., 1986; Katou et al., 1989; Bertario et al., 2001). In the present study, this anomaly was present in 40% of the assessed affected patients. The dense bone islands, presented in 60% of FAP patients, represent a focal increase in bone density without any obvious etiological agent. Based on this unknown origin, the term idiopathic osteosclerosis is also frequently used as a synonym of dense bone islands (McDonnell, 1993; Halse and Molven, 2002; Ledesma et al., 2019). On the other hand, hazy sclerosis associated with the root of a single tooth can occur in unaffected patients as an inflammatory condition in the bone in response to periodontal disease. However, this condition is already accounted for in the DPRS method. Hazy sclerosis associated with the root of a single tooth has a lower score than hazy sclerosis not associated with teeth roots. Therefore, even though it is a common bone disorder in the general population, it is more prevalent in the FAP-affected population (Thakker et al., 1995).

Supernumerary tooth, present in 20% of FAP patients from our sample, represents the only dental anomaly finding of the present study. Most supernumerary teeth are sporadic, although they may also occur in genetic syndromes. Some of them, including FAP, present strong evidence of the association with supernumerary teeth (Lubinsky and Kantaputra, 2016). The reported prevalence of supernumerary tooth in FAP patients varies between 11% and 27%, significantly higher than in the general population (Wijn et al., 2007). Odontomas, which are dental features commonly found in the incisors and premolars region, are reported in FAP patients with frequencies between 9.4% and 83.3%, significantly higher than the prevalence of 0 to 4% in the control groups (Ida et al., 1981; Wijn et al., 2007;

Owosho et al., 2013). Despite the considerable prevalence, there were no cases of odontomas in the studied family.

To estimate and discuss the worldwide prevalence of dento-osseous anomalies in patients with FAP, we performed a pooled prevalence analysis of 18 previously selected studies (Appendices IV-IX). Supplementary data about the inclusion and exclusion criteria for studies are described in detail in Appendix IV. The statistical analysis showed that the worldwide prevalence of bone and dental anomalies in the positive FAP population was 69% and 30%, respectively (Appendix V and Appendix VI). Analyzing the results separately by continent, Asian patients had presented a higher prevalence of dento-osseous anomalies compared to the other continents investigated, with 82% prevalence of bone and 45% dental alterations. In contrast, North American patients had a lower prevalence of dento-osseous anomalies compared to all other continents, showing a prevalence of bone and dental anomalies of 35% and 14%, respectively. Bone lesions were more frequent than dental anomalies on all continents (Appendix VII). There were no studies concerning the population of Africa and Oceania. There is no scientific evidence about the worldwide prevalence of dento-osseous anomalies in the population with FAP. Further investigations, as well as the development of multicentric studies regarding ethnic influence on the prevalence of oral and extra-intestinal manifestations in FAP, are still needed.

In the current study, a heterozygous nonsense variant in *APC* exon 11 (c.1370C>G, p.Ser457*) was identified in the index patient with a diagnosis of classic FAP. This variant has been previously reported although the described dento-osseous anomalies were not associated with this *APC* variant (Michils et al, 2002; Friedl and Arentz, 2005; Stekrova et al, 2007; Lagarde et al, 2010). Although 75% of the *APC* coding region is in exon 15, this stop codon at the beginning of the gene promotes the termination of protein translation that could result in a non-functional apc protein, or nonsensemediated decay of the transcript (Wallis et al., 1999; Nykamp et al., 2020). In several studies, an association between the location of *APC* variants and the phenotype in FAP patients has been described (Davies et al.,1995; Torrezan et al., 2013, Newton et al., 2012). However, only a few studies discuss the genotype related to dental phenotype (Septer et al.,2018; Davies et al., 1995). In particular,

jaw osteomas are the most common lesions described in the genotype-phenotype studies and they are related to classic FAP and variants in exon 15 around codons 1310–1444 (Nieuwenhuis and Vasen, 2007). Thus, we can speculate that the variant found in our FAP patient may be associated with a phenotype with more bone changes than dental manifestations.

Concerning the reliability of the DPRS diagnostic test, Thakker et al. (1995) showed a small reduction of the specificity percentage when ambiguous changes were counted as positive for the diagnosis of FAP. Whereas in this study it occurred only When the minimum changes were counted as positive. However, overall, specificity tended to decrease, and sensitivity tended to increase in the same trend as reported by Thakker et al. (1995). It was demonstrated that the diagnostic reliability of DPRS is higher when only significant and ambiguous changes are counted as positive for the diagnosis. In the reproducibility analysis of the DPRS method, excellent results of Kappa values were demonstrated. These results suggest that the DPRS method is reliable and reproducibly (Viera and Garrett, 2005). Furthermore, DPRS is a method that can be better explored in the dental routine and assist in the early diagnosis of FAP in a non-invasive and efficient manner.

Conclusion

The study has confirmed that patients with FAP have a high prevalence of dentoosseous anomalies, with bone alterations being more frequent than dental manifestations. Among the bone anomalies, dense bone islands were the most frequent lesion identified. Furthermore, the *APC* heterozygous nonsense variant was identified in this particular family. Since the current cross-sectional study is based on one family, we suggest further research with a larger number of individuals with genotype and dento-osseous phenotype to better understand the occurrence of these extraintestinal features in FAP patients. We recommend the referral of FAP patients for dental examination and genetic counseling to perform early diagnosis of dento-osseous anomalies and evaluate the implications of the molecular findings in each particular family.

Figure 1

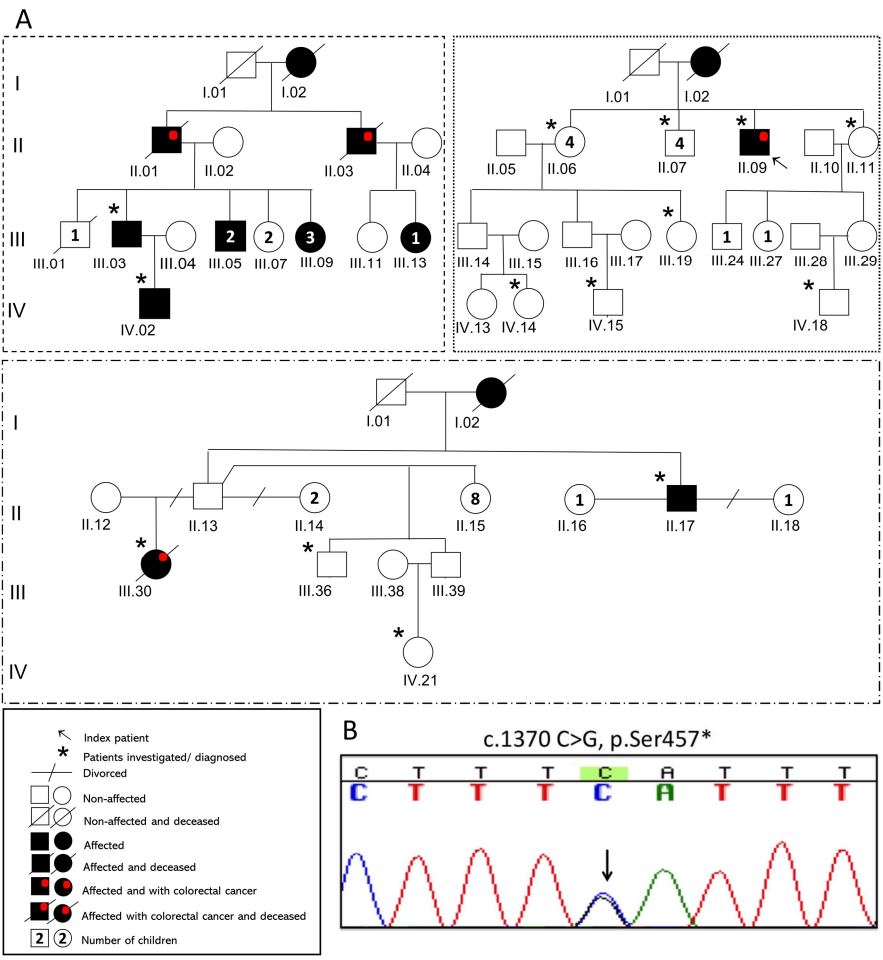

Figure 1- (A) Complete Brazilian family pedigree divided into 4 sections. (B) Sanger sequencing validation of the base substitution first detected by whole-exome sequencing.

Table 1: Demographic characteristics of Familial Adenomatous Polyposis patients

Patients	Inheritance pattern	Sex	Age	Age at FAP diagnosis (y)	Age at CRC diagnosis (y)	Disease	Dento-osseous anomalies	Extraintestinal manifestations
II.09 (index)	AD	M	66	39	39	FAP	Yes	Gastric polyps in the antrum region associated with flat erosions (2 -3 mm)
II.17	AD	M	57	32	No CRC	FAP	No	None reported
III.03	AD	M	41	32	No CRC	FAP	Yes	Gastric polyps in the antrum region with low-grade dysplasia
III.30	AD	F	41	34	39	FAP	Yes	None reported
IV.02	AD	M	14	11	No CRC	FAP	Yes	None reported

Abreviations: AD: autosomal dominant; CRC: Colorectal carcinoma; F: female; FAP: Familial Adenomatous Polyposis; M: male.

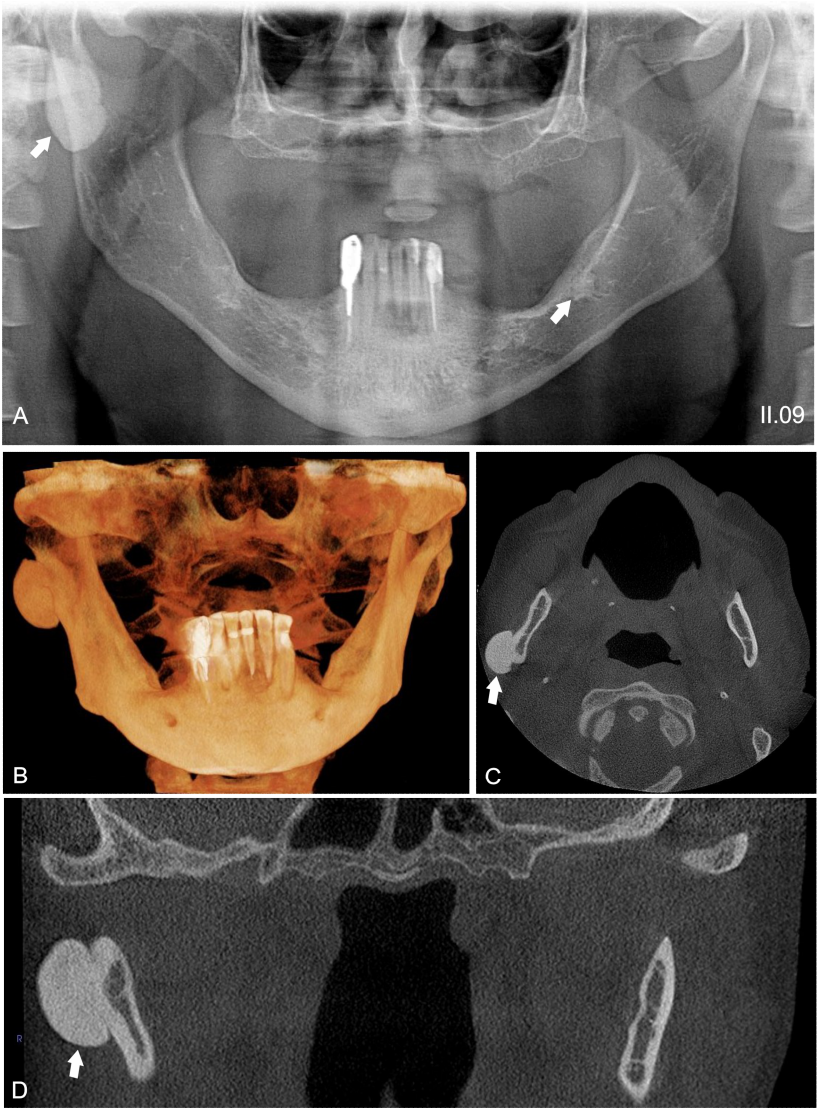

Figure 2- Dento-osseous anomalies found on index patient (white arrows). (A) Patient II.09- Osteoma at the right ramus of the mandible and dense bone island in the body of the mandible on the left side. (B) CBCT 3D reconstruction showing the osteoma. (C, D) CBCT axial and coronal showing the osteoma.

