CLÁUDIO RODRIGUES REZENDE COSTA

CARACTERIZAÇÃO MORFOLÓGICA DE TECIDOS E FIBROBLASTOS GENGIVAIS DE PACIENTES COM SÍNDROME ESMALTE RENAL E SÍNDROME DE RAINE

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FACULDADE DE CIÊNCIAS DA SAÚDE
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Exemplar para a banca de doutorado, apresentado como requisito para a obtenção do título de Doutor em Ciências da Saúde, no Programa de Pós-Graduação em Ciências da Saúde.

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Coorientadora: Dra. Bruna Rabelo Amorim

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2021

« *L'essentiel est invisible pour les yeux* »

Antoine de Saint-Exupéry, Le Petit Prince (1943)

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RESUMO

A fibromatose gengival (FG) pode ser definida como um crescimento progressivo da gengiva caracterizado por um aumento fibroso no tecido conjuntivo, podendo ser observadas como parte das síndromes Esmalte Renal (ERS) e síndrome de Raine (RNS). A ERS e RNS são causadas por variantes patogênicas recessivas nos genes que codificam as proteínas FAM20A e FAM20C e ambas manifestam anomalias orodentais e calcificações ectópicas em tecido conjuntivo. O presente estudo teve com objetivos: 1) realizar revisão sistemática da literatura disponível com o propósito de sintetizar as características clínicas e genéticas de síndromes com fibromatose gengival, 2) analisar as características histopatológicas de gengiva de uma paciente com ERS e dois pacientes de famílias com RNS, não relacionadas, em acompanhamento na Clínica de Atenção Odontológica para Doenças Raras na Unidade de Saúde Bucal do Hospital Universitário de Brasília e 3) estabelecer e caracterizar culturas primárias de fibroblastos (hGFCs) a partir de tecidos doados pelos três pacientes sindrômicos e cinco indivíduos controle. A revisão sistemática identificou a ERS como a síndrome com maior frequência de fibromatose gengival relatada, seguida pelas síndromes de Zimmermann-Laband e síndrome de Costello. A hipertricose, a calcificações ectópicas gengivais e o querubismo foram outras manifestações clínicas descritas nos casos relatados. As variantes patogênicas mais frequentemente descritas foram as do gene *FAM20A*. Os resultados da análise histológica confirmaram o diagnóstico de fibromatose gengival e mostraram a presença de calcificações ectópicas nos tecidos analisados. Além disso, foi demostrado a expressão de FAM20A, FAM20C nos tecidos gengivais de pacientes ERS e RNS assim como a expressão de α-SMA em um paciente RNS. Quanto às culturas primárias de fibroblastos gengivais controle, ERS e RNS, foi observada a diminuição na viabilidade e proliferação das células de ERS e RNS. Além disso, foi demonstrado que os núcleos de fibroblastos de RNS apresentam um aumento significativo de tamanho em comparação com as células controle ($p < 0,0001$). A expressão de FAM20A, FAM20C e α-SMA foi observada no citoplasma celular. Uma subexpressão de ERK nos fibroblastos ERS e RNS em relação ao controle foi observada, sugerindo que a via ERK possa estar alterada nas duas síndromes. Finalmente, este estudo demonstrou a capacidade de formação de nódulos minerais dos fibroblastos gengivais de ERS e RNS. Esses resultados expandem o conhecimento em alguns aspectos da patogênese das síndromes, no entanto, novos

estudos e análises pós-transcricionais são necessários para elucidar os mecanismos que resultam em anormalidades gengivais e para melhor compreender o papel das proteínas FAM20A e FAM20C na fisiopatologia das ERS e RNS.

Palavras-chaves: FAM20A, FAM20C, Síndrome Esmalte Renal, Síndrome de Raine, fibromatose gengival, fibroblastos.

ABSTRACT

Gingival fibromatosis (GF) can be defined as a gingival overgrowth characterized by a fibrous increase of connective tissue and can be seen as part of the Renal Enamel (ERS) and Raine syndrome (RNS) syndromes. ERS and RNS are caused by pathogenic recessive variants in the genes that encode proteins FAM20A and FAM20C and both manifest oro-dental abnormalities and ectopic calcifications in connective tissue. This study aimed to: 1) perform a systematic review of the available literature in order to synthesize the clinical and genetic aspects of syndromes with gingival fibromatosis, 2) to analyze the histopathological characteristics of the gingiva of a patient with ERS and two patients from unrelated families with RNS being followed up at the Oral Care Center for Inherited Diseases, University Hospital of Brasilia and 3) establishe and characterize primary cultures of gingival fibroblasts (hGFCs) from tissues donated by the three syndromic patients and five control individuals. The systematic review identified ERS as the syndrome with the highest frequency of reported gingival fibromatosis, followed by Zimmermann-Laband syndromes and Costello syndrome. Hypertrichosis, ectopic gingival calcifications and cherubism were other clinical manifestations described in the reported cases. The most frequently described pathogenic variants were those of the *FAM20A* gene. The results of the histological analysis confirmed the diagnosis of gingival fibromatosis and showed the presence of ectopic calcifications in the analyzed tissues. In addition, the expression of FAM20A, FAM20C in the gingival tissues of ERS and RNS patients was demonstrated, as well as the expression of α-SMA in an RNS patient. As for the primary cultures of gingival control fibroblasts, ERS and RNS, a decrease in viability and proliferation of ERS and RNS hGFCs was observed. In addition, RNS fibroblast nuclei appeared to have a significant increase in size when compared to control cells ($p < 0.0001$). The expression of FAM20A, FAM20C was also observed in the cell cytoplasm of all hGFCs and α-SMA labelling was observed in ERS and RNS hGFCs. An under expression of ERK in the ERS and RNS fibroblasts in relation to the control was observed, suggesting that the ERK pathway may be altered in both syndromes. Finally, this study demonstrated the capability of ERS and RNS fibroblasts to form mineral nodules. The present results expand the knowledge in some biological aspects of the syndromes pathogenesis, however, further studies and post-transcriptional analyzes are needed to elucidate the mechanisms that result in gingival abnormalities and to further

understand the role of FAM20A and FAM20C proteins in the pathogenesis of ERS and RNS.

Keywords: FAM20A, FAM20C, Enamel Renal Syndrome, Raine Syndrome, Gingival fibromatosis, Fibroblasts.

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LISTA DE ABREVIAÇÕES E SIGLAS

α-MEM – *Alpha Modification of Eagle's Medium*

α-SMA – *Alpha-Smooth Muscle Actin*

ABCC9 – *ATP Binding Cassette Subfamily C Member 9*

ACTB – β-actina

AHSG – Glicoproteína Alpha-2-HS, Fetuin-A

AI – Amelogênese Imperfeita

AIGFS – Síndrome Amelogênese Imperfeita associada a Fibromatose Gengival

ALPL – Fosfatase Alcalina

ATP6V1B1 – *ATPase H+ transporting V1 subunit B2*

BMP – Proteína Morfogenética Óssea

BSP - Sialoproteína Óssea

CARE – *Case Report guidelines*

COL – Colágeno

DMEM – *Dulbecco's Modified Eagle's Medium*

DMP - Proteína da Matriz Dentinária

DSP – Sialofosfoproteína Dentinária

ELMO – *Engulfment and Cell Motility*

ERS – Síndrome Esmalte Renal

FAM20 – *Family with sequence similarity 20*

FAM20A - *Family with sequence similarity 20, member A*

FAM20B - *Family with sequence similarity 20, member B*

FAM20C - *Family with sequence similarity 20, member C*

FG ou GF – Fibromatose Gengival

FGF – Fator de Crescimento Fibroblástico

FGH – Fibromatose Gengival Hereditária

HA – Hidroxiapatita

HET – Heterozigoto

HFS – Síndrome da Fibromatose Hialina

HOM – Homozigoto

JBI - *Joanna Briggs Institute*

KCNH1 – *Potassium Channel Subfamily H Member 1*

KCNK4 – *Potassium Channel Subfamily K Member 4*

KCNQ1 – *Potassium Voltage-Gated Channel KQT-Like Subfamily Q Member 1*

LCM - *Laser Capture Microdissection*

MGP – Proteína Glutâmica da Matriz

MM – Meio indutor mineralizante

MMP – Metaloproteinases

MTT – (brometo de 3-(4,5-dimetil-2-tiazolil)-2,5-difenil-2H-tetrazólio)

OM – Meio indutor osteogênico

OMIM – *Online Mendelian Inheritance in Man®*

OPG – Osteoprotegerina

OPN – Osteopontina

PBS – Tampão fosfato-salino

PM – Meio proliferativo

PRISMA – *Preferred Reporting Items for Systematic Reviews and Meta-Analyses*

PROSPERO - *International Prospective Register of Systematic Reviews*

PXE – Pseudoxantoma elástico

RNS – Síndrome de Raine

RUNX - *Runt-related transcription factor 2*

FBS – Soro Fetal Bovino

SIBLING – *Small Integrin-Binding Ligand, N-linked Glycoprotein*

SR ou RS – Revisão sistemática

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1. MARCO TEÓRICO

1.1 SÍNDROME ESMALTE RENAL:

A síndrome Esmalte Renal (*Enamel Renal Syndrome,* ERS, OMIM #204690) foi relatada pela primeira vez em 1972, sendo denominada como síndrome de Amelogênese Imperfeita associada à Nefrocalcinose (MacGibbon, 1972). A paciente foi diagnosticada com nefrocalcinose sem alterações nos exames sanguíneos e com atraso na erupção, apresentando vários dentes permanentes retidos. Outros estudos foram publicados posteriormente e as manifestações clínicas dos pacientes ERS foram descritas incluindo, além da nefrocalcinose, hipocalciúria e fosfatase alcalina elevada (Delow et al., 1998; Hall et al., 1995). As principais caraterísticas fenotípicas orodentais da síndrome foram a amelogênese imperfeita (AI) hipoplásica e a fibromatose gengival (Jaureguiberry et al. 2012; Koruyucu et al. 2018). No entanto, outras alterações como retenção prolongada dos dentes decíduos, atraso na erupção dos permanentes, microdontia, maloclusão, hamartomas foliculares e calcificações intrapulpares também foram descritas (Paula et al., 2005; Martelli-Junior et al., 2011; Dure-Molla et al., 2014; Nitayavardhana et al., 2020).

Assim, a ERS é também conhecida como Amelogênese Imperfeita e Nefrocalcinose (MacGibbon, 1972; Jaureguiberry et al., 2012) e Amelogênese Imperfeita associada à Fibromatose Gengival (AIGFS, OMIM #614253) (Martelli-Júnior et al., 2008; O'Sullivan et al., 2011), causadas por variantes patogênicas recessivas no gene *FAM20A*. Atualmente, essas duas condições descritas são consideradas uma síndrome única, com expressividade variável (Dure-Molla et al., 2014).

Até 2020, mais de 50 famílias com 100 variantes no gene *FAM20A* foram relatadas na literatura (O'Sullivan et al., 2011; Koruyucu et al., 2018; Nitayavardhana et al., 2020). Os achados genéticos no gene *FAM20A* descrevem variantes recessivas em homozigose ou variantes em heterozigose composta distribuídas em todos os exons (1–11) e do tipo *frameshift, nonsense, missense* e *splice site* (Nitayavardhana et al., 2020).

1.1.1 Etiopatogenia da ERS:

A FAM20, denominada "*family with sequency similarity 20",* é um grupo de proteínas altamente conservadas que regulam a função e diferenciação hematopoiética e óssea, sendo composta pelas proteínas FAM20A, FAM20B e FAM20C, apresentando atividades bioquímicas distintas (Nalbant et al., 2005; Tagliabracci et al., 2012). A FAM20A foi descrita inicialmente como uma quinase que fosforila proteínas envolvidas na formação do esmalte dentário, em calcificações ectópicas pulpares, na formação radicular e na erupção dentária, e também na homeostasia de cálcio e fosfato nos rins (Wang, S. K. et al., 2013, Cui et al., 2015). A proteína FAM20A é expressa principalmente em células hematopoiéticas, de glândulas paratireoides, dentárias e gengivais (Nalbant et al., 2005; O'Sullivan et al., 2011). A FAM20B é uma xilosil quinase que regula a síntese de proteoglicanas (Tagliabracci et al., 2012). Por sua vez, a FAM20C é uma caseína quinase do complexo de Golgi que fosforila a maioria das proteínas secretadas com motivos Ser-X-Glu/pS, envolvidas no processo de biomineralização (Hao et al., 2007; Tagliabracci et al., 2012; Zhang et al., 2018). Uma vez que não possui um sítio catalítico para a sua atividade, a FAM20A tem sido considerada, mais recentemente, uma pseudoquinase em relação dimérica com a FAM20C, formando um complexo e ativando alostericamente a FAM20C e, por conseguinte, otimizando a função quinase da FAM20 C (Cui et al., 2015; Cui et al., 2017; Zhang et al., 2018).

Estudos em modelo animal relataram a importância da proteína FAM20A na modulação dos processos de biomineralização. Em camundongos *knockout Fam20a* foi observado sobrevida dos animais à idade adulta e defeitos de biomineralização. Os animais que sofreram a inativação apresentaram AI grave, calcificações disseminadas das artérias musculares e calcificações pulmonares (Vogel et al., 2012). Ainda em estudo animal, o papel essencial do *Fam20a* no desenvolvimento de tecidos orais foi sugerido com o notável atraso na erupção dentária, hiperplasia gengival, esmalte dentário mais fino, e ameloblastos deficientes originando dentes com AI (Li et al., 2016).

1.2 SÍNDROME DE RAINE

A síndrome de Raine (RNS, OMIM #259775) é uma displasia esquelética rara causada por variantes recessivas no gene *FAM20C*, caracterizada por um aumento da densidade óssea (displasia osteoesclerótica) neonatal, que geralmente leva à morte mais comumente relatada com insuficiência respiratória, devido a malformação torácica e hipoplasia pulmonar, ainda nas primeiras semanas de vida (Raine et al., 1989; Simpson et al., 2007). Essa síndrome tem uma prevalência de 1:1.000.000 (Faundes et al., 2014) e, segundo a *The Human Gene Mutation Database* (HGMD®), já existem mais de 52 casos relatados na literatura com a especificação de diferentes variantes patogênicas avaliadas até o presente.

A RNS foi descrita pela primeira vez em 1989 por Raine et al, que descreveu uma recém-nascida que faleceu logo após o parto por complicações respiratórias por má formação torácica e hipoplasia pulmonar. Além disso, o relato incluía esclerose óssea, microcefalia, depressão da ponte nasal, hipoplasia do terço médio da face, baixa inserção das orelhas, exoftalmia, tórax curto e estreito, hiperplasia gengival, micrognatia e fissura palatina. A letalidade neonatal é frequente e os pacientes não resistem aos primeiros dias ou semanas após o nascimento e outros casos desta síndrome foram publicados relatando a letalidade neonatal (Al Mane et al., 1996; Acosta et al., 2000).

No entanto, outros indivíduos com a síndrome chegaram à infância ou até mesmo à vida adulta, caracterizando a forma menos grave e, portanto, não-letal da doença (Simpson et al., 2009; Acevedo et al., 2015; Elalaoui et al., 2016). A primeira descrição de RNS não-letal foi em 2009 em dois indivíduos que sobreviveram por motivos ainda não esclarecidos (Simpson et al., 2009). Os indivíduos portadores desta forma não-letal da síndrome foram descritos com fenótipo clássico de dismorfismo e osteoesclerose difusa, além de calcificações renais e intracranianas (Simpson et al., 2009; Fradin et al., 2011; Rafaelsen et al., 2013). Até 2020, 43 famílias, apresentando cerca de 34 casos letais e 18 não-letais, com mais de 30 variantes no gene *FAM20C* foram relatadas na literatura (Kingston et al., 1991; Eltan et al., 2020; Mameli et al., 2020). Os achados descrevem variantes recessivas em homozigose ou heterozigose composta distribuídas entre os exons 1 a 10, do tipo *frameshift*, rearranjos, *nonsense*, *missense* e *splice site* (Simpson et al., 2007; Rafaelsen et al., 2013; Mamedova et al., 2019). Na Tabela Suplementar 1, estão

descritas as principais características demográficas e clínico-radiográficas dos casos letais e não letais relatados até o momento.

Além das manifestações clínicas, os indivíduos afetados pela RNS apresentam alterações dentárias graves que foram descritas em relatos de casos não-letais e caracterizadas em dois estudos (Rafaelsen et al., 2013; Acevedo et al., 2015). Em 2013, Rafaelsen et al. relataram dois casos não-letais de RNS em uma família norueguesa e caracterizaram algumas manifestações dentárias como a presença de abscessos periapicais, dentina com defeitos de mineralização, esmalte hipoplásico e edentulismo dos pacientes antes dos 18 anos de idade. Mais tarde, em 2015, Acevedo et al. identificaram duas novas variantes patogênicas recessivas no *FAM20C* em duas famílias brasileiras não relacionadas com história de consanguinidade e caracterizaram mais detalhadamente as manifestações bucais de cinco casos de afetados. Foram observados em ambas as famílias AI hipoplásica, defeitos dentinários, formação incompleta das raízes, e histórico de abcessos recorrentes e lesões periapicais em vários dentes, mucosa gengival e palatina hiperplásica, calcificações ectópicas em gengiva e folículo dentário (Acevedo et al., 2015).

Na primeira família de pais consanguíneos, primos de primeiro grau, de seis irmãos, três irmãos apresentaram diagnostico de RNS, sendo dois do sexo masculino e uma do sexo feminino, com 16, 12 e 10 anos de idade na primeira avaliação no Hospital Universitário de Brasília. Todos os três pacientes apresentavam AI. A mãe informou não ter tido complicações na gestação, relatando que apenas um dos filhos teve problemas respiratórios perinatal, devido a atresia coanal que foi corrigida por meio de cirurgia. Nesta família, foram relatadas surdez neurosensorial, calcificações intracranianas, mas sem envolvimento de complicações renais. Foram observadas manifestações craniofaciais, hipoplasia de face, micrognatia, palato alto, má oclusão e AI hipoplásica nos três indivíduos afetados. Não houve atraso na erupção dentária, e o aspecto de esmalte hipoplásico foi relatado em todos os irmãos afetados. Além disso, abscessos periapicais e periodontais também foram observados em todos os indivíduos afetados. Os tecidos pericoronários de terceiros molares inclusos foram analisados microscopicamente e a presença de calcificações ectópicas nos tecidos moles foram relatadas. Também foram observadas alterações da dentina coronária e radicular com a presença de dentina interglobular, principalmente na dentina circumpulpar. Por meio de sequenciamento de

exoma, foi identificada, na família 1, variante em homozigose em sítio de *splicing* no gene *FAM20C* (c.784+5 G>C, p.Trp202Cys*37). As características de um paciente desta família podem ser vistas na Figura 1.

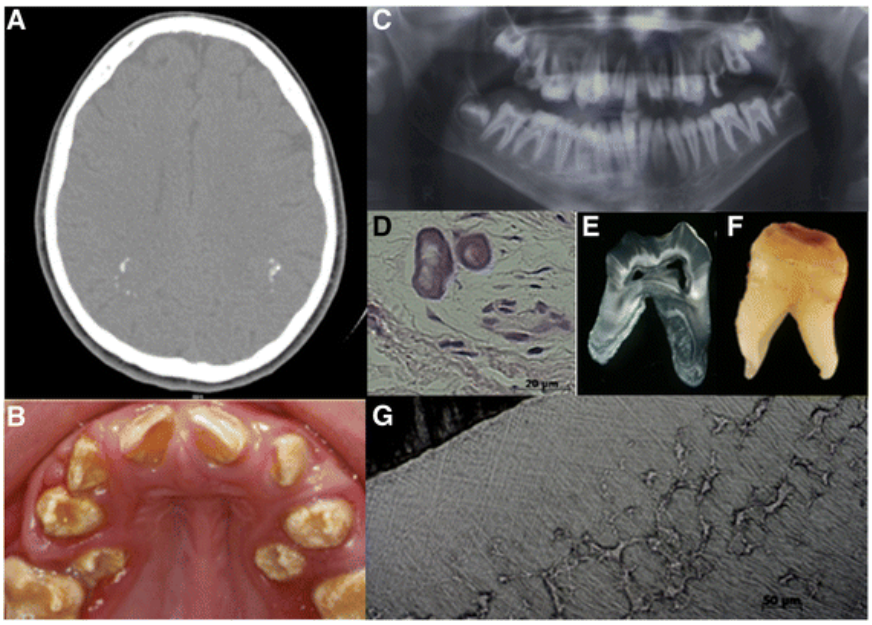

Figura 1. Caracterização fenotípica de um paciente da família 1 descrita por Acevedo et al. (2015). (A) Tomografia computadorizada revelando as calcificações intracranianas; (B) Fotografia intraoral demonstrando alterações dentárias e redução na espessura do esmalte; (C) Radiografia panorâmica demonstrando a ausência de radioluscência entre esmalte e dentina, além de comprometimento pulpar e periodontal; (D) Presença de calcificação ectópica gengival; (E e F) Imagem macroscópica revelando esmalte hipoplásico; (F) Defeitos de dentina interglobular. Fonte: Acevedo et al., 2015.

Na segunda família relatada por Acevedo et al. (2015), os dois de três filhos do casal de primos de primeiro grau foram diagnósticos com RNS, aos 5 e 4 anos de idade. Os pais revelaram que os avós também eram primos de primeiro grau. As crianças apresentaram problemas respiratórios com a necessidade de hospitalização nos primeiros meses de vida. Os dois apresentaram atraso no desenvolvimento, estatura baixa e outras características fenotípicas recorrentes na RNS. Eles apresentaram calcificações intracranianas e craniossinostose da sutura sagital. O irmão mais velho desenvolveu uma grave deficiência visual, comprometendo 90% da visão aos 11 anos de idade, além de

microcalcificações na medula renal. Ambos os irmãos apresentavam mucosa gengival aumentada, dentes permanentes não irrompidos, AI hipoplásica, e histórico de abscessos periapicais recorrentes em decíduos e permanentes. Na avaliação histopatológica de biópsia gengival foi observado uma hiperplasia gengival com presença de calcificações ectópicas no tecido conjuntivo. Na família 2, foi identificada variante *missense* em homozigose no *FAM20C* (c.1487C>T, p.Pro496Leu). As características dos pacientes afetados desta família podem ser vistas na Figura 2.

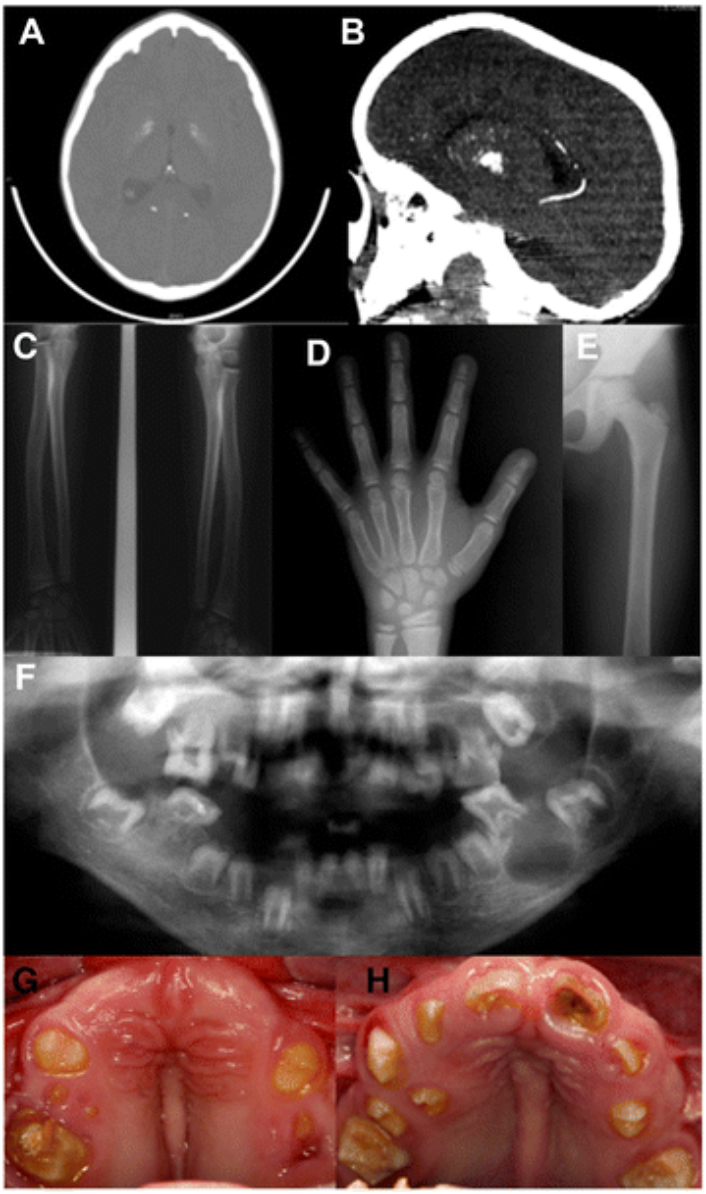

Figura 2. Caracterização fenotípica de pacientes da família 2 descrita por Acevedo et al. (2015). (A e B) Tomografia computadorizada revelando a presença de calcificações intracranianas; (C, D e E) Radiografias demonstrando ossos longos, ossos carpais e falanges submineralizados e um leve arqueamento do rádio; (F) Radiografia dentária mostrando ausência de diferenças de densidade entre esmalte e dentina, formação radicular incompleta, câmaras pulpares aumentadas, e radiolucências apicais associadas aos dentes permanentes; (G e H) Fotografia intraoral demonstrando atraso na erupção e esmalte hipomineralizado e hipoplásico. Fonte: Acevedo et al., 2015.

1.2.1 Etiopatogenia da RNS

A proteína FAM20C é uma quinase localizada no interior do complexo de Golgi, responsável pela fosforilação de um amplo espectro de substratos, que incluem fosfoproteínas, o FGF23 e a família *SIBLING* ("*Small Integrin-Binding Ligand, N-Linked Glycoprotein"*) (Tagliabracci et al., 2012; Xiao et al., 2013; Tagliabracci et al., 2015). As glicoproteínas *SIBLINGs* pertencem à família de fosfoproteínas ligantes do cálcio. Tais proteínas (sialofosfoproteína dentinária - DSP, proteína da matriz dentinária - DMP1, osteopontina - OPN, sialoproteína óssea - BSP) são secretadas durante a formação e mineralização da dentina e do osso, podendo ser responsáveis pelo depósito de fosfato de cálcio (Fisher e Fedarko, 2003). Foi descrito que o gene *FAM20C* é expresso em condrócitos e osteoblastos de ossos longos e em células dentárias, e que a proteína FAM20C sintetizada pode ser detectada no citoplasma de células ósseas e dentárias e na matriz extracelular do osso, incluindo dentina, esmalte e osso alveolar (Hao et al., 2007; Wang et al., 2010). Mais recentemente, estabeleceu que a Fam20c exerce uma atividade de quinase que fosforila os motivos S-x-E/pS das proteínas, identificando mais de 100 fosfoproteínas secretadas como substratos Fam20C. Além disso, sugere que a Fam20c tem participação no controle da biomineralização, homeostase lipídica, cicatrização de feridas e migração e adesão celular (Tagliabracci et al., 2015).

Camundongos *knockout* do *Fam20c* demonstraram semelhanças no fenótipo dentário sugestivo de AI (Vogel et al., 2012). Nos estudos de Vogel et al. (2012), foi relatada taxa de mortalidade de 20% em camundongos Fam20c$^{-/-}$. Foi demonstrado que os camundongos *Fam20c*$^{-/-}$ apresentaram sugestão de deficiência auditiva, anormalidade neurológica, crescimento atrofiado, densidade óssea reduzida e mortalidade precoce. Por análise de microtomografia computadorizada foi observado uma falta total ou quase que total do esmalte dentário. Nesse mesmo trabalho, avaliaram a dentição dos camundongos *Fam20c*$^{-/-}$ e descreveram que os animais apresentavam alterações patológicas no osso alveolar, dentina e polpa. Os dentes molares eram pequenos e em formato de pinos e apresentavam esmalte defeituoso. Todos os dentes dos camundongos *Fam20c*$^{-/-}$ apresentavam-se com anormalidade, e foi comprovada a redução do conteúdo mineral do esmalte em relação à dentina e ao osso. Os dentes frequentemente apresentaram necrose pulpar com o aparecimento de abcessos e a dentina apresentava-se com estrutura

desorganizada e redução dos túbulos dentinários. Além dos achados dentários, a presença de calcificações vasculares nos camundongos *Fam20c-null* também foi relatada.

Wang et al. (2012a) também realizaram estudos em camundongos *knock-out* condicionais e seus achados foram semelhantes aos de Vogel et al. Os incisivos dos camundongos *knockout* eram mais curtos, com menos tecido mineralizado e com câmaras pulpares aumentadas. Além disso, apresentavam uma hipomineralização nos ossos alveolares. À nível ultraestrutural, foi observada falta de estrutura de esmalte, dentina mais fina na porção radicular, perda de túbulos dentinários e menos cemento celular. Também foi relatado um encurtamento das raízes dentárias e osso alveolar mais poroso, além de uma redução na expressão de DMP1 e DSP (Wang et al., 2012a; Wang et al., 2012b).

Recentemente, Liu et al. (2018) realizaram estudo *in vitro* de células dentárias mesenquimais imortalizadas de camundongos. A deleção do *Fam20c* não alterou a morfologia celular, mas resultou em uma menor proliferação e migração das células. Além disso, a deficiência do gene reduziu significantemente a expressão gênica de fosfatase alcalina (*Alpl),* colágeno tipo 1 *(Col1a1), Osterix e* fator de transcrição relacionado ao Runt 2 (*Runx2),* e aumentou significantemente a expressão de fator de crescimento de fibroblasto 23 (*FGF23).* Os estudos sugeriram que a FAM20C afeta a mineralização de forma direta, não somente pela homeostasia do fósforo sérico, mas também alterando a função celular e controle da mineralização.

No entanto, a maioria dos estudos relacionados a alterações nos genes *FAM20A e FAM20C*, tem sido enfatizado com a avaliação de tecidos ósseos e dentários mineralizados em modelos animais. Recentemente, Lignon et al. (2017) caracterizaram o conteúdo mineral das calcificações de pacientes portadores de ERS, porém poucos estudos têm avaliado as alterações gengivais e calcificações ectópicas observadas em pacientes ERS e RNS.