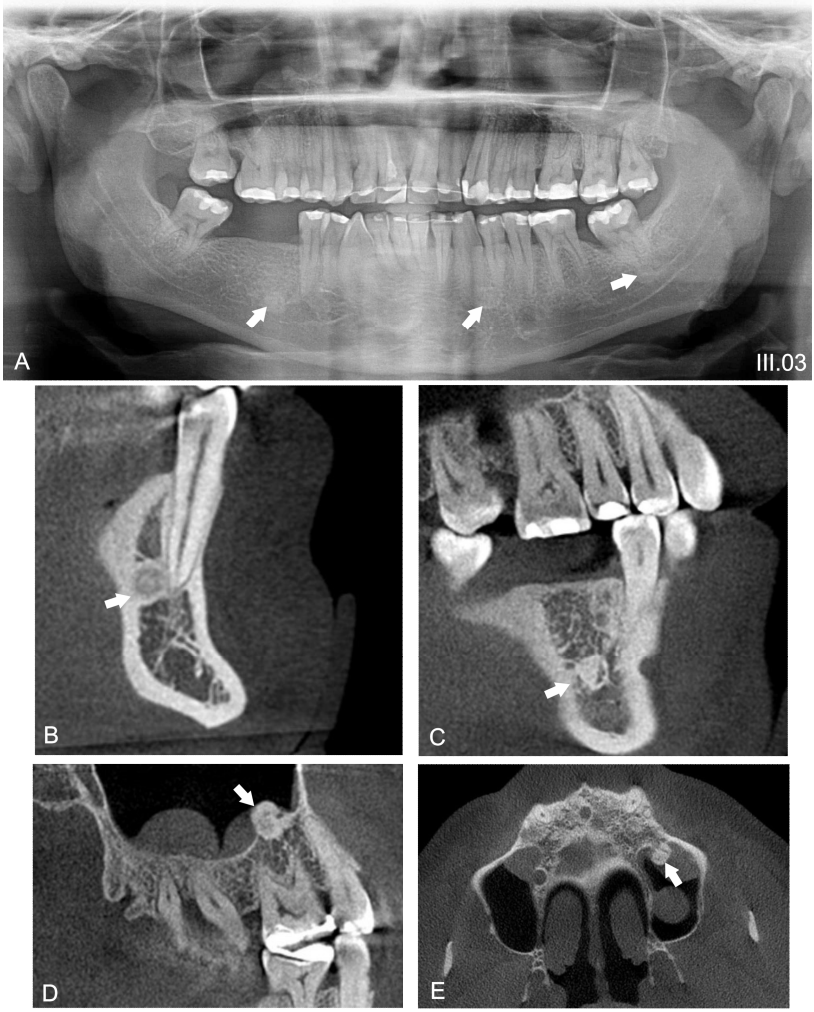

Figure 3- Dento-osseous anomalies in a FAP affected patient (white arrows). (A) Patient III.03- Dense bone island area in the region of the absent lower right first molar, and presence of hazy sclerosis associated with the roots of the lower left first premolar and third molar. (B) CBCT sagittal showing hazy sclerosis associated with the roots of the lower left first premolar. (C) CBCT sagittal demonstrating dense bone island area in the region of the absent lower right first molar. (D, E) CBCT sagittal and axial showing the osteoma in the left maxillary sinus.

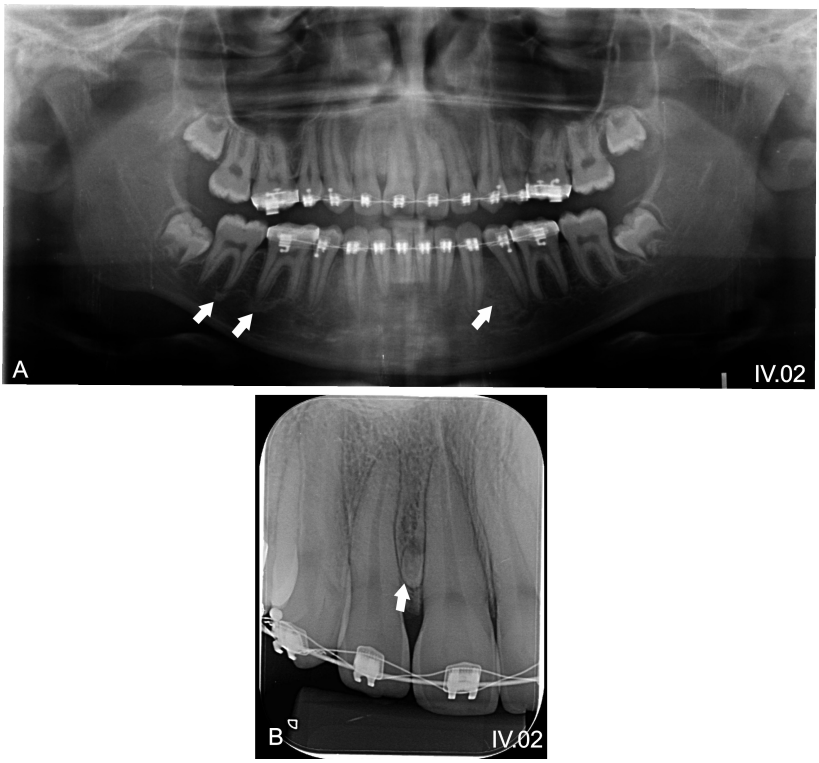

Figure 4- Bone alterations in a FAP affected patient (white arrows). (A) Patient IV.02- Hazy sclerosis area associated with the roots of the lower right first and second molar, and hazy sclerosis not associated with roots between the canine and the second lower left premolar. (B) Periapical radiographs showing an supernumerary tooth between the roots of the right central and lateral incisors on the same patient.

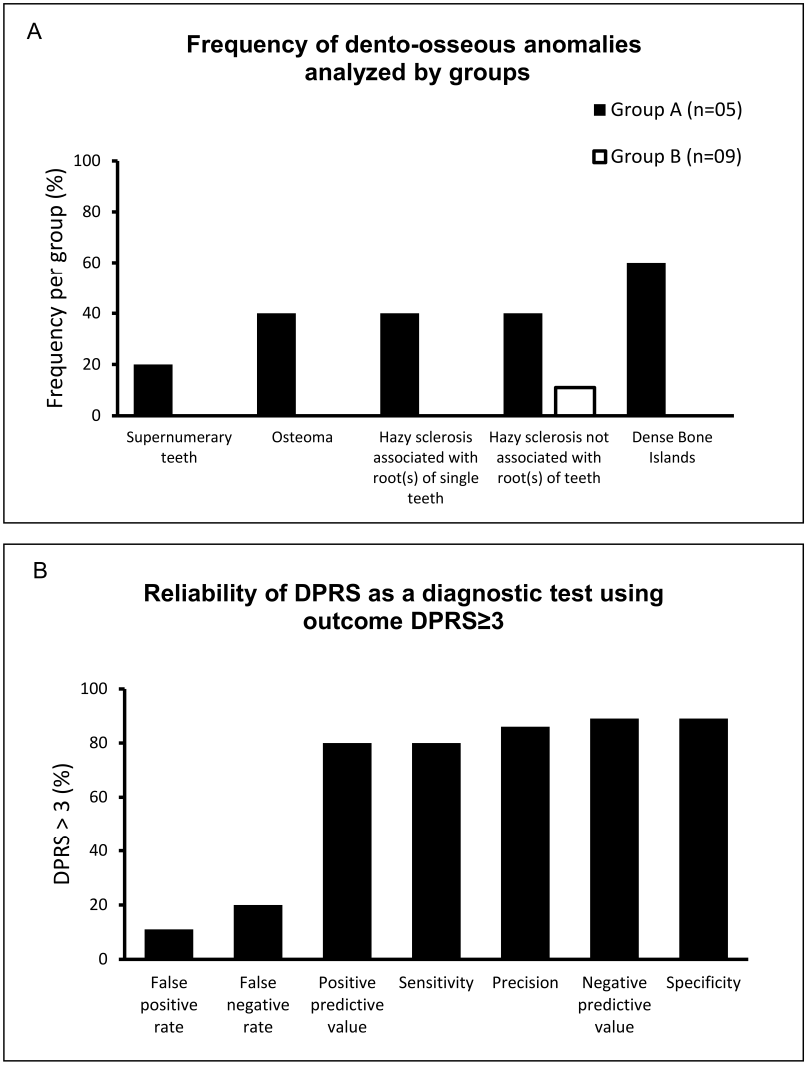

Figure 5- (A) Frequency of dento-osseous anomalies analyzed by groups. (B) Reliability of Dental Panoramic Radiograph Score (DPRS) as a diagnostic test using outcome DPRS ≥3 as a positive result for the diagnosis of FAP.

Table 2: Clinical features of Familial Adenomatous Polyposis population and Dental Panoramic Radiograph Score (DPRS) results.

Patients	FAP	DPRS scores	Osteoma	Hazy sclerosis associated with root(s) of single teeth	Hazy sclerosis associated with root(s) of multiple teeth	Hazy sclerosis not associated with root(s) of teeth	Hazy sclerosis diffuse	Dense bone island	Supernumerary tooth
II.06	ND	0	-	-	-	-	-	-	-
II.07	ND	4	-	-	-	+ (1/ 2.5)	-	-	-
II.09	+	14	+ (1/ 3.5)	-	-	-	-	+ (1/ 1.5)	-
II.11	ND	0	-	-	-	-	-	-	-
II.17	+	0	-	-	-	-	-	-	-
III.03	+	8	+ (1/ 0.5)	+ (2/ -)	-	-	-	+ (1/ 1)	-
III.19	ND	0	-	-	-	-	-	-	-
III.30	+	10	-	-	-	+ (1/ 0.5)	-	+ (2/ 2)	-
III.36	ND	0	-	-	-	-	-	-	-
IV.02	+	8	-	+ (2/ -)	-	+ (1/ 1.5)	-	-	+ (1/ -)
IV.14	ND	0	-	-	-	-	-	-	-
IV.15	ND	0	-	-	-	-	-	-	-
IV.18	ND	0	-	-	-	-	-	-	-
IV.21	ND	0	-	-	-	-	-	-	-

Abreviations: +: present; -: absent; ND: Not determined. FAP: Familial Adenomatous Polyposis. DPRS: Dental Panoramic Radiograph Scores.

Notes: 1) The values inside the parentheses show the number and size in centimeters of the dento-osseous anomalies, respectively. 2) The score values are in accordance with the table provided in Appendix I. 3) The supernumerary teeth and the osteoma respectively seen in the periapical radiograph and CBCT scan were not included in Table 2 as it relates to the DPRS only.