1.3 FIBROMATOSE E CALCIFICAÇÕES ECTÓPICAS GENGIVAIS NA ERS E RNS.

A Fibromatose Gengival (FG) é caracterizada por um epitélio pavimentoso estratificado com papilas alongadas em direção a um tecido conjuntivo fibroso associado a poucos fibroblastos (Martelli-Júnior et al., 2000; Araújo et al., 2003). As formas mais comuns da FG estão relacionadas aos efeitos adversos de medicamentos sistêmicos, como os bloqueadores de canais de cálcio, anticonvulsivantes e imunossupressores. Além disso, a FG pode ser herdada como um traço isolado (fibromatose gengival hereditária) ou como parte de uma síndrome (Nevin et al., 1971; Goldblatt e Singer et al., 1992; Coletta e Graner, 2006; Pehlivan et al., 2009).

A FG não sindrômica geralmente é transmitida como um traço autossômico dominante, embora em casos esporádicos sejam comuns e a herança autossômica recessiva também já tenha sido relatada (Gawron et al., 2016). A condição hereditária isolada na qual o tecido gengival espontaneamente e progressivamente aumenta é identificada como Fibromatose Gengival Hereditária (HGF; OMIM#135300), classificada como uma doença rara que afeta 1:750.000 nascidos vivos, sendo a forma mais comum causada por variantes em heterozigose nos genes *SOS1* (Hart et al., 2002; Pehlivan et al., 2009) e *REST* (Pehlivan et al., 2009; Bayram et al., 2017). As formas sindrômicas de FG também foram relatadas com modos de herança autossômicas dominantes ou recessivas (Kortüm et al., 2015; Bauer et al., 2018; Dourado et al., 2019).

Estudos demonstram que as características histopatológicas da FG são semelhantes independente da sua etiopatogenia, apresentando um epitélio bem estruturado com longas cristas delgadas que se projetam em direção ao tecido conjuntivo, caracterizado histologicamente como feixes densos de fibras de colágeno intercaladas com fibroblastos (Coletta e Graner, 2006; Meng et al., 2007). As características epiteliais ainda são pouco estudadas e divergem de acordo com os estudos publicados, sendo descrito a acantose como um dos principais achados em associação com um infiltrado inflamatório (Nevin et al., 1971; Raeste et al., 1978; Araújo et al., 2003).

Os fibroblastos gengivais também podem ser responsáveis pela síntese e secreção de colágeno tipo I e da remodelação da matriz extracelular (MEC), resultando em um crescimento gengival (Kather et al., 2008). Estudos indicam que o aumento gengival pode

ser causado por distúrbios na remodelação do colágeno, alterações na proliferação dos fibroblastos, e de metaloproteinases da matriz e inibidores teciduais de metaloproteinases, MMPs e TIMPs (Martelli-Júnior et al., 2000; Araújo et al., 2003; Meng *et al.*, 2007). Além disso, a presença de miofibroblastos foi relatada em fibromatoses gengivais hereditárias e medicamentosa, como sendo uma das principais células responsáveis pela síntese e degradação da matriz extracelular (MEC) durante os processos inflamatórios e de reparo tecidual (Bitu et al., 2006; Dill e Iacopino, 1997). No entanto, os mecanismos moleculares responsáveis pela maioria dos casos permanecem indefinidos.

A FG tem sido relatada como manifestação clínica em pacientes com ERS e observada clinicamente como um aumento volumétrico gengival, e microscopicamente observa-se um alongamento das cristas epiteliais e uma lâmina própria com grossos feixes de fibras colágenas distribuídas em várias direções. Além disso, foi também demonstrada a presença de restos epiteliais odontogênicos e calcificações ectópicas focais no tecido conjuntivo gengival (Paula et al., 2005; Martelli-Junior et al., 2008). Análise imuno-histoquímica demonstrou a presença de miofibroblastos distribuídos pela lâmina própria (Martelli-Junior et al., 2008). Dure-Molla et al. (2014) relataram que fibromatose gengival é uma manifestação comum com gravidade variável na síndrome ERS. Estudo recente demonstrou a presença de acantose e inflamação gengival, sugerindo que alterações na transição epitélio mesenquimal podem contribuir para a formação de nódulos minerais na lâmina própria de gengiva de pacientes ERS (Simancas Escorcia et al., 2020).

Acevedo et al. (2015) descreveram as alterações gengivais em pacientes RNS com aspectos muito semelhantes aos pacientes ERS. Em seus estudos, citaram a presença de mucosa gengival aumentada, acantose epitelial e sugeriram a presença de fibromatose gengival, sem mais estudos que elucidassem esses achados. Além disso, também relataram a presença das calcificações ectópicas nas duas famílias investigadas.

A patogênese das calcificações ectópicas gengivas ainda não foi esclarecida. No entanto, o desenvolvimento de calcificações ectópicas em outros tecidos conjuntivos tem sido amplamente estudado tais como as calcificações vasculares. Diversos mecanismos têm sido propostos que incluem a transição das células do músculo liso do miocárdio para um fenótipo calcificado (Reynolds et al., 2004; Chen et al., 2008; Alves et al., 2014). Estudos sugerem que as calcificações podem se iniciar a partir de vesículas de matriz

fisiologicamente parecidas com o que ocorre no tecido ósseo e dentinário (González e Martin, 2001) ou serem formadas pela degradação celular autofágica, expondo cristais de hidroxiapatita para o meio extracelular (Speer e Giachelli, 2004). Além disso, as calcificações vasculares patológicas podem surgir devido ao estresse oxidativo e perda de inibidores de mineralização, que são continuamente expressos em tecido vascular saudável (Cola et al., 2004; Moe et al., 2005). Entretanto, tem sido discutida a hipótese de que as mineralizações ectópicas ocorrem devido ao desequilíbrio nos mecanismos reguladores que previnem ativamente as calcificações patológicas, como por exemplo a regulação da calcificação vascular pelas proteínas MGP, TNF, BMP, FGF23 e Fetuin-A (Proudfoot, 2019).

Além disso, condições genéticas podem levar à ocorrência de calcificações ectópicas em tecidos conjuntivos moles. O pseudoxantoma elástico (PXE, OMIM #264800), por exemplo, é uma desordem do tecido conjuntivo que se caracteriza pela presença de calcificações precoces das fibras elásticas, levando às lesões de pele. Estudos demonstraram nessa síndrome a sobre-expressão de MGP e Fetuína-A presentes na pele apenas de indivíduos afetados, e sugeriram ser proteínas que participam no controle da mineralização de tecidos moles (Vanakker et al., 2010). Além dessas, várias outras proteínas participam do processo de regulação da formação de tecido ósseo atuando como promotores e inibidores de calcificações em tecidos moles, e devem ser melhor investigadas para a compreensão das mineralizações ectópicas (Moe et al., 2003; Mejía et al., 2011; Ossareh, 2011).

Considerando que a FG pode ser uma manifestação de diversas síndromes foi realizada uma revisão sistemática da literatura com o intuito sintetizar a literatura disponível sobre síndromes com fibromatose gengival, relatar o fenótipo, bem como as alterações moleculares. Além disso, a caracterização histológica de tecidos gengivais e a investigação de propriedades biológicas de culturas primárias de fibroblastos gengivais de pacientes brasileiros com ERS e RNS e em acompanhamento no Hospital Universitário de Brasília foi realizada.

HIPÓTESES

1. O diagnóstico de fibromatose gengival nos paciente RNS foi sugerido em estudo prévio. Portanto, espera-se que a análise histopatológica confirme a hipótese diagnóstica de FG.
2. Estudos têm mostrado a presença de miofibroblastos em FG medicamentosa, hereditária e sindrômica. Dessa forma, há a hipótese de que tecidos gengivais dos pacientes ERS e RNS também apresentem miofibroblastos envolvidos na patogênese da fibromatose gengival.
3. Devido as alterações gengivais observadas em pacientes ERS e RNS, espera-se observar expressão das proteínas FAM20A e FAM20C em células epiteliais e fibroblastos.
4. Considerando os estudos que demonstram uma dualidade funcional das proteínas FAM20A e FAM20C, espera-se observar uma colocalização citoplasmática nos fibroblastos gengivais.
5. Devido a importância das proteínas FAM20A e FAM20C na fosforilação de múltiplas proteínas, espera-se que as propriedades biológicas de cultura primária de fibroblastos gengivas em cultura de pacientes ERS e RNS estejam alteradas.
6. Devido ao diagnóstico de calcificações ectópicas em pacientes ERS e RNS, espera-se que as células de fibroblastos gengivais sejam capazes de produzir nódulos minerais em cultura com meios condicionados.

OBJETIVOS

OBJETIVO GERAL

Caracterizar o tecido gengival e a culturas primárias de fibroblastos gengivais de pacientes ERS e RNS em acompanhamento na Clínica de atendimento de Pacientes Portadores de Anomalias Dentárias na unidade de Saúde Bucal, Hospital Universitário de Brasília, Brasília, DF.

OBJETIVOS ESPECÍFICOS

1) Realizar uma revisão sistemática a fim de sintetizar as características clínicas e genéticas de síndromes que apresentam fibromatose gengival;
2) Analisar histopatologicamente as amostras gengivais de pacientes ERS e RNS;
3) Analisar a expressão da FAM20A, FAM20C e α-SMA em cortes transversais de gengiva de pacientes ERS e RNS;
4) Estabelecer cultura primária de fibroblastos gengivais de pacientes ERS e RNS e de indivíduos não sindrômicos (C);
5) Caracterizar a morfologia de fibroblastos gengivais ERS, RNS e C;
6) Avaliar proliferação e migração de fibroblastos gengivais ERS, RNS e C;
7) Avaliar a expressão gênica de *FAM20A* e *FAM20C* em fibroblastos gengivais ERS, RNS e C;
8) Avaliar a expressão da FAM20A, FAM20C e α-SMA em fibroblastos gengivais ERS, RNS e C;
9) Avaliar a expressão da ERK e p-ERK em fibroblastos gengivais ERS, RNS e C.
10) Avaliar o potencial de mineralização de fibroblastos gengivais ERS, RNS e C.

Para a apresentação dos resultados, este trabalho será dividido em duas partes em forma de artigos. O Artigo 1 se refere ao primeiro trabalho denominado "*Syndromes with gingival fibromatosis: a systematic review*", o qual já foi publicado na revista *Oral Diseases*, em abril de 2020. O Artigo 2 se refere ao segundo estudo com o título "*Characterization of gingival tissues and fibroblasts in patients with Enamel Renal syndrome and Raine syndrome*" que será submetido para publicação na revista *Journal of Periodontology*.

2. ARTIGOS

ARTIGO 1

TITLE: Syndromes with gingival fibromatosis: a systematic review.

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ABSTRACT

Objective: The aim of systematic review was to describe the phenotypes and molecular profiles of syndromes with gingival fibromatosis (GF).

Methods: A comprehensive search of PubMed, LILACS, Livivo, Scopus and Web of Science was conducted using key terms relevant to the research questions, and supplemented by a grey literature search. The Methodological Quality and Synthesis of Case Series and Case Reports in association with the Case Series and Prevalence Studies from the Joanna Briggs Institute critical appraisal tools were used for the risk of bias. We followed the PRISMA checklist guidelines.

Results: Eighty-four studies reporting GF as an oral manifestation of a syndrome were identified in this review. Enamel renal syndrome was the most frequently reported syndrome with GF, represented by 54 individuals in 19 studies, followed by Zimmermann-Laband syndrome with 24 individuals in 15 studies and Costello syndrome, which was presented in a case series study with 41 individuals. Among reported cases, other clinical manifestations such as hypertrichosis, ectopic gingival calcification and cherubism were described.

Conclusions: The results emphasize the need of systematic oro-dental-facial phenotyping for future descriptions as well as further molecular analysis in order to better understand the occurrence of syndromic GF.

Key-words: Gingival fibromatosis, Syndrome, Systematic review.

INTRODUCTION

Gingival fibromatosis (GF) can be defined as a gingival overgrowth characterized by a slowly fibrous enlargement of gingiva. Commonly, GF may be observed in patients using anticonvulsant, immunosuppressive and antihypertensive (with calcium channel-blocker activity) agents (Teoh et al., 2019). However, inherited forms of GF have been reported in the literature and can be observed as an isolated trait or as part of several syndromes (Coletta & Graner, 2006; Hart & Hart, 2009). Hereditary gingival fibromatosis (HGF; OMIM#135300) represents the GF nonsyndromic form with an estimated prevalence of 1:750,000 (Hart et al., 2002; Pehlivan et al., 2009).

Several syndromes with GF have been reported in the literature, with enamel renal syndrome (ERS; OMIM#204690), Zimmerman-Laband syndrome (ZLS1 and ZLS2; OMIM#135500 and OMIM#616455) and Ramon syndrome (OMIM 266270) representing the most common ones (Coletta & Graner, 2006; Gawron et al., 2016). GF is usually present in patients with hyaline fibromatosis syndrome and infantile systemic hyalinosis (HFS; OMIM#228600), but in these cases, GF is a result of progressive accumulation of amorphous hyaline material over time, which affects not only the gingiva but also the skin and other visceral organs (Casas-Alba et al., 2018). The clinical and histological features of nonsyndromic and syndromic GF are similar. The main clinical feature is the partial or full coverage of dental crowns. GF is rare at birth and usually begins at the eruption of primary dentition, causing malposition and prolonged retention of teeth. The enlargement is most intense during tooth eruption and minimal growth is observed in adults, however, according to the syndrome, GF may manifest with varying degrees of extension, severity and progression (Dhase et al., 2012; Gawron et al., 2016; Rodríguez-Vázquez et al., 2007). There is a large consensus that GF, regardless whether it is isolated or associated with other diseases characterizing a syndrome, should be surgically removed in a conservative way, with excision of the enlarged gingival tissues and tooth preservation. Although recurrence is unpredictable, it is most often seen in children and teenagers rather than adults (Coletta & Graner, 2016). Although studies indicate that gingival enlargement may be caused by disturbances in collagen remodeling and alterations in fibroblast proliferation as well (Coletta & Graner, 2006; Meng et al., 2007), the molecular mechanisms responsible for most of the reported cases remain undefined (Gawron et al., 2016).

As previous studies have reported GF as a manifestation of several syndromes associated with multiple clinical signs and symptoms and no systematic reviews (SR) have yet been conducted, the aim of this study was to synthesize the available literature concerning the presence of GF in syndromes, emphasizing the phenotype and the molecular etiology when available. An overall understanding of the GF phenotypic spectrum is relevant to clinical diagnosis, genetic counselling and management. In addition, this review may update the criteria used for dental surveillance in syndromic patients consequently improving their quality of life.

METHODS

Protocol and Registration

This SR followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009; Shamseer et al., 2015). Furthermore, the protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO) (Booth et al., 2011).

Eligibility Criteria

The study was undertaken to systematically review syndromes that present with GF and investigate the more frequently reported. A PICOS acronym was used to formulate the questions for this study: P – participants (syndromic patients with GF), I – intervention or exposition (not applicable), C – comparison or control (not applicable), O – outcomes measures (frequency and types of genetic disorders associated with GF), and S – types of studies included (descriptive observational studies – case reports and series of cases).