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Appendix I

Dental panoramic radiograph scoring system.Weighted scores for number and size (where appropriate) for each anomaly are shown.(Thakker *et al.*1995)[17].

	Scoring criteria			
DPR anomaly	No	Score	Size (cm)	Score
Osteoma	0	0	≤0.5	0
	1	4	>0.5 ≤ 2.0	3
	2	7	>2.0 ≤4.0	6
	3-5	9	>4.0 ≤6.0	8
	6-8	11	>6.0	10
	(+1-3)	(+2)		
Dense bone island	0	0	≤0.5	0
	1	2	>0.5 ≤ 2.0	2
	2	4	>2.0	4
	3-5	6		
	6-8	8		
	(+1-3)	(+2)		
Hazy sclerosis associated with root(s) of single teeth	1 área	2	-	-
	>2 áreas	4		
Hazy sclerosis associated with root(s) of multiple teeth	≥1	4	-	-
Hazy sclerosis not associated with root(s) of teeth	1	2	≤1.0	0
	≥2	4	>1.0	2
Hazy sclerosis diffuse	-	12	-	-
Odontomas	0	0	-	-
	1	7		
	2	9		
	(+1)	(+2)		
Supernumerary teeth (unerupted or erupted)	0	0		
	Mesiodens	3		
	1	6		
	2	9		
	(+1)	(+2)		
Unerupted teeth	0	0		
	1	3		
	2	5		
	3	7		
	(+1)	(+2)	-	-

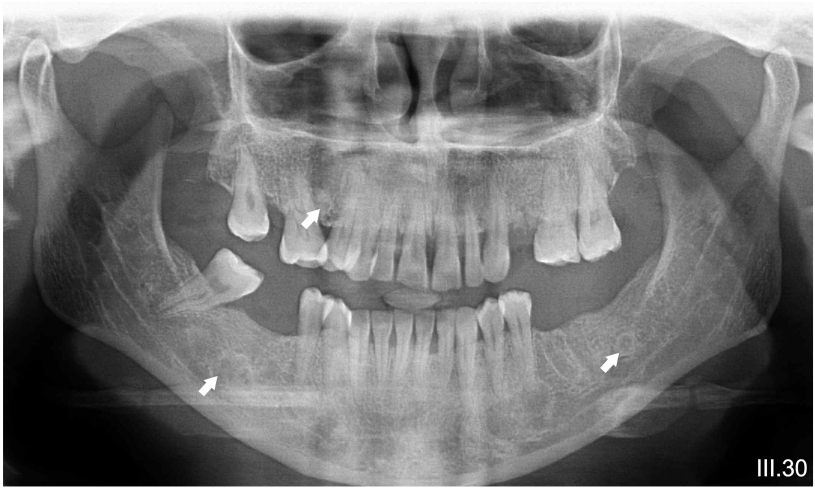
Appendix II

Significance of the dental panoramic radiograph scores (DPRS).

Dental Panoramic Radiograph Score (DPRS)	Outcome
0-2	Normal
3-4	Minimal change(s)
5-6	Ambiguous change(s)
≥7	Significant change(s)

Source: Thakker *et al.* (1995)[17].

Appendix III



Appendix III- Panoramic Radiographs showing dento-osseous anomalies in FAP affected patient (white arrows). Patient III.30- Hazy sclerosis area in the region of the absent upper right second premolar and the presence of dense bone island bilaterally on the body of the mandible

Appendix IV

Detailed data about studies studies for the literature review and the pooled prevalence analysis. Literature review

A literature review was carried out on studies that reported the presence of oral manifestations (dental, bone, and mucosal anomalies) in patients with FAP at any age. Cohort, case-control, and cross-sectional studies published up to August 2020 without language limitation were included. For this, the search conducted on Almeida et al. (2016) until March 2015 was considered, and a new search was performed to include articles posteriorly published using the same inclusion and exclusion criteria. The studies were selected based on individual search strategies for the following databases: Cochrane Library, Embase, Lilacs, PubMed, Scopus, and Web of Science electronic. The grey literature was also assessed on Google Scholar (Appendix VIII and Appendix IX). As result, 18 studies were included (Almeida et al., 2016 = 16 studies; Present study = 02 studies) to the achievement of a pooled prevalence analysis of the disease, using the IBM SPSS Statistics Base 22.0 version for Windows (Brazil, 2020).

Studies that reported the presence of oral manifestations (dental, bone, or mucosal changes) in children or adults diagnosed with FAP were included. No time or language restrictions were imposed. The exclusion criteria were: 1) other colorectal diseases different of FAP; 2) patients without oral manifestations; 3) reviews, letters, personal opinions, book chapters, conference abstracts, posters, patents, case reports, and case-series; 4) different target condition such as correlation between the severity of the dento–osseous phenotype with *APC* mutation; and 5) studies with results not individualized for FAP syndrome. The results are summarized in Appendix V - VII.

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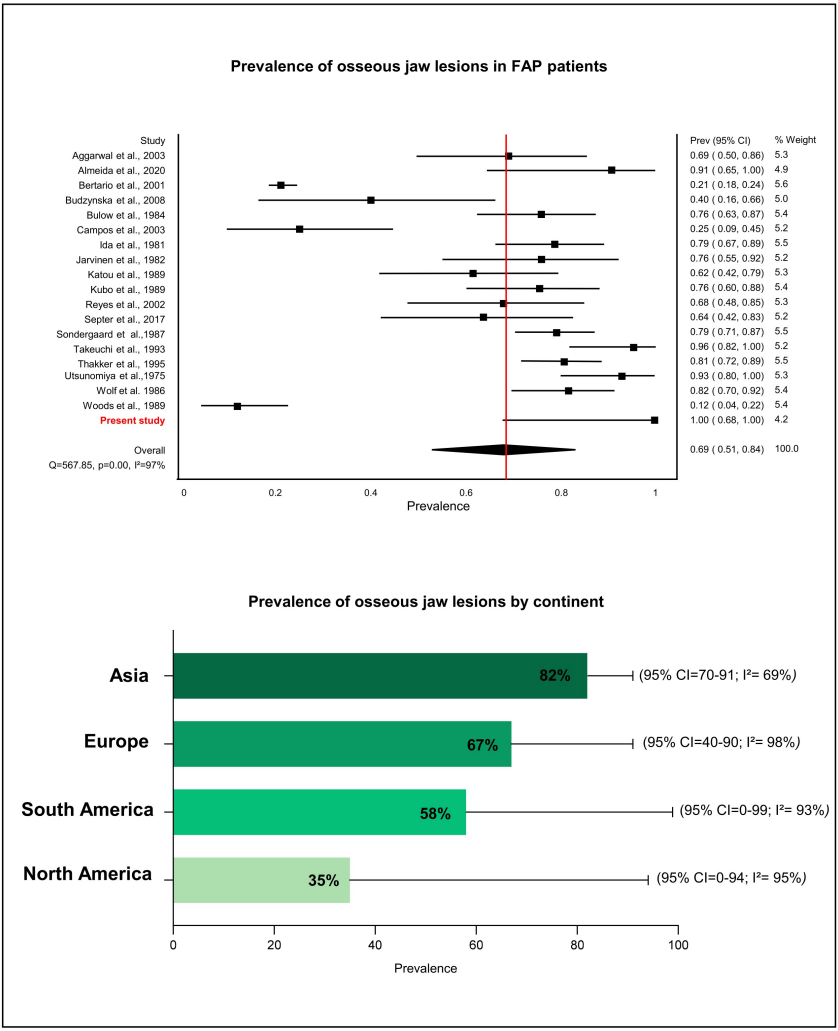
Appendix V

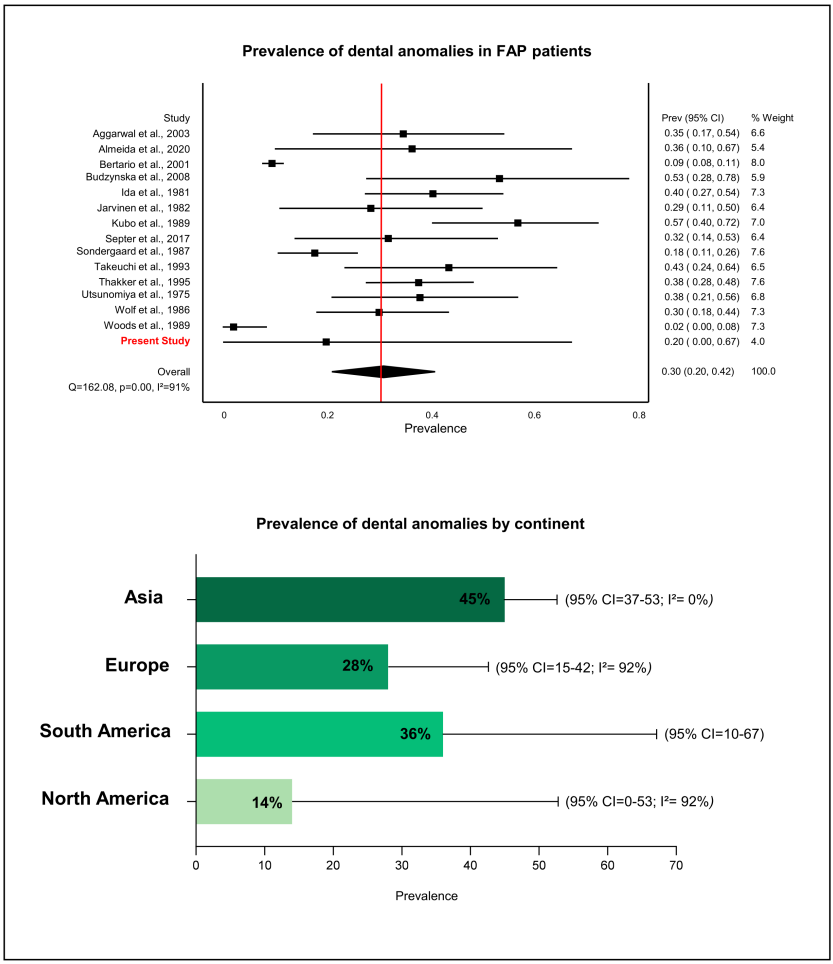
Worldwide prevalence of osseous jaw lesions in patients with FAP. Figure elaborated in the program IBM SPSS Statistics 22.0 for Windows.



Abreviations: CI confidence interval; Prev – prevalence.