Criteria for the exclusion of articles were as follows: 1- Studies that described nonsyndromic GF, 2- Studies that described drug-induced GF, 3- Studies that described inflammatory GF, 4- Studies that did not report GF, 5- Reviews of the literature, theses, personal opinions and conference abstracts, 6- Full articles not found, 7- Papers that were not in Roman alphabet, 8- Duplicate cases from other studies, 9- Studies that did not

report oral involvement, and 10 - Studies that described cases of hyaline fibromatosis or infantile systemic hyalinosis syndrome.

Study Selection

The studies were selected by developing detailed individual search strategies for each of the following bibliographic databases: Latin American and Caribbean Health Science (LILACS), PubMed, Livivo, Scopus, and Web of Science. A gray literature search was conducted using Google Scholar, OpenGray and ProQuest. The survey included all articles published on or before April 17th, 2019. Detailed search strategies are provided in Table S1. Hand searches of bibliographies from included studies and expert-recommended studies have also been added. Duplicate references were removed using reference manager software (EndNote® X7, Thomson Reuters, New York, USA), transferred to and worked in Rayyan (Rayyan®, Qatar Computing Research Institute, Qatar Foundation) (Ouzzani et al., 2016).

Two phases were established to select the studies. In the first phase, two authors (CRRC and SVB) reviewed the titles and abstracts of all references selected as relevant considering all inclusion and exclusion criteria. The next step involved the participation of a third author (ACA), who reviewed all articles where there was no consensus in the initial evaluation to determine which articles would be included in the second phase. The second phase involved two authors (CRRC and SVB) who reviewed the full-text of the publications. Disagreements were resolved by discussion until a consensus was reached among the three reviewers (CRRC, SVB, and ACA).

Data Collection Process and Data Items

Two authors (CRRC and SVB) collected the required information from each included article. A third author (ACA) checked the information. Any disagreements were discussed until a consensus was reached among the three authors (CRRC, SVB, and ACA). The participation of a fourth reviewer (RDC) was requested if a consensus could not be reached.

Risk of bias in Individual Studies

For the quality assessment of each study, the Methodological Quality and Synthesis of Case Series and Case Reports proposed by Murad et al. 2018, in association with the Case Series and Prevalence Studies from the Joanna Briggs Institute (JBI) critical appraisal tools were used (Munn et al., 2015; Moola et al., 2017). Therefore, this quality assessment was applied to all selected articles. The quality assessment of the studies was assessed by the same two authors (CRRC and ACA). The methodology of the selected studies was evaluated through the Joanna Briggs Institute Critical Appraisal Tools for Studies Reporting Prevalence Data for Use in Systematic Reviews – referred to as "Check-list for Case Series and Case Reports". The authors scored each item as "yes," "no", "unclear" or "not applicable" when assessing the quality of each included study.

RESULTS

Study Selection

In the first phase of the selection, 3,329 articles were identified from six electronic databases. After removing duplicates, 2,367 articles remained. Evaluation of the titles and abstracts resulted in the exclusion of 2,068 articles, leaving 299 articles. Using Grey Literature, we identified 227 articles, nine of which were included. One additional article was identified and included from reference lists, and two additional articles were added from expert review, resulting in the selection of 311 articles for the second phase.

After the selection process, texts were completely read and evaluated. Then, 227 studies were excluded based on the exclusion criteria (Table S2), leaving 84 articles for final analysis (Figure S1).

Study Characteristics

The present SR included 218 individuals affected by syndromes that contained GF in the clinical spectrum, representing 82 case reports (173 individuals) and 2 case series (45 individuals). The studies were published between 1958 (McIndoe & Smith, 1958) and 2019 (Debnath et al., 2019; Dourado et al., 2019) and the characteristics of studies are summarized in Table S3. The geographic distribution of the individuals was:

59 from Asia, 52 from Latin America, 49 from North America, 33 from Europe, 8 from Africa and 6 from Oceania (Figure S2).

Different diagnostic methods were used for the assessment of GF in syndromic patients. All reports included clinical examinations, whereas radiographic assessment was reported in 64 studies and histopathological confirmation was presented only in 52 studies. The extent of GF was observed to be generalized in 77 studies, localized in six articles (Giansanti et al., 1973; Chacon-Camacho et al., 2011; Chadwick et al., 1994; Goodwin et al., 2014; Hallet et al., 1995; Ishita et al., 2016) and not determined in one study (Pachajoa et al., 2018). The degree of severity was also assessed, with 15 studies reporting mild fibromatosis (Debnath et al., 2019; Dourado et al., 2019; Giansanti et al., 1973; Goodwin et al., 2014; Abo-Dalo et al., 2008; Afifi et al., 2015; Ashkenazi et al., 2014; Feitosa et al., 2011; Hartsfield et al., 1985; Holzhausen et al., 2003; Landoulsi et al., 2012; Mangino et al., 2003; Martelli-Júnior et al., 2008; O'Connell et al., 2014; Laouina & Zupan, 2017), 25 studies reporting moderate fibromatosis (Afifi et al., 2016; Bhesania et al., 2015; Castori et al., 2013; Cho et al., 2012; de la Tranchade et al., 2003; Goldblatt & Singer, 1992; Jaouad et al., 2015; Kaisare, 2007; Kang et al., 2018; Kantaputra et al., 2017; Kissi et al., 2006; et al., Lee et al., 1993; Lin et al., 2010; Martins et al., 2010; Mérgabané et al., 2016; Molano et al., 1996; Oikarinen et al., 1990; Paula et al., 2005; Pêgo et al., 2017; Poulter et al., 2015; Prasad et al., 2012; Singer et al., 1993; Torres et al., 2018; Wang et al., 2013; Winter & Simpkiss, 1974), three studies revealing moderate-severe fibromatosis (Acevedo et al., 2015; Cabral et al., 2013; Kortüm et al., 2015), 40 studies describing severe fibromatosis (McIndoe & Smith, 1958; Chacon-Camacho et al., 2011; Hallet et al., 1995; Ishita et al., 2016; Landoulsi et al., 2012; Anderson et al., 1969; Bakaeen & Scully, 1991; Balaji & Balaji, 2017; Bauer et al., 2018; Benazza et al., 2005; Cuestas-Carnero & Bornancini, 1988; Davalos et al., 2011; Elavarasu et al., 2017; Gita et al., 2014; Guevara-Sanginés et al., 2002; Harrison et al., 1998; Haytac & Ozcelik, 2007; He & Ping, 2012; Hungund et al., 2013; Johnson et al., 1986; Kanagotagi et al., 2015; Kantaputra et al., 2014; Kim et al., 2007; Koch et al., 1992; Kulkarni et al., 2011; Kundoor et al., 2016; Laband et al., 1964; Ooya et al., 1988; Pina-Neto et al., 1986; Queiroz et al., 2005; Ramon et al., 1967; Roginsky et al., 2009; Roopa et al., 2016; Roquebert et al., 2008; Shah et al., 2004; Stefanova et al., 2003; Sunil et al., 2016; Tang et al., 2014; Tommiska et al., 2017; Yalçin et al., 1999) and one study not determining the severity of this condition (Pachajoa et al., 2018). The most frequently

dental alterations identified in the syndromic patients were amelogenesis imperfecta (AI), delayed or failed tooth eruption, retained teeth, root dilacerations, dental agenesis, taurodontism, supernumerary teeth and dental pulp calcifications. The oro-dental features of syndromic patients are summarized in Table S4.

Gingival ectopic calcifications were a common feature associated with GF in the reported syndromes. A total of 18 individuals with gingival ectopic calcifications had AI, being present in six individuals with ERS (Debnath et al., 2019; O'Connell et al., 2014; Beshania et al., 2015; Kantaputra et al., 2017; Pêgo et al., 2017; Torres et al., 2018); five individuals with Raine syndrome (RS; OMIM#259775) (Acevedo et al., 2015); four individuals with GF and dental abnormalities (Martelli-Júnior et al., 2008); one individual with GF, AI and tooth eruption defects (Molano et al., 1996); one individual with autosomal recessive rough hypoplastic AI (Ooya et al., 1988); and in one individual with rough hypoplastic AI, dental follicular hamartomas and gingival hyperplasia (Roquebert et al., 2008). In most of these studies, ectopic calcifications were reported in other oral tissues as well, including dental pulps in 35 individuals (Debnath et al., 2019; Dourado et al., 2019; Ashkenazi et al., 2014; Martelli-Júnior et al., 2008; O'Connell et al., 2014; Kang et al., 2018; Kantaputra et al., 2017; Paula et al., 2005; Pêgo et al., 2017; Wang et al., 2013; Acevedo et al., 2015; Kantaputra et al., Ooya et al., 1988; Roquebert et al., 2008) and dental follicles in 8 individuals (de la Tranchade et al., 2003; Paula et al., 2005; Acevedo et al., 2015; Roquebert et al., 2008) (Figure S3).

Results of Individual Studies

This SR describes the names of syndromes as reported by the authors. A total of 24 different syndromes were identified (Table S3 and Table S5). Fifty-four studies (152 individuals) reported syndromes with described phenotypes and known molecular etiologies in the OMIM database, 11 studies (19 individuals) reported syndromes with described phenotypes but unknown molecular etiologies, and 11 studies (47 individuals) reported syndromic phenotypes not characterizing a specific syndrome. The syndromes with the largest number of affected individuals were ERS with 54 reported individuals (24.8%), Costello syndrome with 41 reported individuals (18.8%) and Zimmermann-Laband syndrome with 24 reported individuals (11%).

When assessing the systemic and oral manifestations of the reported syndromes, those associated with hypertrichosis and any ectopic calcifications in connective tissues were the most frequently reported. Other syndromes were associated with hearing loss (Hatsfield et al., 1985; Cuestas-Carnero & Bornancini, 1988; Davalos et al., 2011; Harrison et al., 1998; Roginsky et al., 2009; Roquebert et al., 2008; Shah et al., 2004), facial dysmorphism (Chacon-Camacho et al., 2011; Ishita et al., 2016; Goldblatt & Singer, 1992; Lee et al., 1993; Martins et al., 2010; Acevedo et al., 2015; Kortüm et al., 2015; Elavarasu et al., 2017; Harrison et al., 1998; Kanagotagi et al., 2015; Kulkarni et al., 2011; Kundoor et al., 2016; Tang et al., 2014; Yalçin et al., 1999) and hormonal alterations (de la Tranchade et al., 2003; Stefanova et al., 2003) (Table S3).

Molecular profile of GF in syndromes

The molecular profile of the different studies is summarized in Table 1. One study with congenital generalized hypertrichosis terminalis with gingival hyperplasia (Afifi et al., 2015) reported 17q24.2-q24.3 chromosomal microdeletion, another study with Zimmermann–Laband (Kim et al., 2007) reported a chromosomal translocation t(3;8)(p21.2;q24.3), and another paper (Stefanova et al., 2003) reported a patient with the same syndrome but carrying a different translocation t(3;17)(p14.3; q24.3). Seven gene mutations have been reported in *ABCC9* in Cantu syndrome (Pachajoa et al., 2018; Afifi et al., 2016), *ATP6V1B1* in Zimmermann–Laband syndrome (Bhesania et al., 2015; Abo-Dalo et al., 2008), *FAM20A* in AIFGS and ERS (Dourado et al., 2019; Jaouad et al., 2015; Kantaputra et al., 2017; Pêgo et al., 2017; Poulter et al., 2015; Wang et al., 2013; Cabral et al., 2013; Kantaputra et al., 2014), *FAM20C* in RS (Acevedo et al., 2015), *KCNH1* in Temple-Baraitser syndrome and Zimmermann–Laband syndrome (Kortüm et al., 2015; Mégarbané et al., 2016), *KCNK4* in facial dysmorphism, hypertrichosis, epilepsy, intellectual disability/developmental delay and gingival overgrowth (Bauer et al., 1991), and *KCNQ1* in pituitary hormone deficiency and maternally inherited gingival fibromatosis (Tommiska et al., 2017).

Risk of bias within studies

The results of risk of bias are described in Table S6 and Table S7. Two case series did not clearly report the demographics of the participants, and in one of these case series,

it was unclear whether patient inclusion was consecutive or not. None of these studies reported the demographic characteristics of the presenting site and this may have had an impact on the external validity of the included studies. The remaining 82 studies were case reports. Twenty-eight studies described clearly the patient demographics, the clinical history was described in 50 studies, and the histopathological features were reported in 33 studies. However, only 54 studies highlighted had relevant information for clinical practice.

Risk of bias across studies

The methodological quality of included studies is depicted in Figure S4. High risk of bias was identified in this analysis due to the lack of description of the demographic characteristics for the inclusion of patients in the case reports and case series. In addition, the studies included in this SR have failed to provide a detailed description of the diagnostic methods and results in the case reports. This SR described rare syndromes and, therefore, the included studies were highly heterogeneous. Although the representative sample of each study was not a cause for concern, this caused the lack of statistical data for additional analysis.

DISCUSSION

This SR investigated the available literature on the presence of GF in syndromic individuals. The results suggest that the clinical examination is sufficient for the diagnosis of GF, however the inclusion of histopathological examination may aid in the diagnosis. The names of the syndromes used for data compilation were the names reported by the study authors. Some articles, especially the oldest ones, did not present the updated denomination of syndromes or did not use the current OMIM database nomenclature (www.omim.org). Moreover, many of these reports did not include molecular analysis. This was especially noted in the oldest reports of two separate autosomal recessive conditions: amelogenesis imperfecta and gingival fibromatosis syndrome (AIGFS) and ERS. Currently, these conditions are considered a single entity since recessive mutations

in the *FAM20A* gene are responsible for both syndromes (Jaureguiberry et al., 2012; O'Sullivan et al., 2011; de La Dure-Molla et al., 2014). At present, the effect of *FAM20A* mutations on gingival overgrowth and associated ectopic calcifications is unknown. Considering that all patients with ERS have GF and frequently the initial diagnosis is performed by a dentist, the management of these patients may be challenging.

Several syndromes in this SR also reported hypertrichosis as a common finding as well as GF. Hypertrichosis may be defined by the patient's history as acquired by the use of medications (anticonvulsant and immunosuppressant) or congenital and is a clinical feature in several rare syndromes due to the development of hair follicle dysfunction (Poonia et al., 2018; Saleh & Cook, 2018). Further studies are necessary in order to better understand if GF and hypertrichosis are associated features. Twelve cases of Ramon syndrome and cherubism were included in this SR. Besides GF, individuals with Ramon syndrome also presented with maxillary fibrous dysplasia, epilepsy, mental deficiency, hypertrichosis, stunted growth, rheumatoid arthritis, and retinal changes. At present, the molecular etiology of Ramon syndrome is unknown, but a recent report described a mutation in the *ELMO2* gene in a girl diagnosed with Ramon syndrome (Mehawej et al., 2018).

Concerning the molecular analysis of the reported syndromes, chromosomal abnormalities were determined in congenital generalized hypertrichosis terminalis with gingival hyperplasia syndrome (Afifi et al., 2015) and Zimmermann-Laband syndrome (Kim et al., 2007; Stefanova et al., 2003). In addition, seven mutated genes were reported as being disease causative and according to their function can be divided in two groups. The first group consisted of syndromes with pathogenic variants in genes encoding proteins responsible for regulating proton pumps and ion channels (*ABCC9*, *ATP6V1B1*, *KCNH1*, *KCNK4* and *KCNQ1*), mainly potassium (K+) channels. Most of the reported variants in potassium channel encoding genes (*ABCC9*, *KCNH1*, *KCNK4*, *KCNQ1*) were gain of function mutations and interestingly gingival overgrowth was a major feature in the affected individuals as well as hypertrichosis. Although it has been demonstrated that ion channels play a role in cell proliferation, the role of these ion channels in gingiva development and pathology is unknown (Urrego et al., 2014; Bauer et al., 2018). The other group was composed of ERS and RS caused by recessive mutations in the *FAM20A* (Dourado et al., 2019; Jaouad et al., 2015; Kantaputra et al., 2017; Pêgo et al., 2017; Poulter et al., 2015; Wang et al., 2013; Cabral et al., 2013; Kantaputra et al., 2014) and

FAM20C (Acevedo et al., 2015) genes, respectively. The proteins encoded by these genes are part of the FAM20 protein family, which is a highly conserved group of secreted kinases (FAM20A, FAM20B and FAM20C) that regulates various cellular functions and biomineralization (Nalbant et al., 2005; Tagliabracci et al., 2012; Zhang et al., 2018). This SR showed that the occurrence of gingival ectopic calcifications was exclusively reported in ERS and RS, suggesting that GF is associated with an impairment of mineralization control of soft tissues in these syndromes. In order to better understand the underlying pathogenesis of GF, further studies should be conducted to understand the effects of these channels and kinases on the development of gingival overgrowth.

Although rare diseases are uncommon at the individual level, they cumulatively represent a significant public health problem. Further efforts are necessary in order to improve the diagnosis, follow-up and quality of life of syndromic patients. Case and series reports must include combined systematic phenotyping and molecular analysis of the patients in order to better determine phenotypic specificity that may provide insights into the pathophysiology and genotype-phenotype correlations. Management of the oro-dental features in these syndromes can be challenging and requires a multi-disciplinary specialized health team in particular the potential role of periodontists in the assessment and accurate determination of gingival conditions is relevant.

Limitations

Some limitations should be considered. The number of different syndromes and their heterogeneity were a strong limitation. Although considered high strength evidence, the present SR emphasizes the importance of the inclusion of case reports when reviewing rare disease manifestations, as a single individual or a few individuals may be representative. They are often the first source of evidence for new therapies, drug effects, and rare syndromes (Jenicek, 2008; Sayre et al., 2017). However, the risk of bias shows that the articles do not follow all guidelines to appropriately report the cases of syndromic patients. Therefore, the results suggest that a better description of case reports and case series should be made and the use of the Case Report (CARE) guidelines that provides a checklist could assist researchers in publishing complete and meaningful clinical information (Murad et al., 2018).

CONCLUSION

This SR describes syndromes with GF in their clinical spectrum, highlighting a high association of GF with ectopic gingival calcification in ERS, hypertrichosis in Zimmermann-Laband syndrome and bone alterations of the jaws in Ramon syndrome and cherubism. It is important to alert clinical geneticists and dentists for a complete oro-dental-facial examination, since in some cases the initial diagnosis is reached based on the characterization of the oral manifestations.

Acknowledgements

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Figure 1. Flow diagram of literature search and selection criteria adapted from Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Moher et al., 2009).

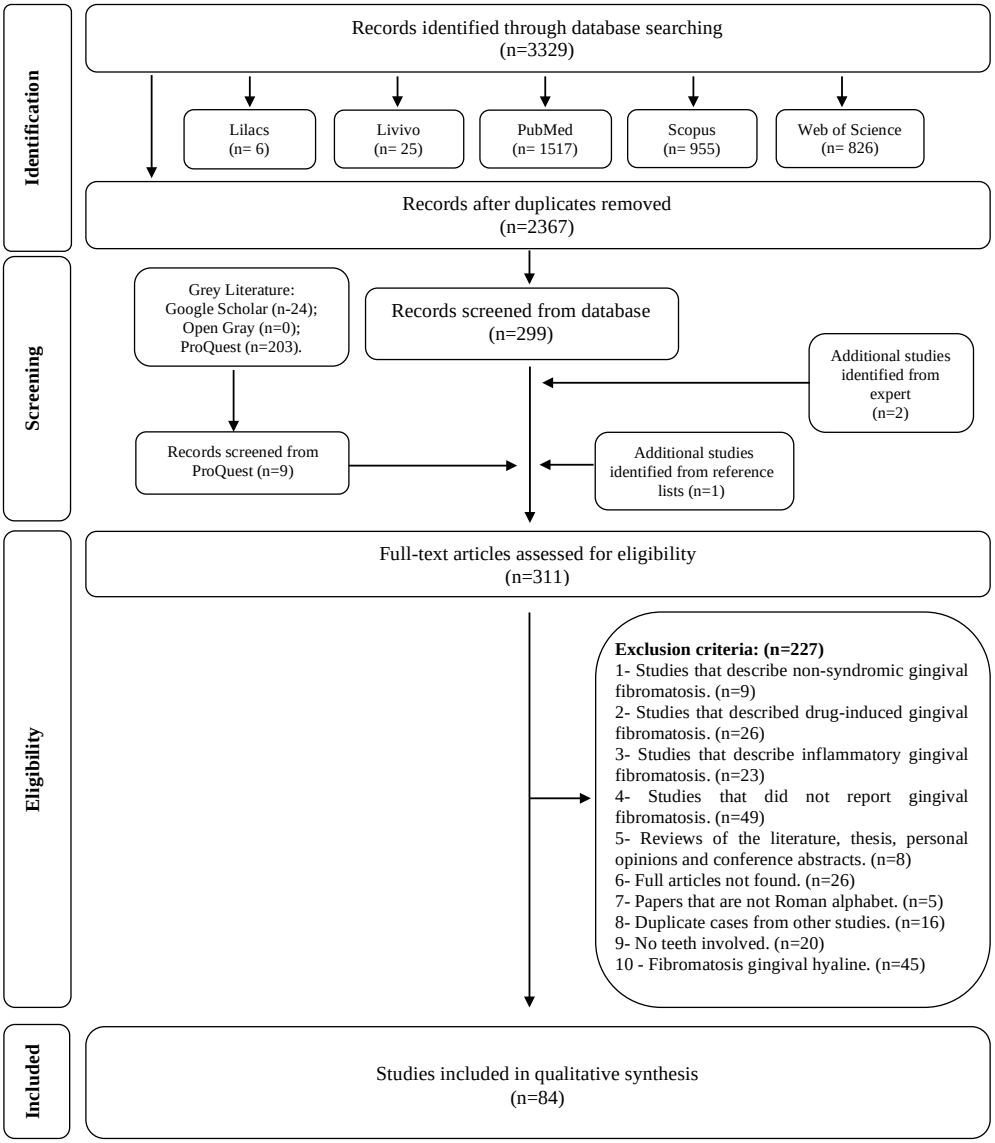


Table 1. Molecular profile of syndromic cases with gingival fibromatosis.

Author Year	Syndrome Name #OMIM	Inheritance	Causative Gene	Variants	Zygosity	Type of Mutations	Effect
Abo-Dalo et al., 2008	Zimmermann-Laband/ OMIM#135500 OMIM#616455	*AD*	*KCNH1*[a]	c.974C>A[a] c.1066G>C[a] c.1055C>A[a] c.1147G>C[a]	HET	Missense[a]	p.Ser325Y[a] p.Val356Leu[a] p.Ser352Tyr[a] p.Val383Leu[a]
			KCNH1[a]	c.1042G>A[a] c.1123G>A[a]	HET	Missense[a]	p.Gly348Arg[a] p.Gly375Arg[a]
			ATP6V1B2[a]	c.1454G>C[a]	HET	Missense[a]	p.Arg485Pro[a]
Acevedo et al., 2015.	Raine / OMIM#259775	*AR*	*FAM20C*	c.1487C>T	HOM	Missense	p.Pro496Lys
				c.784+5G>C	HOM	Splicing	p.Trp202Cysfs*37
Afifi et al. 2015	Congenital generalized hypertrichosis terminalis with gingival hyperplasia / OMIM#135400	AR	17q24.2- q24.3 chromosomal microdeletion	arr[hg19] 17q24.2q24.3(67,012,238–67,938,237)x1 or (67,012,239–67,938,238)x1	HET	Microdeletion	NA
Afifi et al. 2016	Cantu / OMIM#239850	*AD*	*ABCC9*	c.3460C>T	HET	Missense	p.Arg1154Trp
Bauer et al., 2018	Facial dysmorphism, hypertrichosis, epilepsy, intellectual/developmental delay, and gingival overgrowth syndrome / OMIM#618381	*AD*	*KCNK4*	c.515C>A	ND	Missense	p.Ala172Glu
				c.730G>C	ND	Missense	p.Ala244Pro
				c.515C>A	ND	Missense	p.Ala172Glu
Cabral et al., 2013	Autosomal Recessive Gingival Hyperplasia and dental anomalies	*AR*	*FAM20A*	c.174-175ins29	HOM	Duplication	NR
Castori et al., 2013	Zimmermann-Laband/ OMIM#135500 OMIM#616455	*AD*	*KCNH1*[a]	c.1054C>G[a] c.1135C>G[a]	HET[a]	Missense[a]	p.Leu352Val[a] p.Leu379Val[a]
			ATP6V1B2[a]	c.1454G>C[a]	HET[a]	Missense[a]	p.Arg356Pro[a]
Cho et al., 2011	AIGFS / OMIM#204690	*AR*	*FAM20A*	c.34_35delCT	HOM	Nonsense	p.Leu12Alafs*67
				c.813-2A>G	HOM	Splicing	p.Arg271Serfs*70
				c.1175_1179delGGCTC	HOM	Deletion	p.Arg392Profs*22

				c.[590-2A>G]; [826C>T]	COMPOUND HET	Splicing	p.Asp197_Ile214delinsVal p.Arg276*
Dourado et al., 2019	Enamel Renal Syndrome / OMIM#204690	*AR*	*FAM20A*	c.1447delG	HOM	Nonsense	p.Glu483-Lysfs*24
Jaouad et al., 2015	AIGFS / OMIM#204690	*AR*	*FAM20A*	c.34_35delCT	HOM	Deletion	p.Leu12Alafs*67
Kantaputra et al., 2014	Enamel Renal/ OMIM#204690	*AR*	*FAM20A*	c.34_35delCT	HOM	Deletion	p.Leu12AlafsX67
				c.1482_1483insAC	HOM	Insertion	p.Leu495ThrfsX13
Kantaputra et al., 2017	Enamel Renal / OMIM#204690	*AR*	*FAM20A*	c.349_367delCTGGCCAGCCAGGAG GCGC;	HOM	Deletion	p.Leu117Cysfs*22;
				c.915_918delCTTT c.976_977insG	HET	Deletion Insertion	p.Phe305Leufs* p.Glu326Glyfs*54
Kortüm et al., 2015	Zimmermann-Laband / OMIM#135500 OMIM#616455	*AD*	*KCNH1*	c.1399A>G[a]	HET	Missense	p.Ile467Val
				c.1480A>G[a]	HET	Missense	p.Ile494Val
				c.1405G>A[a]	HET	Missense	p.Gly469Arg
				c.1486G>A[a]	HET	Missense	p.Ile494Val
Mégarbané et al., 2016	Temple-Baraitser / OMIM#611816	*AD*	*KCNH1*	c.1042G > A	HET	Missense	p.Gly348Arg
Pachajoa et al., 2018	ND		*ABCC9*	c.3625T>C	HET	Missense	p.Tyr1209Hys
Pêgo et al., 2016	Enamel Renal Syndrome / OMIM#204690	*AR*	*FAM20A*	c.406C>T	HOM	Nonsense	p.Arg136*
Poulter et al., 2015	Enamel Renal Syndrome / OMIM#204690	*AR*	*FAM20A*	c.1294G>A	HET*	Missense	p.Ala432Thr
				c.988T>C duplication spanning chr17:66,543,172-66,597,963	COMPOUND HET	Missense	p.Cys330Arg
Tommiska et al., 2017	Pituitary hormone deficiency and maternally inherited gingival fibromatosis	*AD*	*KCNQ1*	c.347G>T	HET	Missense	p.Arg116Leu
				c.1106C>T	HET	Missense	p.Pro369Leu
Wang et al., 2013	Enamel Renal / OMIM#204690	*AR*	*FAM20A*	c.992G>A	HOM	Missense	p.Gly331Asp
				c.720-2A>G	HOM	Splicing	p.Gln241_Arg271del
				c.406C>T c.1432C>T	HOM	Nonsense	p.Arg136* p.Arg478*

Abbreviations: OMIM: Online Mendelian Inheritance in Man®; #: OMIM's description – phenotype description, molecular basis known; AIGFS: Amelogenesis Imperfecta and Gingival Fibromatosis Syndrome; NA: not applicable; NR: Not reported; *KCNH1*: Potassium Channel Subfamily H Member 1; ATP6V1B2: ATPase H+ transporting V1 subunit B2; *FAM20C*: Family with sequence similarity 20, member C; *ABCC9*: ATP Binding Cassette Subfamily C Member 9; NR: Not related; *KCNK4*: Potassium Channel Subfamily K Member 4; *FAM20A*: Family with sequence similarity 20, member A; *KCNQ1*: Potassium Voltage-Gated Channel KQT-Like Subfamily Q Member 1; HOM: Homozigozy; HET: Heterozigozy.

*Variant in homozygous state in cDNA

[a] published in Kortüm, F., Caputo, V., Bauer, C. K., Stella, L., Ciolfi, A., Alawi, M., ... & Grammatico, P. (2015). Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome. *Nature genetics*, *47*(6), 661.

Appendix to the manuscript

Syndromes with gingival fibromatosis: a systematic review.

Table S1. Search strategy.