Appendix VI

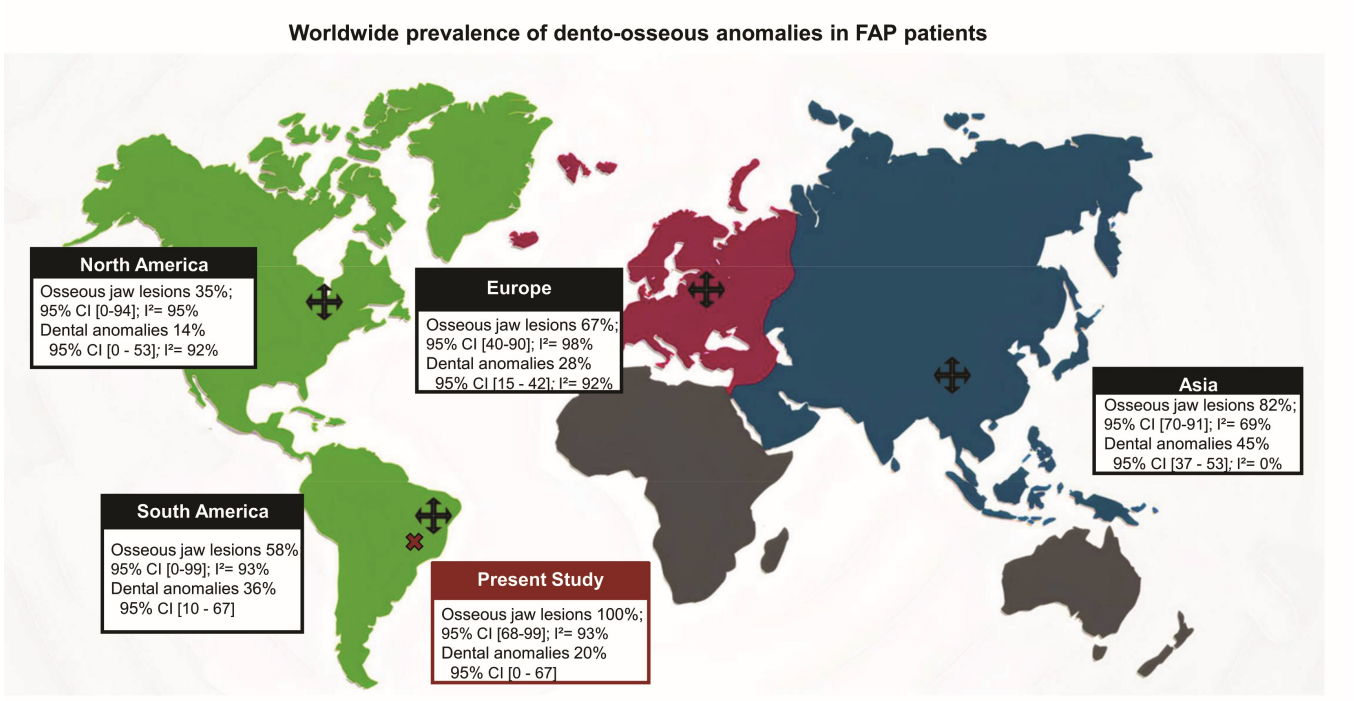
Worldwide prevalence of dent anormalies in patients with FAP. Figure elaborated in the program IBM SPSS Statistics 22.0 for Windows.



Abreviations: CI –confidence interval; Prev – prevalence.

Appendix VII

Worldwide prevalence of dento-osseous anomalies in FAP. Abreviations: CI –confidence interval



Appendix VIII

Summary table of studies found from 15 March 2015 to 11 August 2020.

Electronic databases	SR search 12.03.2015	Current 11.08.2020	New studies 12.03.2015 – 11.08.2020
Pubmed	466	662	164
Web of Science	341	407	114
Lilacs	09	20	01
Cochrane	23	39	21
Scopus	576	720	115
Google Scholar	49	49	34

SR: sistematic review.

Appendix IX

Search for applications in the databases.

Electronic databases	Search	Results
Pubmed	((("Adenomatous Polyposis Coli"[Mesh] OR "Familial Adenomatous Polyposis" OR "Gardner Syndrome" OR "Gardner's Syndrome" OR "Familial Colon Cancer" OR "Familial Colorectal Cancer" OR "Hereditary Colon Cancer" OR "Hereditary Colorectal Cancer" OR "Hereditary Gastrointestinal Disease" OR "Hereditary Polyposis Conditions" OR "Familial Adenomatosis Coli")) AND (("Oral Manifestations"[Mesh] OR "Oral Manifestations" OR "Oral Findings" OR "Maxillofacial Manifestations" OR "Dento Osseous Changes" OR "Dento Osseous Anomalies" OR "Dento Osseous Alterations" OR "Osteomatosis of the Jaw" OR "Osteomatosis" OR "Osteomas" OR "Oral Alterations" OR "Head and Neck" OR "Oral Cavity" OR "Oral" OR "Bucal" OR "Diffuse Sclerosis" OR "Odontomas" OR "Supernumerary Teeth" OR "External Manifestations" OR "Dento-Maxillary Stigmas" OR "Radiological Findings" OR "Radiopaque Lesions")))	662
Web of Science	ALL=("Familial Adenomatous Polyposis*" OR "Gardner Syndrome*" OR "Gardner's Syndrome*" OR "Familial Colon Cancer*" OR "Familial Colorectal Cancer*" OR "Hereditary Colon Cancer*" OR "Hereditary Colorectal Cancer*" OR "Hereditary Gastrointestinal Disease*" OR "Hereditary Polyposis Conditions*" OR "Familial Adenomatosis Coli*")ALL=("Oral Manifestations*" OR "Oral Findings*" OR "Maxillofacial Manifestations*" OR "Dento Osseous Changes*" OR "Dento Osseous	407

	Anomalies*" OR "Dento Osseous Alterations*" OR "Osteomatosis of the Jaw" OR "Osteomatosis*" OR "Osteomas*" OR "Oral Alterations*" OR "Head and Neck*" OR "Oral Cavity*" OR "Oral*" OR "Buccal*" OR "Diffuse Sclerosis*" OR "Odontomas*" OR "Supernumerary Teeth*" OR "External Manifestations*" OR "Dento-Maxillary Stigmas*" OR "Radiological Findings*" OR "Radiopaque Lesions*")	
Lilacs	"Síndrome de Gardner" OR "Polipose Adenomatose do Colo" OR "Poliposis Adenomatosa del Colon" AND "Manifestações Bucais" OR "Manifestaciones Bucales" OR "Anomalías Dentarias" OR "Anormalidades Dentárias" OR "Pólipos do Colo"	20
Cochrane	"Familial Adenomatous Polyposis" OR "Gardner Syndrome" OR "Gardner's Syndrome" OR "Familial Colon Cancer" OR "Familial Colorectal Cancer" OR "Hereditary Colon Cancer" OR "Hereditary Colorectal Cancer" OR "Hereditary Gastrointestinal Disease" OR "Hereditary Polyposis Conditions" OR "Familial Adenomatosis Coli" AND "Oral Manifestations" OR "Oral Findings" OR "Maxillofacial Manifestations" OR "Dento Osseous Changes" OR "Dento Osseous Anomalies" OR "Dento Osseous Alterations" OR "Osteomatosis of the Jaw" OR "Osteomatosis" OR "Osteomas" OR "Oral Alterations" OR "Head and Neck" OR "Oral Cavity" OR "Oral" OR "Buccal" OR "Diffuse Sclerosis" OR "Odontomas" OR "Supernumerary Teeth" OR "External Manifestations" OR "Dento-Maxillary Stigmas" OR "Radiological Findings" OR "Radiopaque Lesions"	39
Scopus	(TITLE-ABS-KEY ("Oral Manifestations" OR "Oral Findings" OR "Maxillofacial	720

	Manifestations" OR "Dento Osseous Changes" OR "Dento Osseous Anomalies" OR "Dento Osseous Alterations" OR "Osteomatosis of the Jaw" OR "Osteomatosis" OR "Osteomas" OR "Oral Alterations" OR "Head and Neck" OR "Oral Cavity" OR "Oral" OR "Buccal" OR "Diffuse Sclerosis" OR "Odontomas" OR "Supernumerary Teeth" OR "External Manifestations" OR "Dento-Maxillary Stigmas" OR "Radiological Findings" OR "Radiopaque Lesions")) AND (TITLE-ABS-KEY ("Familial Adenomatous Polyposis" OR "Gardner Syndrome" OR "Gardner's Syndrome" OR "Familial Colon Cancer" OR "Familial Colorectal Cancer" OR "Hereditary Colon Cancer" OR "Hereditary Colorectal Cancer" OR "Hereditary Gastrointestinal Disease" OR "Hereditary Polyposis Conditions" OR "Familial Adenomatosis Coli")) AND (LIMIT-TO (DOCTYPE , "ar") OR LIMIT-TO (DOCTYPE , "re") OR LIMIT-TO (DOCTYPE , "ip"))	
Google Scholar	"Familial Adenomatous Polyposis" AND "Gardner Syndrome" AND "Oral Manifestations"	49

Annex I Approval of the Research Ethics Committee

FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - CEP/FS-UNB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Caracterização das anomalias dento-ósseas e investigação de variações de sequência no gene APC em pacientes com Polipose Adenomatosa Familial

Pesquisador: Fabiana Tolentino de Almeida

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 3

CAAE: 12696913.0.0000.0030

Instituição Proponente: Faculdade de Ciências da Saúde da Universidade de Brasília

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 493.502

Data da Relatoria: 04/12/2013

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

BRASILIA, 13 de Dezembro de 2013

Assinador por:
Natan Monsores de Sá
(Coordenador)

Annex II Informed Consent

Termo de Consentimento Livre e Esclarecido - TCLE

O (a) Senhor(a) está sendo convidado(a) a participar do projeto: **"Caracterização das anomalias dento-ósseas e investigação de variações de sequência no gene *APC* em pacientes com Polipose Adenomatosa Familial**".

O objetivo desta pesquisa é avaliar as anomalias dento-ósseas e investigar as variações de sequência no gene *APC* em pacientes com Polipose Adenomatosa Familial e seus Familiales. Espera-se com essa pesquisa estabelecer uma relação da anomalia dento-óssea com a mutação genética (genótipo/fenótipo). O(a) senhor(a) receberá todos os esclarecimentos necessários antes e no decorrer da pesquisa e lhe asseguramos que seu nome não aparecerá sendo mantido o mais rigoroso sigilo através da omissão total de quaisquer informações que permitam identificá-lo(a). A sua participação será através de uma entrevista sobre seu estado de saúde geral, realização de exame clínico intrabucal não invasivo com espátula de madeira, realização de radiografia panorâmica da face e coleta de sua saliva. Para coletar a saliva, o senhor (a) realizará um bochecho e irá cuspir em um tubo. Quando forem visualizadas alterações dento-ósseas pela radiografia panorâmica da face, que necessitarem de avaliação em três dimensões, será realizada tomografia computadoriza por feixe cônico que limita a dose de radiação à região da maxila e mandíbula. Os exames radiográficos serão realizados com o equipamento de radioproteção necessário (avental de chumbo e protetor de tireoide). Todos os procedimentos serão realizados em uma única consulta. Após conclusão da pesquisa, seus dados serão armazenados e, caso sejam utilizados em uma pesquisa futura, o senhor(a) será contatado para nova autorização.

A participação é voluntária e o senhor (a) não terá nenhum custo e não receberá nenhuma remuneração. O senhor(a) pode se recusar ou desistir da pesquisa em qualquer momento, sem qualquer forma de prejuízo. Em qualquer momento, o senhor (a) poderá ter acesso aos resultados e eles poderão ser divulgados na Universidade de Brasília e/ou em eventos e revistas científicas, sempre mantendo o sigilo de sua

participação. Os dados e materiais utilizados na pesquisa ficarão sobre a guarda do pesquisador.

Se o(a) senhor(a) tiver qualquer dúvida em relação à pesquisa, por favor entrar em contato com a pesquisadora responsável Dra. Fabiana Tolentino de Almeida pelo telefone (61) 91249647. Poderá entrar em contato também com as discentes do estudo, Karen Ariely Rocha Arruda e Ana Gabriela Costa Normando, pelo número de telefone (61) 98115-0934 e (61) 99995-1617 respectivamente.