Databases	**Research**
LILACS	(tw:("Fibromatosis Gingival" OR "Fibromatose Gengival" OR "Fibromatosis, Gingival")) AND (tw:(Síndrome OR syndrome))
Pubmed	("Hereditary gingival fibromatosis" OR "Gingival fibromatosis" OR "gingival enlargement" OR "gingival hyperplasia" OR "gingival overgrowth" OR "hereditary gingival hyperplasia" OR "hypertrophic gingiva" OR "Gingival enlargement" OR "gingival hyperplasia" OR "elephantiasis gingivae" OR "familial elephantiasis" OR "gigantism of the gingiva" OR "congenital macrogingivae" OR Hyperthricose OR "hypertrichosis"[MeSH Terms] OR "Fibromatosis, Gingival"[Mesh] OR "Gingival Fibromatosis" OR "Fibromatoses, Gingival" OR "Gingival Fibromatoses" OR "Fibromatosis Gingivae" OR "Gingival Overgrowth"[Mesh] OR "Gingival Overgrowths" OR "Overgrowth, Gingival" OR "Overgrowths, Gingival") AND (Syndrome OR "Syndrome"[Mesh] OR Syndromes OR "Syndrome fibromatosis" OR "syndromes fibromatosis" OR Syndromic OR Congenital OR "congenital abnormalities"[Mesh] OR abnormalities)
Livivo	TI=("Hereditary gingival fibromatosis" OR "Gingival fibromatosis" OR "gingival enlargement" OR "gingival hyperplasia" OR "gingival overgrowth" OR "hereditary gingival hyperplasia" OR "hypertrophic gingiva" OR "Gingival enlargement" OR "gingival hyperplasia" OR "elephantiasis gingivae" OR "familial elephantiasis" OR "gigantism of the gingiva" OR "congenital macrogingivae" OR Hyperthricose OR "hypertrichosis" OR "Fibromatosis, Gingival" OR "Gingival Fibromatosis" OR "Fibromatoses, Gingival" OR "Gingival Fibromatoses" OR "Fibromatosis Gingivae" OR "Gingival Overgrowth" OR "Gingival Overgrowths" OR "Overgrowth, Gingival" OR "Overgrowths, Gingival") AND TI=(Syndrome OR Syndromes OR "Syndrome fibromatosis" OR "syndromes fibromatosis" OR Syndromic OR Congenital OR "congenital abnormalities" OR abnormalities)

Scopus	TITLE-ABS("Hereditary gingival fibromatosis" OR "Gingival fibromatosis" OR "gingival enlargement" OR "gingival hyperplasia" OR "gingival overgrowth" OR "hereditary gingival hyperplasia" OR "hypertrophic gingiva" OR "Gingival enlargement" OR "gingival hyperplasia" OR "elephantiasis gingivae" OR "familial elephantiasis" OR "gigantism of the gingiva" OR "congenital macrogingivae" OR hyperthricose OR "hypertrichosis" OR "Fibromatosis, Gingival" OR "Gingival Fibromatosis" OR "Fibromatoses, Gingival" OR "Gingival Fibromatoses" OR "Fibromatosis Gingivae" OR "Gingival Overgrowth" OR "Gingival Overgrowths" OR "Overgrowth, Gingival" OR "Overgrowths, Gingival") AND TITL-ABS(syndrome OR syndromes OR "Syndrome fibromatosis" OR "syndromes fibromatosis" OR syndromic OR congenital OR "congenital abnormalities" OR abnormalities) AND (LIMIT TO (DOCTYPE , "ar") OR LIMIT-TO(DOCTYPE , "ip"))
Web of Science	TS=("Hereditary gingival fibromatosis" OR "Gingival fibromatosis" OR "gingival enlargement" OR "gingival hyperplasia" OR "gingival overgrowth" OR "hereditary gingival hyperplasia" OR "hypertrophic gingiva" OR "Gingival enlargement" OR "gingival hyperplasia" OR "elephantiasis gingivae" OR "familial elephantiasis" OR "gigantism of the gingiva" OR "congenital macrogingivae" OR Hyperthricose OR "hypertrichosis" OR "Fibromatosis, Gingival" OR "Gingival Fibromatosis" OR "Fibromatoses, Gingival" OR "Gingival Fibromatoses" OR "Fibromatosis Gingivae" OR "Gingival Overgrowth" OR "Gingival Overgrowths" OR "Overgrowth, Gingival" OR "Overgrowths, Gingival") AND TS=(Syndrome OR Syndromes OR "Syndrome fibromatosis" OR "syndromes fibromatosis" OR Syndromic OR Congenital OR "congenital abnormalities" OR abnormalities)
ProQuest	TI("Hereditary gingival fibromatosis" OR "Gingival fibromatosis" OR "gingival enlargement" OR "gingival hyperplasia" OR "gingival overgrowth" OR "hereditary gingival hyperplasia" OR "hypertrophic gingiva" OR "Gingival enlargement" OR "gingival hyperplasia" OR "elephantiasis gingivae" OR "familial elephantiasis" OR "gigantism of the gingiva" OR "congenital macrogingivae" OR Hyperthricose OR "hypertrichosis" OR "Fibromatosis, Gingival" OR "Gingival Fibromatosis" OR "Fibromatoses, Gingival" OR "Gingival Fibromatoses" OR "Fibromatosis Gingivae" OR "Gingival Overgrowth" OR "Gingival Overgrowths" OR "Overgrowth, Gingival" OR "Overgrowths, Gingival") AND TI(Syndrome OR Syndromes OR "Syndrome fibromatosis" OR "syndromes fibromatosis" OR Syndromic OR Congenital OR "congenital abnormalities" OR abnormalities)
Google Scholar	allintitle: fibromatosis gingival syndrome
OpenGrey	"fibromatosis gingival" AND syndrome

Table S2. Excluded articles and reasons for exclusion (n=227)

Reference number	Author, year	PMID	Reasons for exclusion
1.	Abe et al., 1973	4526785	1
2.	Abo-Dalo et al., 2007	17937436	8
3.	Ahluwalia et al., 1998	10530201	2
4.	Akca et al., 2004	15259977	4
5.	Akozsoy, 1963	14011577	6
6.	Alavandar, 1965	5216911	6
7.	Aldred and Crawford, 1987	2433666	3
8.	Alessio et al., 2010	20634169	10
9.	Al-Malik et al., 2005	20634169	3
10.	Al-Najjadah et al., 2003	14501336	4
11.	Altug et al., 2009	19782615	4
12.	Anand and Roshna, 2006	16820812	4
13.	Anavi et al., 1989	2806737	2
14.	Atabek et al., 2005	16267629	9
15.	Atasu et al., 1999	10204452	6
16.	Basman et al., 2017	28955595	3
17.	Bagavad et al., 2014	24963260	3
18.	Balbi et al., 1993	8510618	5
19.	Banjar et al., 1997	10128298	10
20.	Baratieri et al., 1974	4142897	2
21.	Bazopoulou-Kyrkanidou et al., 1990	2250229	2
22.	Bazopoulou-Kyrkanidou et al., 1998	9682988	10
23.	Belford et al., 1991	1880654	10
24.	Bhatsange et al., 2018	29568175	3
25.	Bhavsar et al., 1991	1820429	4
26.	Bondeson et al., 1993	8213906	4
27.	Bramswig et al., 2015	26264464	2
28.	Brandão et al., 2009	20191183	10
29.	Brennan et al., 1999	10204448	2
30.	Breton et al., 1988	3043641	4
31.	Browmick et al., 2001	11585376	1
32.	Brownstein et al., 2001	11497014	4
33.	Burzymshi et al., 1975	806047	3
34.	Byars et al., 1961	24545452	4
35.	Byars and Sarnat, 1944	24545452	3
36.	Canabarro et al., 2008	19081899	3
37.	Canargiu et al., 2009	19633638	4
38.	Canun et al., 2003	12503107	4
39.	Capoglu et al., 2002	12450310	5
40.	Cha and Byn, 1977	04944739	10
41.	Chaikin, 1965	5215210	8
42.	Chigot et al., 1978	014079	2
43.	Chodirker et al., 1986	3789014	2
44.	Cinotti et al., 2013	23637089	4
45.	Cooper et al., 2014	24700710	4

46.	Cooper et al., 2015	26621776	4
47.	Czeschik et al., 2013	23307537	9
48.	Dávalos et al., 2005	16261693	9
49.	Denadai et al., 2012	22383261	10
50.	Deshmukh et al., 2007	17883060	10
51.	De Coster et al., 2003	12969232	4
52.	De Grouchy et al., 1972	4537727	4
53.	De Pina Neto et al., 1988	9557893	4
54.	De Pina Neto et al., 1998	3228149	8
55.	Digilio et al., 2008	17726614	9
56.	Dos Santos Neto et al., 2011	21493880	1
57.	Douzgou et al., 2009	19625955	2
58.	Dowling et al., 2003	12973667	10
59.	Drugowick et al., 2007	17539732	2
60.	Dumic et al., 1999	9933906	4
61.	Durán Padilla et al., 2001	11421107	4
62.	Davalos et al., 2011	21614982	8
63.	El-Maaytah et al., 2010	20863945	10
64.	Elia et al., 1995	8747594	2
65.	Ennibi et al., 2013	23838247	4
66.	Eronat et al., 2009	19761290	4
67.	Familusi et al., 1976	1086595	6
68.	Fauth et al., 2016	26545172	9
69.	Favia et al., 2015	26946876	3
70.	Fayad et al., 1987	3544844	10
71.	Fazekas and Szegoe, 1964	14236786	6
72.	Fehlow, 1990	2377378	6
73.	Finlay et al., 1983	6189506	10
74.	Fryns et al., 1996	9297447	4
75.	Galili et al., 1974	4361095	9
76.	Garcia-Gonzalez et al., 2012	22504422	9
77.	Gardner, 1971	4996613	5
78.	Gawron et al., 2018	29989318	8
79.	Gazit et al., 1989	2740092	10
80.	Gellis et al., 1975	1121964	6
81.	Glover et al., 1991	1702887	10
82.	Göhlich-Ratmann et al., 2000	11102931	1
83.	Grothe et al., 2008	18505648	6
84.	Haddad et al., 1997	9538423	10
85.	Haidar et al., 2017	28148224	8
86.	Hallet et al., 1995	7600221	8
87.	Hamada et al., 1979	1997703	10
88.	Hamada et al., 1980	7218556	10
89.	Han et al., 2019	30907493	8
90.	Harutunian et al., 2011	20711158	3
91.	Haustein et al., 2011	21491119	4
92.	Henderson et al., 1996	8957853	3
93.	Herenger et al., 2015	26188272	4
94.	Higgs et al., 2015	25714557	4

95.	Hoff and Vissink, 2000	11385817	3
96.	Horning et al., 1985	3859642	2
97.	Huttunen et al., 2018	29740400	5
98.	Ishikawa and Mori, 1973	4124008	4
99.	Il'ina et al., 1988	3205658	7
100.	Ito et al., 1974	4549384	7
101.	Iivonen et al., 2018	29703730	4
102.	Jaouad et al., 2014	25186005	10
103.	Jaureguiberry et al., 2012	23434854	8
104.	Jones et al, 1977	890092	8
105.	Jorgenson, 1971	5173226	6
106.	Kasaboglu et al., 2004	15287422	5
107.	Kalgaonkar et al., 2017	28892992	10
108.	Kan and Rogers, 1989	2748477	10
109.	Karabulut et al., 2005	15725846	10
110.	Karaçal et al., 2005	15953379	10
111.	Katz et al., 2002	12064501	1
112.	Kasaboglu et al., 2004	15287422	6
113.	Kelly et al., 1975	1121970	4
114.	Keser et al., 1999	11206353	10
115.	Kiraz et al., 2013	23610866	4
116.	Kirel et al., 2017	29483803	9
117.	Zirzioglu et al., 2009	19680205	5
118.	Kiss et al., 1990	2113016	4
119.	Koehne et al.,	27239697	1
120.	Kondoh et al., 2001	11285076	9
121.	Korol et al, 2008	18380572	3
122.	Koruyucu et al, 2018	29439260	8
123.	Krasuska-Sławińska et al., 2015	26207694	9
124.	Kratz and Morin, 1987	3469398	6
125.	Kurban et al., 2012	22310962	4
126.	Landing et al., 1986	2434938	10
127.	Lacombe et al., 1994	7811425	10
128.	Larralde et al., 2001	11737684	10
129.	Leung et al., 1989	2627233	9
130.	Lim et al., 2005	15690301	10
131.	Lindemann et al., 2001	11272247	10
132.	Li, 1983	6414667	7
133.	Mastrangelo et al., 2016	27267311	2
134.	Mackler et al., 1972	4506719	2
135.	Malathi et al, 2006	16933730	10
136.	Malfait et al., 2004	15389701	3
137.	Mallet et al., 2010	20470917	10
138.	Mancini et al., 1999	10026396	10
139.	Mantri et al., 2016	27688461	10
140.	Marakoglu et al., 2005	16080296	6
141.	Mattoo, 1997	9203202	6
142.	McCleary, 1972	4341138	6
143.	Mbibi et al., 2015	26521326	2

144.	Mehta et al., 2008	18583796	2
145.	Melinkeri et al., 2002	12185512	3
146.	Menni et al., 1990	2279608	3
147.	Miles et al., 1987	3477765	4
148.	Miyake et al., 1995	8599473	10
149.	Morey and Higgins, 1990	2301476	9
150.	Muniz et al., 2006	17014642	10
151.	Murube, 2011	21338565	5
152.	Nabai et al., 1978	604368	9
153.	Nath et al., 2018	29482692	4
154.	Nazif et al., 1978	282544	2
155.	Nibali et al., 2012	22532955	1
156.	Nofal et al., 2009	19344977	10
157.	Oata et al., 1969	5266095	7
158.	Oikawa et al., 1979	283203	4
159.	Olczak-Kowalczyk et al., 2011	21806909	10
160.	O'Neill et al., 1989	2663869	10
161.	Ozdemir et al., 2010	20831140	4
162.	Patel and Ambani, 1980	6118467	2
163.	Pavone et al., 1997	9268094	2
164.	Pfeiffer et al., 1992	1740896	2
165.	Piattelli et al., 1996	8708973	10
166.	Purwar et al., 2016	26885413	3
167.	Radhakrishnan and Rajan, 2003	15164660	8
168.	Rahman et al., 2002	12214284	10
169.	Raine et al., 1989	2614802	9
170.	Raja et al., 2018	18793596	8
171.	Rangel Rivera et al., 2015	26294158	10
172.	Ray et al., 1966	5963212	5
173.	Reddy et al., 2011	22048596	1
174.	Regen et al., 2010	21070714	4
175.	Requena Caballero et al., 1987	2449004	2
176.	Reveillon et al., 2015	25614612	3
177.	Ritter et al., 2013	23633095	3
178.	Robertson et al., 1998	9674908	4
179.	Rodriguez-Vazquez et al., 2007	18330840	9
180.	Salinas, 1982	7115915	6
181.	Sarikayakar et al., 1981	6951993	9
182.	Sarkar et al., 2016	27307675	2
183.	Sawaki et al., 2012	22838235	9
184.	Schaller et al., 1997	9206714	10
185.	Sciubba and Niebloom, 1986	2430247	10
186.	Seki et al., 2010	20614039	1
187.	Shah et al., 1986	3462252	1
188.	Shapiro et al., 1990	2201708	6
189.	Shashi et al., 1999	10507724	1
190.	Siala-Sahnoun et al., 2016	26874853	4
191.	Slootweg and Beemer, 1987	2442332	3
192.	Smith et al., 1993	7919864	6

193.	Snyder, 1965	14339411	2
194.	Soni et al., 2016	27753005	4
195.	Sood et al., 2000	10803876	4
196.	Skrinjaric et al., 1983	6584024	1
197.	Skrinjaric et al., 1989	2533254	1
198.	Stirrups and Inglis., 1980	6928303	4
199.	Stoyle et al., 2018	30159147	4
200.	Swartz, 1967	5228706	6
201.	Swenson, 1984	6596411	5
202.	Takagi and Yamamoto, 1990	2081939	7
203.	Takenoshita et al., 1993	8492208	3
204.	Tay et al., 2001	11841646	9
205.	Tehranchinia and Rahimi, 2010	20627857	4
206.	Thomas et al., 1992	1507052	2
207.	Torbus et al., 2002	12053598	4
208.	Toksavul et al., 2004	14765635	6
209.	Török et al., 1983	6192385	10
210.	Turgut et al., 2001	11642534	4
211.	Unal et al., 2009	19774849	4
212.	Ungari et al., 1979	514742	6
213.	Vally et al., 1990	2406675	1
214.	Vanbuggenhout et al., 1995	8775419	4
215.	Vashi et al., 2001	11453806	3
216.	Verloes et al., 1993	8319710	4
217.	Vogel and Matarasso, 1978	294878	6
218.	Vontobel, 1973	4773221	6
219.	Wallach et al., 1985	4091435	6
220.	Willert, 1954	14356683	1
221.	Winik et al., 1998	9700376	10
222.	Winstock et al., 1964	5212882	8
223.	Witkop, 1971	4950923	5
224.	Xin et al., 2010	20018682	4
225.	Yamashita and Hayashi, 1985	3921362	4
226.	Yaprak et al., 2012	23091740	1
227.	Yesudian et al., 1984	6083983	10
228.	Yen et al., 1989	2918542	4

Reasons for exclusion: Studies that describe non-syndromic gingival fibromatosis (1) (n=16); Studies that described drug-induced gingival fibromatosis (2) (n=24); Studies that describe inflammatory gingival fibromatosis (3) (n=22); Studies that did not report gingival fibromatosis (4) (n=51); Reviews of the literature, thesis, personal opinions and conference abstracts (5) (n=10); Full articles not found (6) (n=23); Papers that are not Roman alphabet (7) (n=5); Duplicate cases from other studies (8) (n=14); No teeth involved (9) (n=18); Fibromatosis gingival hyaline (10) (n=45).