Esta pesquisa não oferece nenhum risco a(o) senhor(a). Espera-se com esse estudo, obter uma melhor compreensão das alterações dento-ósseas associadas à Polipose Adenomatosa Familial.

Este projeto foi Aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Ciências da Saúde da Universidade de Brasília. As dúvidas com relação à assinatura do TCLE ou os direitos do sujeito da pesquisa podem ser obtidas através do telefone: (61) 3107-1947.Este documento foi elaborado em duas vias, uma ficará com o pesquisador responsável e a outra com o sujeito da pesquisa.

Assinatura do paciente ou responsável

Assinatura do pesquisador responsável

Brasília, ___ de _____de _____

Annex III Archives of Oral Biology - Guide for Authors

Journal Guidelines for Archives of Oral Biology

GUIDE FOR AUTHORS

Editors-in-Chief:

Professor S W Cadden, Dundee, Scotland
Dr Fionnuala T. Lundy, Northern Ireland, UK

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• immunology
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• microbiology
• biology of dental caries and periodontal disease
• forensic dentistry
• neuroscience
• salivary biology
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Introduction
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Phenotypic dento-osseous characterization of a Brazilian family with Familial Adenomatous Polyposis

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ABSTRACT

Objective: To perform a phenotypic characterization of the dento-osseous anomalies in a Brazilian family with Familial Adenomatous Polyposis (FAP) and to investigate the adenomatous polyposis coli (*APC)* causative variant.

Design: The study included a family of 14 individuals (Group A: affected; Group B: non-affected). The frequency of radiographic findings in both groups was evaluated according to the Dental Panoramic Radiograph Score (DPRS) diagnostic method. The accuracy and reproducibility of DPRS were tested. The DNA was isolated from the index patient's saliva and submitted to whole-exome and Sanger sequencing approach.

Results: DPRS $\geq$ 7 was observed in 80 % of Group A but in none of Group B. The most common findings in Group A were dense bone islands (60 %), hazy sclerosis (40 %), osteomas (40 %), and supernumerary tooth (20 %). DPRS has proved to be a reliable method while DPRS $\geq$ 5 and DPRS $\geq$ 7 were taken as positive for FAP, and reproducible diagnosis test considering that the evaluators correctly identified the affected patients (Kappa agreement>0.8, $p = 0.002$). A nonsense heterozygous mutation in the *APC* gene (c.1370C > G; p.Ser457*) of the index case was detected.

Conclusion: FAP patients have a higher frequency of dento-osseous anomalies ($p = 0.005$). Bone abnormalities were more prevalent than dental anomalies ($p = 0.001$). Thus, FAP patients should be referred for dental examination and genetic counseling to perform early diagnosis of dento-osseous anomalies and evaluate the implications of the molecular findings in each particular family.

1. Introduction

Familial Adenomatous Polyposis (FAP- MIM175100) is an autosomal dominant inherited syndrome predisposing to colorectal cancer. FAP is caused by variants in the Adenomatous Polyposis Coli (*APC*) tumor suppressor gene, mapped to the long arm of chromosome 5 (5q21) band q21 (MIM 611731). Pathogenic variants in the *APC* gene can cause two distinct phenotypes: the classic FAP and the Attenuated Familial Adenomatous Polyposis (Knudsen et al., 2010). The encoded APC protein is a nucleus-cytoplasmic shuttling protein, known to antagonize the Wnt signaling pathway by the formation of a cytoplasmic complex that targets β-catenin for degradation (Zeineldin et al., 2014); therefore, the loss of APC function contributes to the increase of cytoplasmic β-catenin levels. The β-catenin protein is related to the process of cell adhesion and gene transcription (Amado et al., 2012). In high concentrations, β-catenin is translocated to the nucleus, where it binds to transcription factors of target genes. The dysregulated signaling pathway induces cell proliferation and the formation of intestinal adenomas, resulting in malignant transformation of normal cells (Fujii et al., 2019; Nusse and Clevers, 2017; Yang et al., 2014).

When pathogenic variants in the *APC* gene are not identified in FAP patients, a second gene must be investigated, the *MUTYH* gene

Abbreviations: APC, Adenomatous Polyposis Coli; CBCT, Cone-Beam Computed Tomography; DPRS, Dental Panoramic Radiograph Score; FAP, Familial Adenomatous Polyposis; FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value.

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(chromosome 1p34.1; MIM 604933). *MUTYH* pathogenic variants cause an attenuated phenotype similar to Attenuated Familial Adenomatous Polyposis that is called MAP, *MUTYH*-Associated Polyposis, and has an autosomal mode of inheritance (Nielsen et al., 2009; Torrezan et al.2013).

In general, FAP patients develop multiple intestinal polyps around the second decade of life. Approximately 50 % of patients with classic FAP have these colorectal adenomas by the age of 15 years, and this percentage increases to 95 % by the age of 35 years (Half et al., 2009). The attenuated form of the disease called Attenuated Familial Adenomatous Polyposis is clinically characterized by the development of less than 100 colorectal adenomatous and by a delay in the polyps' onset, on average of up to 20–30 years (Hernegger et al., 2002). In addition to the development of adenomatous polyps in the colon and rectum, classic FAP patients may present extracolonic manifestations, including gastric and duodenal adenomas, congenital hypertrophy of the retinal pigment epithelium desmoid tumors, fibromas, and dento-osseous anomalies (odontomas, osteomas, dense bone islands, and supernumerary tooth) (Campos et al., 2015; Gardner, 1962). Although commonly noted in FAP, extraintestinal manifestations are rare in Attenuated Familial Adenomatous Polyposis patients, and affected individuals are likely to have a reduced lifetime risk of colorectal cancer, less than in 100 % of cases as in FAP (Jasperson et al., 2010). The diagnosis and follow-up of FAP are achieved by periodic colonoscopy examination and, if indicated, prophylactic or elective colectomy (Syngal et al., 2015).

The presence of both intestinal polyps and dental anomalies in patients with classic FAP were first described by Gardner (Gardner, 1951). Gardner Syndrome is a term which has been used to refer to this phenotypic characterization of FAP. Bone alterations in the jaw and dental anomalies have been reported in 65 % and 30 % of patients respectively and may precede the development of intestinal polyps and the colorectal cancer (Almeida et al., 2016; Almeida et al., 2020; Septer et al., 2018).

Thakker et al. (1995) developed a diagnostic method using panoramic radiographs to identify high-risk FAP patients called Dental Panoramic Radiograph Score (DPRS). This method has demonstrated a significant association between the presence of substantial changes and the presence of FAP, allowing the early diagnosis of the disease (Aggarwal et al., 2003; Thakker et al., 1995). Although the literature presents several studies showing the phenotypic characterization of FAP, studies still lack on showing the genotypic correlation with the clinical data of patients with FAP. Thus, the present study aimed to perform a phenotype characterization of a family with FAP in order to detail the dento-osseous anomalies using the DPRS diagnostic method to determine its diagnostic accuracy. In addition, genetic analyses using whole-exome sequencing and Sanger sequencing validation were performed.

2. Material and methods

This project was approved by the Research Ethics Committee of the Health Sciences Faculty, University of Brasília with a Certificate of Presentation for Ethical Appreciation number 12696913.0.0000.0030 and it is in accordance with the Declaration of Helsinki.

The study includes one Brazilian family with FAP consisting of 14 individuals divided into 2 groups, Group A comprised of FAP-confirmed patients (n = 5), and Group B with family members without FAP diagnosis (n = 9). The diagnosis of FAP was based on confirmation in medical records, and the evaluated criteria were the number of polyps and the age at diagnosis of FAP. This cross-sectional study performed a phenotype characterization of a family with FAP syndrome by the description of radiographic findings using the Dental Panoramic Radiograph Score (DPRS) diagnostic method. Also, we evaluated the proportion of dento-osseous anomalies in FAP patients (Group A) comparing to non-FAP family members (Group B). Medical and dental records were reviewed for contributory history. Furthermore, we carried out a genetic analysis to investigate the causative variant in the family.

All patients were submitted to the following procedures: signature of informed consent; in cases of underage patients, the consent form was signed by the legal responsible; clinical examination with anamnesis, including extra and intraoral physical examination; and panoramic radiography for diagnosis. Cone-beam Computed Tomography (CBCT) was performed exclusively in patients with bone and dental alterations that needed further investigation. For genetic analysis, DNA was isolated from the index patient's saliva.

2.1. Phenotype characterization

All patients from a family with classical FAP were evaluated at the School of Dentistry and Pharmacy Center of the University Hospital of Brasília (Brasília, Brazil). Since the index patient referred other affected patients in the family, all family members were contacted and invited to participate in the study.

Familial adenomatous polyposis is a clinical diagnosis that is typically based on the presence of more than 100 colorectal adenomas, age at diagnosis of FAP and occurrence of colorectal cancer (Friedl & Arentz, 2005). Thereby, anamnesis included an investigation for colorectal cancer and other malignancies as well as family history. The analysis of medical records available at the University Hospital of Brasília confirmed all information such as the presence of FAP and colorectal cancer. In order to confirm the autosomal dominant inheritance, a heredogram was performed using GenoPro2018 - version 3.0.1.4 (Brazil, 2019).

The extraoral physical examination evaluated facial asymmetry, skin, scalp, and lymph nodes. The intraoral examination thoroughly assessed the oral cavity (oral mucosa and teeth). A panoramic radiograph was performed on all patients, using the Kodak 8.000C Digital Panoramic and Cephalometric System (Trophy, France), to diagnose and/or monitor the dento-osseous anomalies. CBCT was performed on an I-CAT Platinum device (Imaging Sciences International, United States) with the following technical parameters: 120kVp, 8 mA, and voxel size of 0.2 mm. Advanced imaging was acquired to assist in the diagnosis and extension of the lesions.

For the assessment of dento-osseous anomalies, panoramic radiographs were blindly analyzed by two Oral and Maxillofacial Radiologists (OMR) evaluators, following the validated DPRS criterion by Thakker et al. (1995). This method considers the nature, extent, frequency, and location of dento-osseous anomalies noted on panoramic radiographs of FAP patients compared to unaffected individuals (Thakker et al. 1995). The DPRS considers four possible outcomes: 1- normal changes, 2- minimal changes, 3- ambiguous changes, and 4- significant changes. Each outcome is associated with a final score, which was obtained by adding the individual scores for each dento-osseous anomaly (Appendix I and II). The size and quantity of these anomalies are taken into consideration to reflect the clinical level of significance if this anomaly occurs in isolation.

To determine the diagnostic accuracy of this tool, several parameters were established (specificity, sensitivity, false positive and negative rate, positive and negative predictive accuracy, and precision) from the final DPRS values for Groups A and B.

Aiming to measure the agreement between the evaluators and the reproducibility of the DPRS, the reliability test was carried out twice by the same OMR, using the same images within a 1-month interval.

2.2. Genetic analysis

Genomic DNA from the index patient was isolated from buccal mucosa cells in saliva as previously described (Aidar & Line, 2007; Almeida et al., 2020).

Whole-exome sequencing was performed in the sequencing facility Macrogen (Seoul, Korea) using Sure select V6 for captures and sequenced in Illumina platform. Candidate variants were filtered against

exome data in publicly available databases, including the 1000 Genomes Project, ExAC, UCSC common Single Nucleotide Polymorphisms database, ClinVar, and results of "*in silico*" algorithms (softwares Polyphen, SIFT, MutationTaster, CADD, Human Splicing Finder). Variants were filtered out if they had low quality, insertions/deletions in regions of homopolymers, and allelic imbalance greater than 80:20. Furthermore, the pathogenicity of variants was determined based on the latest American College of Medical Genetics guidelines (Richards et al., 2015).

To validate the heterozygous nonsense variant detected by the whole-exome sequencing, the Polymerase chain reaction and Sanger sequencing of *APC* exon 11 was carried out according to standard protocols. Primers were designed using ExonPrimer software (http://ihg.gsf.de/ihg/ExonPrimer.html).

2.3. Statistical analysis

Fisher's exact test was applied to investigate the association between patients with FAP / non-FAP and the presence of dento-osseous anomalies through the DPRS results. The Cohen's Kappa Coefficient (k), a quantitative measure of the magnitude of agreement, was applied to analyze the intra and inter reliability between the OMR while evaluating the images. The parameters specificity, sensitivity, false positive (FP) and negative rate (FN), positive predictive value (PPV) and negative predictive value (NPV) accuracy, and precision were determined for the diagnostic study. Analysis of the data was conducted using The Statistical Package for the Social Sciences (version 22; SPSS, IBM, Armonk, NY). P-values below 0.05 indicate statistical significance.

3. Results

A total of five affected and nine unaffected family members (Fig. 1A) were included in the study showing the autosomal dominant inheritance usually present in the classic FAP form. Two of the five patients in Group A (II.09 and III.30), developed colorectal cancer in the third decade of life, and the patient III.30 progressed to liver and lung metastasis. However, these individuals were already diagnosed at a late stage with adenocarcinoma. Patient III.03 was diagnosed with FAP in 2011 at 32 years old, and five years later developed gastric adenomas with low-grade dysplasia. Patient IV.02 was diagnosed in childhood (11 years old) and although had less than 100 intestinal adenomas at that date, five years later the intestinal polyps counted more than 100. Patient II.17 presented tubular adenomas with moderate rectal dysplasia nine years after the initial diagnosis of FAP. The treatment performed was total proctocolectomy with ileal pouch and protective ileostomy. The demographics of the affected individuals are summarized in Table 1.

3.1. Genetic findings

Whole-exome sequencing was performed in the index patient (II.09). The overall targeted nucleotide coverage was 99.8 % at $\geq \times$ 20. An

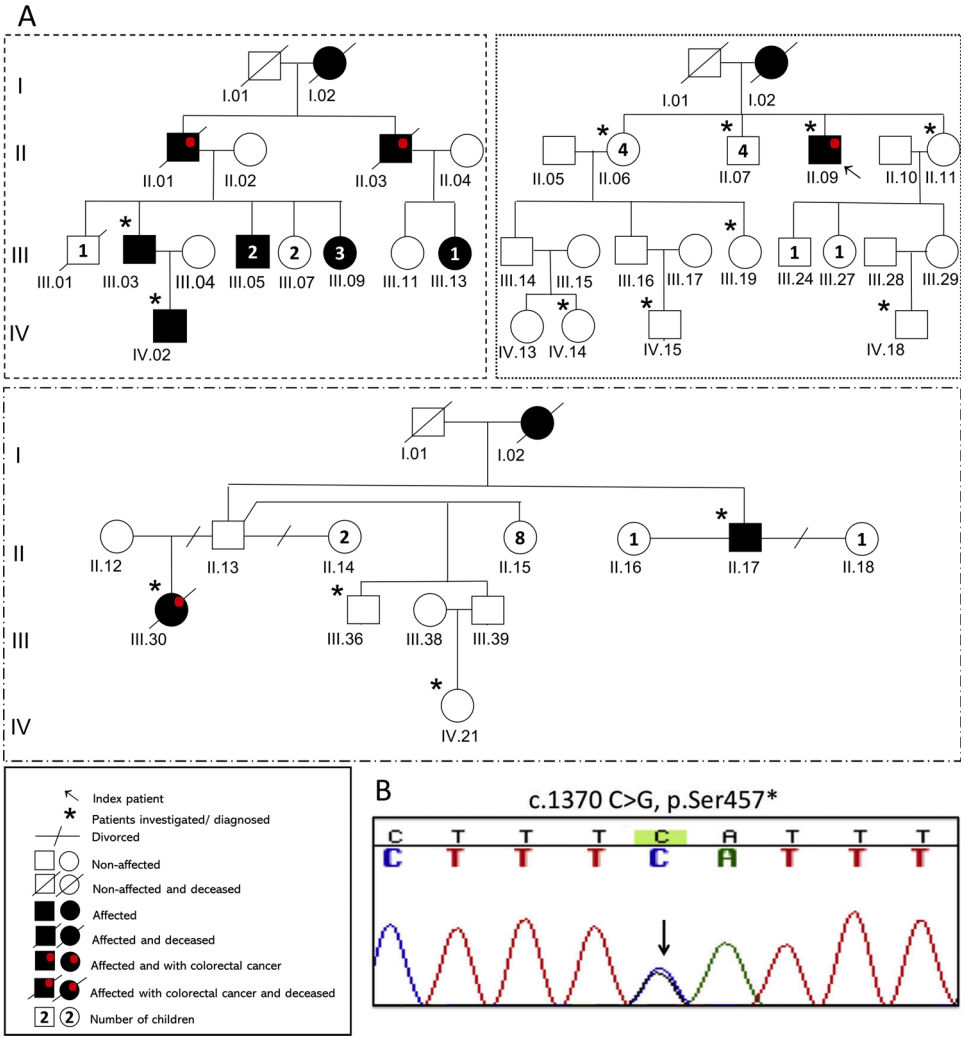

Fig. 1. (A) Complete Brazilian family pedigree divided into 4 sections. (B) Sanger sequencing validation of the base substitution first detected by whole-exome sequencing.

Table 1
Demographic characteristics of Familial Adenomatous Polyposis patients.

Patients	Inheritance pattern	Sex	Age	Age at FAP diagnosis (y)	Age at CRC diagnosis(y)	Disease	Dento-osseous anomalies	Extraintestinal manifestations
II.09 (index)	AD	M	66	39	39	FAP	Yes	Gastric polyps in the antrum region associated with flat erosions (2 −3 mm)
II.17	AD	M	57	32	No CRC	FAP	No	None reported
III.03	AD	M	41	32	No CRC	FAP	Yes	Gastric polyps in the antrum region with low-grade dysplasia
III.30	AD	F	41	34	39	FAP	Yes	None reported
IV.02	AD	M	14	11	No CRC	FAP	Yes	None reported

Abbreviations: AD: autosomal dominant; CRC: Colorectal carcinoma; F: female; FAP: Familial Adenomatous Polyposis; M: male.

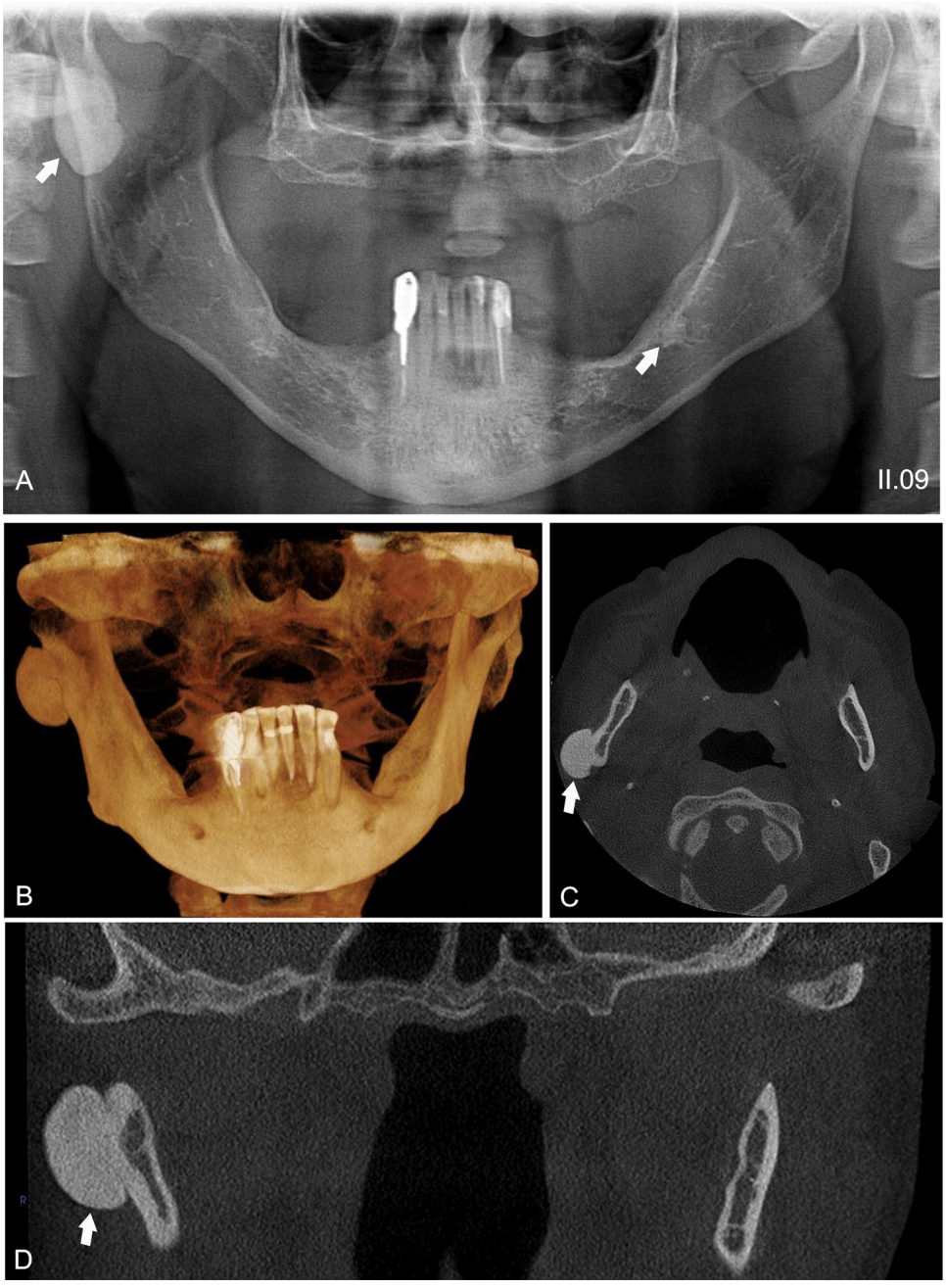

Fig. 2. Dento-osseous anomalies found on index patient (white arrows). (A) Patient II.09- Osteoma at the right ramus of the mandible and dense bone island in the body of the mandible on the left side. (B) CBCT 3D reconstruction showing the osteoma. (C, D) CBCT axial and coronal showing the osteoma.

heterozygous nonsense variant was identified at exon 11 (NM_000038.5: c.1370C > G, p.Ser457*) and confirmed by Sanger sequencing (Fig. 1B). This variant was predicted to be deleterious and disease causing by SIFT, Polyphen2, and Mutation Taster with a CADD score of 38 and predicted pathogenic according to ACMG guidelines.

3.2. Clinical and radiographic findings

Of all the individuals analyzed by the DPRS method, 80 % of Group A (n = 5) was classified with significant changes, DPRS ≥ 7, and 20 % was classified with normal changes, DPRS 0-2. In Group B (n = 9), only one patient was classified with minimal changes (Score 3–4) and all the other eight patients were classified with normal changes (Score 0–2). There was an association between DPRS 0-2, and absence of FAP (p = 0.005), and DPRS≥ 7 was related to group A (p=0.005).