Figure S1. Geographic distribution of patients syndromic with gingival fibromatosis.

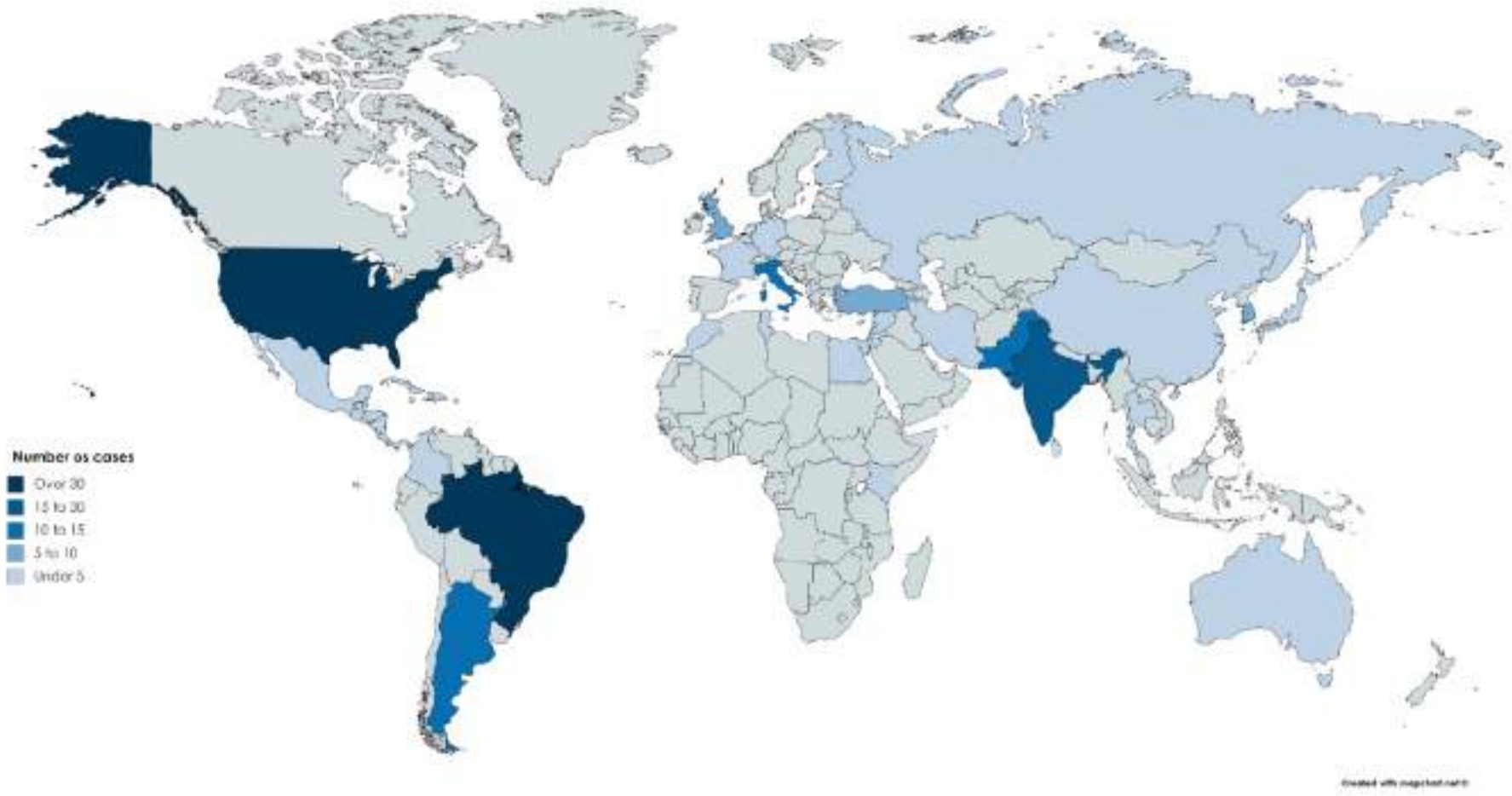


Table S3. Descriptive summary of included articles (n=84).

Author Year	Country	Geographic Origin	Type of study Case report/ Series of cases	Syndrome Name	Sex M/F	Age y/m	Affected Individuals (n)	Consanguinity	Inheritance AR/AD/X-linked	Molecular basis/ Causative Gene	Main Findings
Abo-Dalo et al., 2008	Germany	Australia (England and German ancestry)/ Australia/ France	Case report	Zimmermann–Laband	2M/ 2F	10-24y	4	NR	NR	NR	The study reports four new cases, out of a series of 12 cases with Zimmermann-Laband syndrome, which were associated with gingival fibromatosis. Two individuals had hypertrichosis. All individuals had abnormalities in the hands and feet, such as phalanges and aplastic / hypoplastic nails, and mental retardation. In addition were also found: aplastic phalanges of second and fifth left, and fifth right fingers and all toes except first, bulbous flat nose with 'bifid' nasal tip, synophrys in the 1st individual analysed; Hypoplastic terminal phalanges of hands and feet in the 2nd and 3rd individuals; macroglossia in the 1st and 3rd individuals; Full cheeks, bulbous nose tip, thick floppy ears in the 4th individual; and hypertrichosis in the 1st and 3rd individuals.
Acevedo et al., 2015	Brazil	Brazil	Case report	Raine	4M/ F	6-16y	5	Yes	AR	*FAM20C*	The study reports 5 new cases of Raine syndrome in 2 different families. All individuals were described with exophthalmos and midface hypoplasia. The 3rd individual from family 1 and the 2 individuals from family 2 had intracranial calcification. Seizures and choanal atresia were showed in the 1st individual of family 1 and the 1st individual of family 2. Microencephaly was not reported in the 3rd individual of family 1. Dysplastic ears and hearing loss were described in the 3 individuals of family 1. Craniosynostosis, depressed nasal bridge, low set ears, short stature, short limbs, undermineralized long bones, and clinodactyly were described in the 2 individuals of family 2. Visual impairment and renal calcification were described in the 1st individual of family 2.

Afifi et al. 2015	Egypt	Egypt	Case report	Congenital generalized hypertrichosis terminalis with gingival hyperplasia	F	5y	1	Yes	AR	17q24.2- q24.3 chromosomal microdeletion	Coarse face with bilateral epicanthal folds, large ears, depressed nasal bridge, bulbous columella, wide nostrils, flat broad philtrum, macrostomia, high palate, macroglossia, long uvula, prominent premaxilla, spacing of teeth, deep overbite, generalized gingival hyperplasia with hyperpigmentation, hypertrichosis.
Afifi et al. 2016	Egypt	Egypt	Case report	Cantu	F	6 y	1	No	NR	*ABCC9*	Coarse acromegaloid-like facial appearance, thick, arched, bushy eyebrows; thick eyelids; thick lips, large hands, generalized hypertrichosis except palms and soles, gingival hyperplasia, everted lower lips, macrostomia, macroglossia and multiple frenula.
Anderson et al., 1969	UK	UK	Case report	Hereditary gingival fibromatosis	F	13y	1	No	NR	NR	Mental retardation, hypertrichosis of face, limbs and back, bushy eyebrows, hyperelastic skin, deep palate, gingival overgrowth with unerupted teeth.
Ashkenazi et al., 2014	Israel	Israel	Case report	Enamel renal	M	13y	1	Yes	Probable AR	NR	Nephrocalcinosis, hypoplastic amelogenesis imperfecta, intrapulpal calcifications, retention of primary teeth, delayed eruption of permanent teeth, enlarged dental-follicles, misshaped roots of permanent teeth, gingival overgrowth, severe localized alveolar bone loss and severe malocclusion.
Bakaeen & Scully, 1991	UK	Jordan	Case report	Zimmermann-Laband	M/F	7/14 y	2	Yes	Probable AR	NR	The 2 individuals were described with an enlarged nose, hypoplastic terminal phalanges of fingers and toes and joint hyperflexibility in the hands and feet. The girl had onychodystrophy of most nails.
Balaji & Balaji, 2017	India	India	Case report	Gingival fibromatosis with hypertrichosis	3F	6-10y	3	No	NR	NR	Three unrelated individuals with hypertrichosis and gingival fibromatosis and no other systemic alteration was reported.
Bauer et al., 2018	Germany	Italy	Case report	Facial dysmorphism, hypertrichosis, epilepsy, intellectual disability/ developmental delay, and gingival overgrowth	2M/ F	11m-5y	3	NR	Simplex case, *de novo*	KCNK4	Generalized hypertrichosis occur in all affected individuals as well as intellectual disability and seizures. The craniofacial changes found common to all individuals were: bushy eyebrow, traight eyebrow, short philtrum, everted upper lip, thin upper lip, prominent upper vermilion and micrognathia/receding chin.
Benazza et al., 2005	Morocco	Morocco	Case report	Cherubism	M	7 y	1	Yes	Probable AR	NR	Perturbed consciousness, hyperplasic gingiva, broad cheeks due to facial swelling, maxillary

											and mandibular enlargemente, gingival hyperplasia, unerupted teeth.
Bhesania et al., 2015	India	India	Case report	Enamel renal	M	21 y	1	No	NR	NR	Nephrolithiasis, hypocitraturia, gingival fibromatosis, and eruption anomalies.
Cabral et al., 2013.	USA	Pakistan	Case report	Autosomal recessive gingival hyperplasia and dental anomalies	10M / 2F	NR	12	Yes	AR	*FAM20A*	Twelve members of a six generation family had severe gingival hyperplasia and dental anomalies that included delayed tooth eruption or failure of tooth development. None of the family members had renal abnormalities.
Castori et al., 2013	Italy	Italy	Case report	Zimmermann-Laband	M/ F	5-12y	2	No	NR	NR	Intellectual disability, hypertrichosis, coarse face, anonychia, short neck, aplastic/hypoplastic nails and terminal phalanges were common findings to both individuals. The boy had scoliosis and seizures.
Chacon-Camacho et al., 2011	Mexico	Mexico	Case report	Zimmermann-Laband	F	8 y	1	No	NR	NR	Dysmorphic face, hypertelorism, lower eyelids eversion, thick eyebrows and eyelashes, low-set, cupped and thick ears, bulbous soft nose, thick vermillion of lips, unilateral leucoma and polar cataract, colpocephaly, seizures, intellectual disability, generalized hypertrichosis, nail hypoplasia, hemihyperthophy/hyperplasia of the right side, segmented dark pigmentation; post axial polydactyly, joint hyperextensibility, vertebra anomalies; hemivertebra, urogenital anomalies, bifid uvula; gingival overgrowth, supernumerary teeth.
Chadwick et al., 1994	UK	UK/ Pakistan	Case report	Zimmermann-Laband	F/M	9y/8y	2	No/Yes	NR/Probable AR	NR	The report describes two unrelated individuals with hypertrichosis, aplasia/hypoplasia of phalanges or nails, thick lips, broad nose, dental eruption delayed, macroglossia, gingival enlargement, anterior open bite. The patient 1 had hepatomegaly and the patient 2 had mental retardation, large ears and supernumerary tooth.
Cho et al., 2011	Korea	Korea	Series of cases	AIFGS	2M/ 2F	NR	4	3 Yes/ 1 No	AR	*FAM20A*	Generalized hypoplastic enamel, delayed dental eruption, root dilacerations, pulp calcifications, gingival hyperplasia were reported in all cases. No renal abnormalities were reported.
Cuestas-Carnero &	Argentina	Argentina	Case report	Fibromatosis and hypertrichosis	4M/ F	8-67 y	5	NR	NR	NR	The hypertrichosis was described in mainly face and arms in all patients, but also observed on

Bornancini, 1988											scalp and back. Intellectual impairment was described in two patients.
Davalos et al., 2011	Mexico	Mexico	Case report	Zimmermann-Laband	F	9 y	1	No	NR	NR	Soft bulbous nose, thick floppy ears, thick lips, thick eyelashes and eyebrows, hypertrichosis mainly arms, legs and back, bilateral hearing loss, joint hyperlaxity, hypoplastic terminal phalanges, nail hypoplasia on hands and 5th toes, hepatomegaly. General oral mucosa enlarged since birth, gingival overgrowth confirmed at 11 months.
Debnath et al., 2019	India	India	Case report	Enamel renal	M	17y	1	NR	NR	NR	Pulpal and gingival calcifications, enamel hypoplasia, agenesis of kidney and renal calcifications.
de la Tranchade et al., 2003	France	France	Case report	Amelogenesis imperfecta and nephrocalcinosis	F	15y	1	NR	NR	NR	The case reports a patient with enamel agenesis of the primary and permanent dentition, delayed or absent eruption of the permanent dentition, coronal resorption of unerupted teeth and gingival enlargement and calcifications. Renal symptoms include medullary nephrocalcinosis with evolution to a renal failure.
Dourado et al., 2019	Brazil	Brazil	Case report	Enamel renal	7M/ 4F	6-25y	11	7 Yes/ 4 No	AR	*FAM20A*	All reported individuals from 5 Brazilian families reported hypoplastic amelogenesis imperfecta, microdontia, intra-pulpal calcifications, impacted posterior teeth with hyperplastic pericoronal follicles, gingival fibromatosis, ectopic calcifications on gingival and pericoronal tissues, and nephrocalcinosis.
Elavarasu et al., 2017	India	India	Case Report	Cherubism	M	11 y	1	NR	NR	NR	Facial dysmorphism due to bilateral swelling of mandible, multiple osteolytic lesions in mandible, gingival overgrowth.
Feitosa et al., 2011	Brazil	Brazil	Case Report	Cowden	F	23 y	1	NR	NR	NR	Multiple papules on nose and midfacial skin, fibrous lesions on the back, breast and leg, multipapular lesions on gingiva, tongue and palate. Polypoid lesions also observed in jugal mucosa, generalized gingival overgrowth.
Giansanti et al., 1973	USA	USA	Case Report	Gingival fibromatosis, hypertelorism, anti-mongoloid obliquity, multiple telangiectases and café au lait pigmentation	F	36 y	1	NR	NR	NR	Abnormality of metaphises, cranio-facial disproportion, frontal and maxillary sinuses were quite large, hypoplastic maxilla and zygoma, prognathism, small nose, telangiectases over most of the body and large vascular hamartoma behind right ear, Two spots of cafe au lait pigmentation were show on the right buttock and the right small of the back of approximate diameter of 3 cm.