The radiographs of patients in Group A showed that 20 % of them presented supernumerary tooth, 40 % showed osteomas; 40 % had hazy sclerosis (not so defined increase in bone density) associated with the root of a single tooth; 40 % had hazy sclerosis not associated with teeth roots; 60 % had dense bone islands (well-defined radiodensities with irregularly shaped margins) (Figs. 2–4). Group B showed that only 11.1 % of the group presented hazy sclerosis not associated with teeth roots and the others did not present any dento-osseous anomalies. Images from the patient index II.09 (Fig. 2A-D) showed area of dense bone island in the mandible, and an osteoma located at the right condyle neck (this finding was confirmed on the CBCT). Patient III.03 (Fig. 3A–E) presented areas of dense bone island and hazy sclerosis associated with the teeth roots. In addition, an osteoma located in the left maxillary sinus was detected on the CBCT scan. However, the osteoma was not considered in the DPRS evaluation, because it could not be seen in the panoramic radiograph. Patient IV.02 (Fig. 4A–B), 14 years old, presented hazy sclerosis associated and non-associated with the teeth roots. Besides, a supernumerary tooth localized between the right lateral and central incisors, as seen in the periapical radiograph (Fig. 4B). The supernumerary was not considered in the DPRS evaluation, but even so, the patient presented DPRS score = 8 significant changes. Patient III.30, the only female, presented hazy sclerosis and dense bone island bilaterally in the mandible (Appendix III). It is important to reinforce that the DPRS was calculated according to the dento-osseous alterations detected on the panoramic radiograph, therefore alterations identified in other

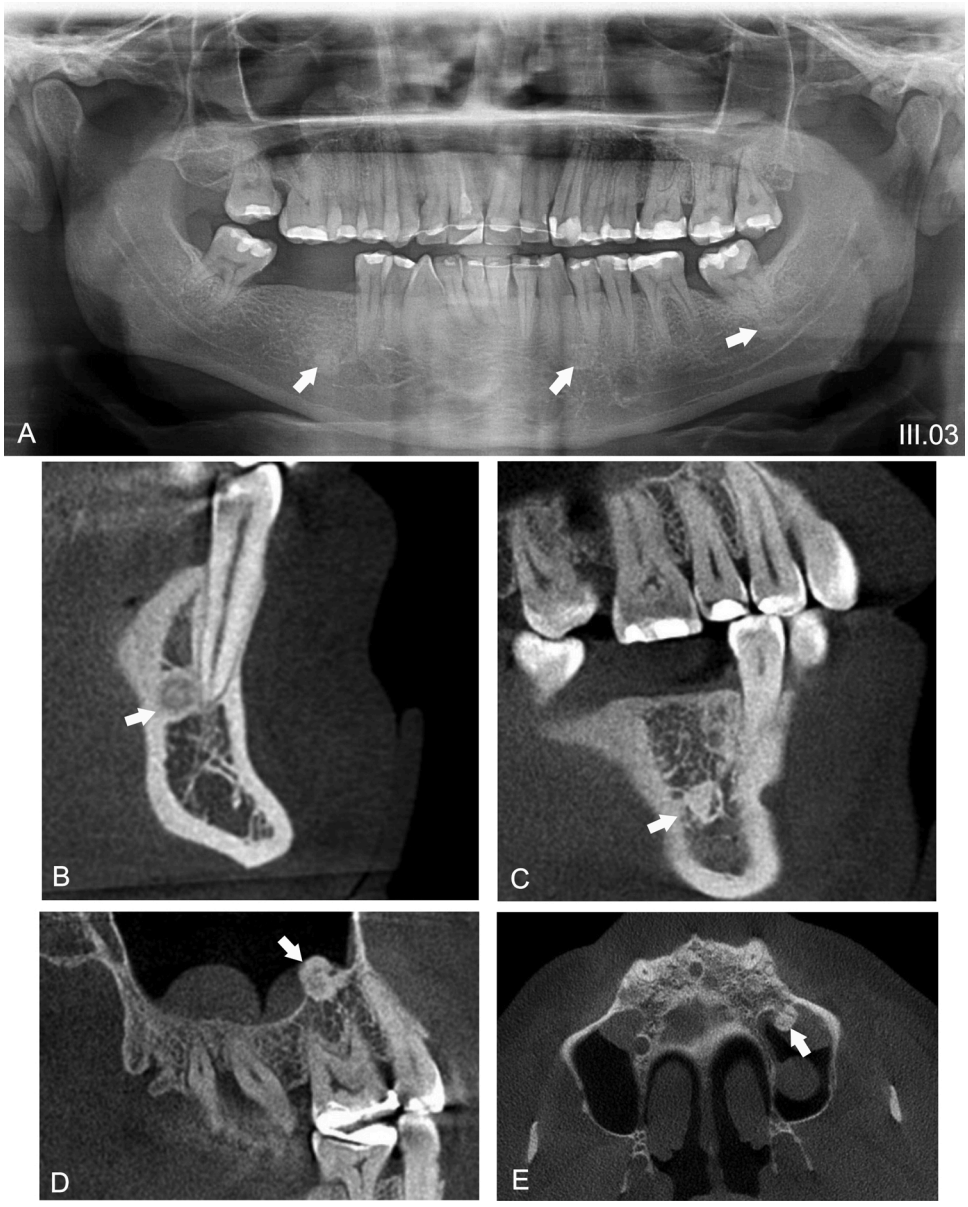

Fig. 3. Dento-osseous anomalies in a FAP affected patient (white arrows). (A) Patient III.03- Dense bone island area in the region of the absent lower right first molar, and presence of hazy sclerosis associated with the roots of the lower left first premolar and third molar. (B) CBCT sagittal showing hazy sclerosis associated with the roots of the lower left first premolar. (C) CBCT sagittal demonstrating dense bone island area in the region of the absent lower right first molar. (D, E) CBCT sagittal and axial showing the osteoma in the left maxillary sinus.

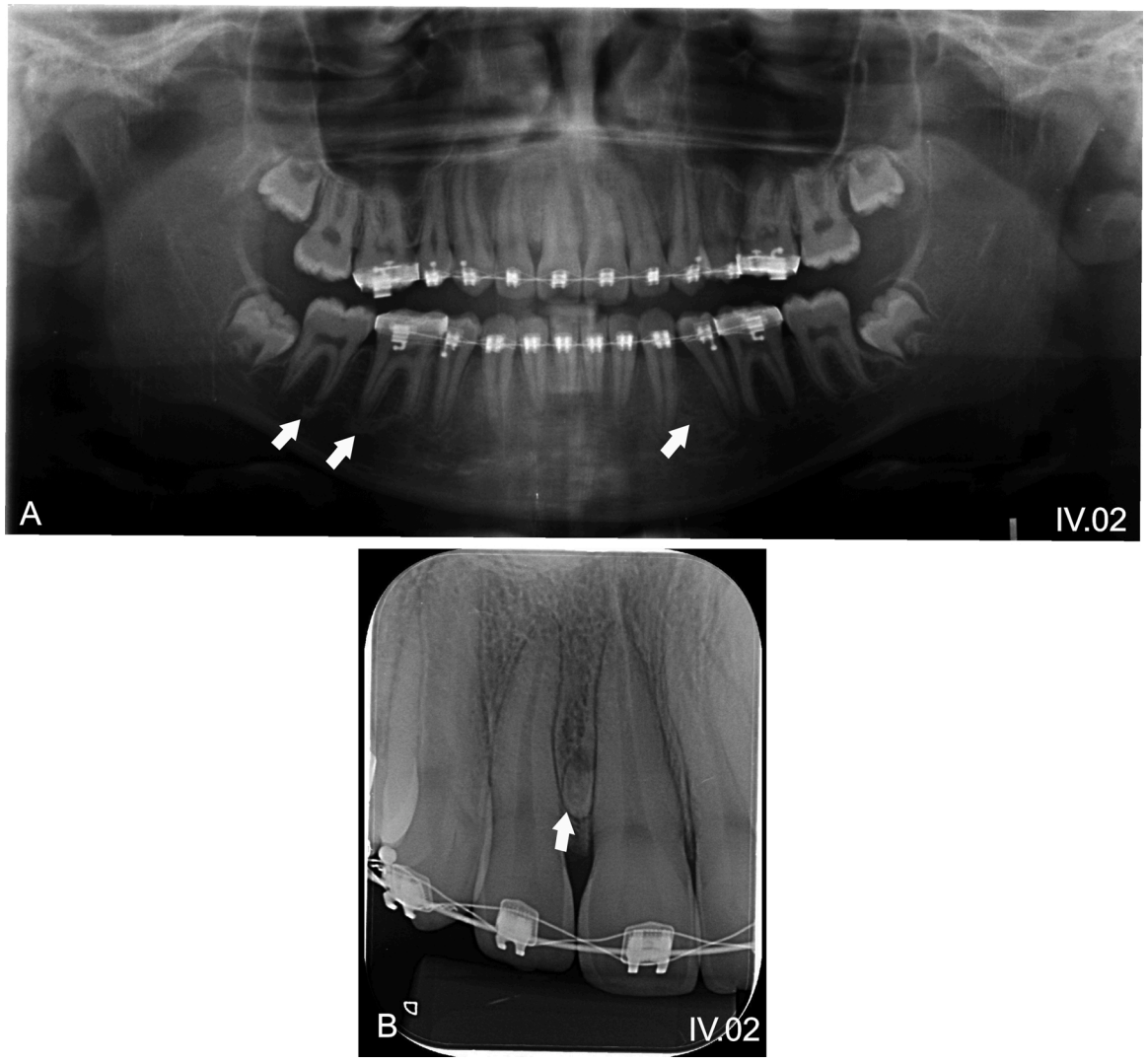

Fig. 4. Bone alterations in a FAP affected patient (white arrows). (A) Patient IV.02- Hazy sclerosis area associated with the roots of the lower right first and second molar, and hazy sclerosis not associated with roots between the canine and the second lower left premolar. (B) Periapical radiographs showing an supernumerary tooth between the roots of the right central and lateral incisors on the same patient.

images modalities were not considered for this purpose. The frequency of anomalies in each group is detailed in Fig. 5A. Table 2 describes the dento-osseous changes found in all patients in this study and their respective DPRS.

3.3. Diagnostic capability of the DPRS

The reliability of the DPRS test evaluated specificity, sensitivity, FP and FN rates, PPV, NPV, and precision for different outcomes. When significant changes (DPRS ≥ 7) were counted as positive for the diagnosis of FAP and all other categories were classified as negative, 100 % of specificity and PPV, 93 % of precision, 90 % of NPV, 80 % of sensitivity and 20 % FN rate were obtained. When the significant and ambiguous changes (DPRS 5-6) were counted as positive for the diagnosis of FAP and the other categories were classified as negative, we obtained the same results as the previous classification and the FP rates remained zero. When the significant, ambiguous, and minimal changes (DPRS 3-4) were counted as positive for the diagnosis of FAP and only normal changes (DPRS 0-2) were excluded, there was a reduction in specificity, NPV, precision, and PPV to 89 %, 89 %, 86 %, and 80 % respectively, and an increase in the FP rates to 11 % (Fig. 5B). Therefore, the results of this analysis suggest that it is reliable to diagnose as FAP the individuals who have DPRS values relative to significant and ambiguous changes and that it is not reliable to take as positive for FAP diagnostics DPRS results equal to or below 3–4, including all the changes (minimal, ambiguous, and significant changes). Concerning all the dependent variables evaluated, the inter, $k = 1$ ($p = 0.000$), and intra-reliability values, $k_1 = 0.81$ ($p = 0.002$) $k_2 = 1$ ($p = 0.000$) respectively to evaluators 1 and 2, were excellent.

4. Discussion

The early diagnosis of individuals affected by FAP is essential in preventing the development of colorectal cancer and consequent improvement in the prognosis of the disease (Aggarwal et al., 2003; Dinarvand et al., 2019; Septer et al., 2018). For this reason, it is necessary to better understand the clinical phenotype of this condition including the extraintestinal manifestations.

In the present study, the difference between the frequency of dento-osseous anomalies observed in the affected group compared to the group of undiagnosed family members was significant ($p = 0.005$). These results demonstrate that dento-osseous changes may be present in groups of people without FAP, but these changes are not significant in this population. These anomalies appear in a higher proportion in affected

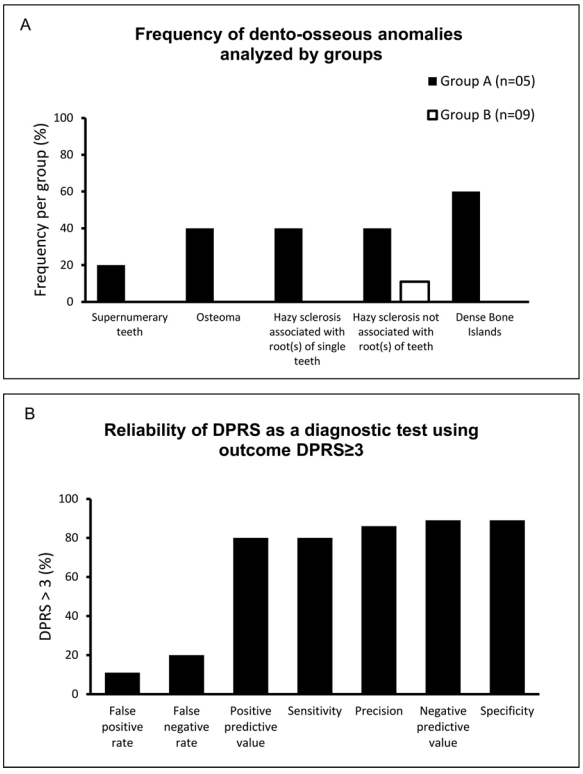

Fig. 5. (A) Frequency of dento-osseous anomalies analyzed by groups. (B) Reliability of Dental Panoramic Radiograph Score (DPRS) as a diagnostic test using outcome DPRS $\geq$ 3 as a positive result for the diagnosis of FAP.

patients; 4: 5 Group A patients with FAP compared to 1: 9 Group B. In this study, as described by others (Aggarwal et al., 2003; Almeida et al., 2016; Almeida et al., 2020; Thakker et al., 1995), a higher frequency of bone alterations (osteomas, islands of bone condensation, and diffuse sclerosis) was observed in comparison to the presence of dental anomalies in affected patients ($p = 0.001$).

Numerous studies demonstrated that the presence of osteomas can be an important marker of FAP, highlighting the importance of knowledge about this anomaly and its potential in early diagnosis of FAP (Almeida et al. 2016; Bertario et al., 2001; Bülow et al., 1984; Ida et al., 1981; Katou et al., 1989; Septer et al., 2018; Wolf et al., 1986). In the present study, this anomaly was present in 40 % of the assessed affected patients. The dense bone islands, presented in 60 % of FAP patients, represent a focal increase in bone density without any obvious etiological agent. Based on this unknown origin, the term idiopathic osteosclerosis is also frequently used as a synonym of dense bone islands (Halse & Molven, 2002; Ledesma et al., 2019; McDonnell, 1993). On the other hand, hazy sclerosis associated with the root of a single tooth can occur in unaffected patients as an inflammatory condition in the bone in response to periodontal disease. However, this condition is already accounted for in the DPRS method. Hazy sclerosis associated with the root of a single tooth has a lower score than hazy sclerosis not associated with teeth roots. Therefore, even though it is a common bone disorder in the general population, it is more prevalent in the FAP-affected population (Thakker et al., 1995).

Supernumerary tooth, present in 20 % of FAP patients from our sample, represents the only dental anomaly finding of the present study. Most supernumerary teeth are sporadic, although they may also occur in genetic syndromes. Some of them, including FAP, present strong evidence of the association with supernumerary teeth (Lubinsky & Kantaputra, 2016). The reported prevalence of supernumerary tooth in FAP patients varies between 11 % and 27 %, significantly higher than in the general population (Wijn et al., 2007). Odontomas, which are dental features commonly found in the incisors and premolars region, are reported in FAP patients with frequencies between 9.4 % and 83.3 %, significantly higher than the prevalence of 0–4% in the control groups (Ida et al., 1981; Owosho et al., 2013; Wijn et al., 2007). Despite the considerable prevalence, there were no cases of odontomas in the studied family.

To estimate and discuss the worldwide prevalence of dento-osseous anomalies in patients with FAP, we performed a pooled prevalence analysis of 18 previously selected studies (Appendices IV-IX). Supplementary data about the inclusion and exclusion criteria for studies are described in detail in Appendix IV. The statistical analysis showed that the worldwide prevalence of bone and dental anomalies in the positive FAP population was 69 % and 30 %, respectively (Appendix V and Appendix VI). Analyzing the results separately by continent, Asian patients had presented a higher prevalence of dento-osseous anomalies compared to the other continents investigated, with 82 % prevalence of bone and 45 % dental alterations. In contrast, North American patients had a lower prevalence of dento-osseous anomalies compared to all other continents, showing a prevalence of bone and dental anomalies of

Table 2
Clinical features of Familial Adenomatous Polyposis population and Dental Panoramic Radiograph Score (DPRS) results.

Patients	FAP	DPRS scores	Osteoma	Hazy sclerosis associated with root(s) of single teeth	Hazy sclerosis associated with root(s) of multiple teeth	Hazy sclerosis not associated with root(s) of teeth	Hazy sclerosis diffuse	Dense bone island	Supernumerary tooth
II.06	ND	0	–	–	–	–	–	–	–
II.07	ND	4	–	–	–	+ (1/ 2.5)	–	–	–
II.09	+	14	+ (1/ 3.5)	–	–	–	–	+ (1/ 1.5)	–
II.11	ND	0	–	–	–	–	–	–	–
II.17	+	0	–	–	–	–	–	–	–
III.03	+	8	+ (1/ 0.5)	+ (2/ -)	–	–	–	+ (1/ 1)	–
III.19	ND	0	–	–	–	–	–	–	–
III.30	+	10	–	–	–	+ (1/ 0.5)	–	+ (2/ 2)	–
III.36	ND	0	–	–	–	–	–	–	–
IV.02	+	8	–	+ (2/ -)	–	+ (1/ 1.5)	–	–	+ (1/ -)
IV.14	ND	0	–	–	–	–	–	–	–
IV.15	ND	0	–	–	–	–	–	–	–
IV.18	ND	0	–	–	–	–	–	–	–
IV.21	ND	0	–	–	–	–	–	–	–

Abbreviations: +: present; -: absent; ND: Not determined. FAP: Familial Adenomatous Polyposis. DPRS: Dental Panoramic Radiograph Scores.
Notes: 1) The values inside the parentheses show the number and size in centimeters of the dento-osseous anomalies, respectively. 2) The score values are in accordance with the table provided in Appendix I. 3) The supernumerary teeth and the osteoma respectively seen in the periapical radiograph and CBCT scan were not included in Table 2 as it relates to the DPRS only.

35 % and 14 %, respectively. Bone lesions were more frequent than dental anomalies on all continents (Appendix VII). There were no studies concerning the population of Africa and Oceania. There is no scientific evidence about the worldwide prevalence of dento-osseous anomalies in the population with FAP. Further investigations, as well as the development of multicentric studies regarding ethnic influence on the prevalence of oral and extra-intestinal manifestations in FAP, are still needed.

In the current study, a heterozygous nonsense variant in *APC* exon 11 (c.1370C > G, p.Ser457*) was identified in the index patient with a diagnosis of classic FAP. This variant has been previously reported although the described dento-osseous anomalies were not associated with this *APC* variant (Friedl & Arentz, 2005; Lagarde et al., 2010; Michils et al., 2002; Stekrova et al., 2007). Although 75 % of the *APC* coding region is in exon 15, this stop codon at the beginning of the gene promotes the termination of protein translation that could result in a non-functional APC protein, or nonsense-mediated decay of the transcript (Nykamp et al., 2020; Wallis et al., 1999). In several studies, an association between the location of *APC* variants and the phenotype in FAP patients has been described (Davies et al., 1995; Newton et al., 2012; Torrezan et al., 2013). However, only a few studies discuss the genotype related to dental phenotype (Davies et al., 1995; Septer et al., 2018). In particular, jaw osteomas are the most common lesions described in the genotype-phenotype studies and they are related to classic FAP and variants in exon 15 around codons 1310–1444 (Nieuwenhuis & Vasen, 2007). Thus, we can speculate that the variant found in our FAP patient may be associated with a phenotype with more bone changes than dental manifestations.

Concerning the reliability of the DPRS diagnostic test, Thakker et al. (1995) showed a small reduction of the specificity percentage when ambiguous changes were counted as positive for the diagnosis of FAP. Whereas in this study it occurred only when the minimum changes were counted as positive. However, overall, specificity tended to decrease, and sensitivity tended to increase in the same trend as reported by Thakker et al. (1995). It was demonstrated that the diagnostic reliability of DPRS is higher when only significant and ambiguous changes are counted as positive for the diagnosis. In the reproducibility analysis of the DPRS method, excellent results of Kappa values were demonstrated. These results suggest that the DPRS method is reliable and reproducibly (Viera & Garrett, 2005). Furthermore, DPRS is a method that can be better explored in the dental routine and assist in the early diagnosis of FAP in a non-invasive and efficient manner.

5. Conclusion

The study has confirmed that patients with FAP have a high prevalence of dento-osseous anomalies, with bone alterations being more frequent than dental manifestations. Among the bone anomalies, dense bone islands were the most frequent lesion identified. Furthermore, the *APC* heterozygous nonsense variant was identified in this particular family. Since the current cross-sectional study is based on one family, we suggest further research with a larger number of individuals with genotype and dento-osseous phenotype to better understand the occurrence of these extraintestinal features in FAP patients. We recommend the referral of FAP patients for dental examination and genetic counseling to perform early diagnosis of dento-osseous anomalies and evaluate the implications of the molecular findings in each particular family.

Authors contributions

Karen Arruda: Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing- original draft, Visualization. **Ana Gabriela Normando:** Conceptualization, Methodology, Validation, Writing- Review & Editing, Supervision. **Camila Pacheco-Pereira:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing- Review & Editing, Supervision. **Juliana Amorim dos Santos:** Conceptualization, Validation, Writing- Review & Editing, Supervision. **Paulo Yamaguti and Juliana Mazzeu:** Conceptualization, Validation, Investigation, Data curation, Writing- Review & Editing, Visualization, Supervision. **Fabiana Almeida:** Conceptualization, Methodology, Validation, Investigation, Data acuration, Writing- Review & Editing, Supervision. **Ana Carolina Acevedo:** Investigation, Writing- Review & Editing, Supervision, Project administration. **Eliete Guerra:** Writing- Review & Editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.archoralbio.2021.105206.

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