Gita et al., 2014	India	India	Case Report	Jones	M	14 y	1	NR	NR	NR	Neurosensorial hearing loss, generalized gingival overgrowth, tooth agenesis.
Goldblatt & Singer, 1992	Australia	Australia	Case Report	Autosomal recessive gingival fibromatosis with distinctive facies	M/F	9/13 y	2	No	AR	NR	All cases described craniofacial dysmorphism that included macrocephaly, bushy eyebrows with synophrys, hypertelorism, downslanting palpebral fissures, flattened nasal bridge, hypoplastic nares and cupid bow mouth with prominent lips and arched palate. No associated generalized hypertrichosis was observed.
Goodwin et al., 2014	USA	USA	Series of cases	Costello	21M / 20F	1-35y	41	NR	NR	NR	The craniofacial and oro-dental features of all patients included macrocephaly, bitemporal narrowing, convex facial profile, full cheeks, and large mouth. Additionally, malocclusion with anterior open bite and posterior crossbite, enamel hypomineralization, delayed tooth development and eruption, thickening of the alveolar ridge, and high palate and gingival hyperplasia were reported.
Guevara-Sanginés et al., 2002	Mexico	Mexico	Case Report	Congenital generalized terminal hypertrichosis with gingival hyperplasia	F	7 y	1	No	NR	NR	Coarse facies, curly and copious eyelashes and eyebrows, bilateral epicanthus, prominent nasal bridge, bulging nasal ala. Generalized hypertrichosis, Also, wide hands with thick hyperextensible fingers but did not have acromegaly.
Hallett et al., 1995	Australia	Italy	Case Report	Klippel-Trénaunay-Weber	M	6 y	1	No	NR	NR	Coarse facial with midfacial hypoplasia, flat nasal bridge, macrocephaly, prominent occiput, cutis marmorata telangiectatica, loose skin, narrow chest, hemihypertrophy, short upper limbs, small hands and macrodactyly, lymphoedema on dorsum of hands and feet, moderate delayed development, intellectual disability and had no speech at 61/2 yrs hepatosplenomegaly, hypoglycemia, intracranial calcification, severe generalized overgrowth with nodular aspect.
Harrison et al., 1998	UK	UK	Case Report	Prune-belly	M	4 y	1	No	NR	NR	Soft dysmorphic facies with moderate frontal bossing, large mouth, broad nasal bridge, high palatal vault and enlargement of the maxillary tuberosities. Large umbilical hernia secondary to a significant anterior abdominal muscular defect, splenomegaly and bilateral hydronephroses, neuropathic, hypotonic, thick-walled and bilateral intraperitoneal testes, talipes, gross enlargement of the attached gingivae.

Hartsfield et al., 1985	USA	USA	Case report	Jones	3M/ 2F	10-33y	5	No	AD	NR	The report describes a family with progressive sensorineural hearing loss and gingival fibromatosis.
Haytac & Ozcelik, 2007	Turkey	Turkey	Case Report	ND	M	9/ 42 y	2	NR	NR	NR	The 1st individual had moderate hearing deficit, broad nose, thick floppy ears, thick eyelashes and eyebrows, short stubby fingers and toes with hypoplasia of the terminal phalanges, hypoplasia of the nails on the thumbs, mild hypertrichosis mainly face and upper limbs. The 2nd individual had mild mental retardation and prolonged retention of primary teeth, without hypertrichosis.
He & Ping, 2012	China	China	Case Report	ND	M	21 y	1	No	NR	NR	Mental retardation, thick and sharply arched eyebrows, telecanthus, lapsus palpebrae superioris, saddle nose and brachyrhinia and thick lips, osteofibrosis of maxillary alveolar bone, congenital cataracta and concomitant esotropia and myopia, gingival overgrowth.
Holzhausen et al., 2003	Brazil	Brazil	Case Report	Zimmermann-Laband	F	13 y	1	No	NR	NR	Thick floppy ears with a low set, bulbous soft nose, prominent maxillae, thick lips, thick eyelashes and eyebrows, mild hirsutism on arms and legs, hypertelorism, telecanthus, and a short neck, hypoplasia of toenails, deformed terminal phalanges of the toes and thumbs and hyperextensibility of the metacarpophalangeal joints, anterior open bite, supranumerary teeth.
Hungund et al., 2013	India	India	Case Report	Cherubism	M	11 y	1	No	NR	NR	Painless bilateral swelling of jaws, multilocular osteolytic lesions, gingival overgrowth.
Ishita et al., 2016	India	India	Case report	Ambras	M	4y	1	Yes	Probable AR	NR	Congenital generalized hypertrichosis, dysmorphic facial features, increased intercanthal distance, and bushy, concrescent eyebrows with long eye lashes. Depressed nasal bridge, round tip nose, thick lips and gingival hyperplasia.
Jaouad et al., 2015	Morocco	Morocco	Case report	AIFGS	F	11y	1	Yes	AR	*FAM20A*	Hypoplastic amelogenesis imperfecta, generalized gingival hyperplasia, retention of four first molars and pulpal obliteration of both permanent superior molars. No nephrocalcinosis was diagnosed.
Johnson et al., 1986	USA	USA	Case report	ND	F	13y	1	NR	NR	NR	Gingival hyperplasia covering all teeth, hypertrichosis, macroglossia and frontal bossing.
Kaisare, 2007	India	India	Case Report	Niemann-Pick	M	25 y	1	Yes	Probable AR	NR	Microcephaly, two episodes of seizures with no medication, moderate mental retardation, dysarthria, ataxia, dystonia, supranuclear vertical gaze paresis, mild spasticity, thick lips, spaced

											teeth, macroglossia, generalized gingival enlargement.
Kanagotagi et al., 2015	India	India	Case report	Gingival fibromatosis with distinctive facies	M/F	7/28/33/60 y	4	NR	NR	NR	Macrocephaly, dysmorphic face with evident protusion of the lips, unable to close his lips, macrocephaly, hypertelorism, bushy eyebrows with synophrys, hypoplastic nares and cupid's bow mouth, gingival enlargement.
Kang et al., 2018	Korea	Korea	Case Report	Gingival fibromatosis with abnormal root development	F	6 y	1	No	NR	NR	Generalized gingival overgrowth and delayed root development.
Kantaputra et al., 2014	Thailand	Turkey	Case Report	Enamel renal	M/F	17/14 y	2	No	AR	*FAM20A*	Hypoplastic amelogenesis imperfecta, no renal alterations in patient 1 and nephrocalcinosis in patient 2.
Kantaputra et al., 2017	Thailand	Thailand/ Singapore/ Turkey	Case report	Enamel renal	2M/F	12/11/11y	3	Yes/No/No	AR	*FAM20A*	All patients had hypoplastic amelogenesis, unbroken teeth, with large dental follicles and intrapulpal calcifications. Nephrocalcinosis was reported only in the Thai boy that the parents were consanguineous.
Kim et al., 2007	USA	USA	Case report	Zimmermann–Laband	M	10y	1	NR	NR	Chromosomal translocation: t(3;17)(p14.3;q24.3).	Gingival hypertrophy, hypertrichosis, hypertelorism, strabismus, distinctive facies with large facial bones and mandibles, large ears, marked hypertrophy of the nose and large protruding upper lip. Also, enlarged fingers and toes and enlarged phallus were reported.
Kissi et al., 2006	Morocco	Morocco	Case Report	Zimmermann-Laband	F	26 y	1	NR	NR	NR	Congenital cardiopathy, hypertrophy of nose and lips, flap ears and macroglossia, myopy, skeletal anomalies (clavicular and phalanges hypoplasia), gingival fibromatosis.
Koch et al., 1992	Germany	Germany	Case report	Zimmermann–Laband	F	8y	1	No	NR	NR	Gingival hypertrophy, dystrophic nails, hypoplasia of phalanges, no neurological symptoms, reduced visual acuity with atypical retinitis pigmentosa.
Kortüm et al., 2015	Germany	Germany/ Italy	Case report	Zimmermann–Laband	2F	12y	2	No	*De novo*	*KCNH1*	The two girls had intellectual disability, seizures, coarse face, scoliosis and hypertrichosis. Patient 1 was described with aplastic / hypoplastic nails and terminal phalanges.
Kulkarni et al., 2011	India	India	Case Report	ND	M	6 y	1	No	NR	NR	Generalized hypertrichosis, dysmorphic face, broad forehead, thick and abundant eye lashes, a broad nose with depressed nasal bridge and labial fullness with a prominent jaw, high arched palate and squared dental arches, short neck and short broad hands, generalized gingival hyperplasia.
Kundoor et al., 2016	India	India	Case report	Ambras	F	38y	1	Yes	Probable AR	NR	Generalized hypertrichosis, dysmorphic facial features such as triangular facies and coarse

											features, hypertelorism, a wide and prominent nasal root, a large interalar distance, round nasal tip, and anteverted nostrils. Also, generalized gingival hyperplasia and high arch palate were reported.
Laband et al., 1964	Trinidad	India	Case report	ND	M/F	5y-38y	6	No	Probable AD	NR	All 6 members of the reported family showed enlargement of the soft tissue of ears and nose, skeletal abnormalities mainly absence or reduction in size of toenails and thumbnails with associated shortening of the terminal phalanx and an an abnormal degree of mobility of metacarpophalangeal and shoulder joints, pes cavus, hypoplastic nails and splenomegaly.
Landoulsi et al., 2012	Tunisia	Tunisia	Case report	ND	M	7y	1	No	NR	NR	Hypertrichosis, thick eyebrows, flattened nose, low set ears, mental retardation, epilepsy and severe gingival overgrowth covering upper teeth. Also, delayed tooth eruption of lower teeth was reported.
Laouina & Zupan, 2017	Morocco	Morocco	Case report	Enamel renal	M	11y	1	Yes	Probable AR	NR	Congenital heart defects, asthma, hypoplastic amelogenesis imperfecta, tooth crowns were shorter and crown resorption of unerupted third molars, dental delayed eruption, nonerupted teeth had complete root formation but root dilacerations was observed as well as gingival enlargement. No signs of nefrocalcinosis.
Lee et al., 1993	Korea	Korea	Case report	Hypertrichosis universalis congenita	F	6y	1	NR	NR	NR	Generalized hypertrichosis with mild gingival fibromatosis, dysmorphic facial, thick eyebrows were very thick and long and coarses eyelashes. The nose was broad and flat, and lips were thick and patulous. Also, macrotia was described in both ears and tongue papillary hypertrophy was reported.
Lin et al., 2010	China	China	Case Report	Zimmermann-Laband	F	12 y	1	NR	NR	NR	Mild cognitive impairement, mild hirsutism, thick eyebrows, telecanthus, bulbous nose and thick lips, proeminent maxilla and mandible, macroglossia, wide and deep tongue fissures, supernumerary teeth, deformed terminal phalanges of toes and splenomegaly.
Mangino et al., 2003	Italy	Italy	Case report	Congenital generalized hypertrichosis terminalis with gingival hyperplasia	4M/ 3F	NR	7	No	NR	NR	The 7 members of a three generation were described with hypertrichosis, synophris and bushy eyebrows.

Martelli-Júnior. et al., 2008	Brazil	Brazil	Case report	Gingival fibromatosis and dental abnormalities	M/F	13y-19y	4	Yes	Probable AR	NR	Four individuals were reported with gingival calcifications and dental anomalies that includes hypoplastic amelogenesis imperfecta, intra pulpal calcifications in erupted and unerupted teeth, pericoronal radiolucencies, retention of deciduous teeth, dental agenesis and microdontia. One of the four patients also had mental retardation.
Martins et al., 2010	Brazil	Brazil	Case Report	Barber-Say	F	7y	1	Yes	Probable AR	NR	Hypertrichosis, redundant and thick skin, telangiectasis, and absence of nipples.facial dysmorphism, low hairline, hypertelorism, eyelid atrophy, sparse eyelashes and eyebrows, telecanthus, bulbous nose, hypoplastic, nasal alae, and low-set ears of abnormal shape, macrostomia, thin upper lip, hypotonic tongue, broad alveolar ridges, tooth shape anomalies, delayed tooth eruption, gingival enlargement covering partially upper teeth.
McIndoe & Smith, 1958	UK	UK	Case report	ND	2M/ 1F	8y-48y	3	Yes	NR	NR	Hypertrichosis and gingival fibromatosis.
Mégarbané et al., 2016	France	Lebanon	Case report	Temple-Baraitser	M	15m	1	No	AD	*KCNH1*	Limbs and facial defects, coarse face, mild hypertelorism, epicanthal folds, broad depressed nasal bridge, short columella, broad mouth, narrow and high palate, intellectual disability, gingival enlargement. The authors suggest that Temple-Baraitser syndrome and Zimmermann Laband syndrome are a same entity cause by *KCNH1* mutations.
Molano et al., 1996	Colombia	Colombia	Case Report	Fibromatosis gingival, amelogenesis imperfecta, tooth eruption defects	F	11y	1	No	NR	NR	Severe delay in growth, hypoplastic amelogenesis imperfecta, dental anomalies of shape and size, dental eruption delay, gingival overgrowth.
O'Connell et al., 2013	UK	Kenya	Case report	Enamel renal	F	7 y	1	NR	NR	NR	Unremarkable medical history, hypoplastic amelogenesis imperfecta, follicular hamartoma, gingival calcifications, gingival overgrowth.
Oikarinen et al., 1990	UK	UK	Case Report	Gingival fibromatosis and growth hormone deficiency	F	8 y	1	No	NR	NR	Fibrous gingival overgrowth covering all the maxillary and mandibular incisors and canines except for the tips of the lower medial incisors.
Ooya et al., 1988	Japan	Japan	Case report	Autosomal recessive rough hypoplastic amelogenesis imperfecta	F	12y	1	NR	Probable AR	NR	Hypoplastic amelogenesis imperfecta, delayed tooth eruption, gingival calcifications and fibromatosis.

Pachajoa et al., 2018	Colombia	Colombia	Case report	ND	1F	8y	1	No	NR	*ABCC9*	Congenital generalized hypertrichosis, acromegaloid facial features, synophyrs, long eyelashes, broad lips, bulbous nose, broad mouth and no skeletal abnormalities or cardiac manifestations. The authors report a novel de novo heterozygous mutation in ABCC9 in a patient with a phenotype that differs from Cantu syndrome and related disorders.
Paula et al, 2005	Brazil	Brazil	Case report	Enamel renal	M	13 y	1	Yes	Probable AR	NR	Bilateral nephrocalcinosis, dental anomalies that include hypoplastic amelogenesis imperfecta, retention of deciduous teeth, eruption delay, yellow discoloration of teeth, pericoronal hamartomas, overgrowth of gingival tissue, gingival calcifications.
Pêgo et al., 2016	Brazil	Brazil	Case report	Enamel renal	F	10y	1	Yes	AR	*FAM20A*	Hypoplastic amelogenesis imperfecta, gingival hyperplasia, delayed tooth eruption, pericoronal dental pulpal and cerebral calcifications, hypertrichosis, characterized by thick eyebrows and eyelashes and excessive amount of hair in the forehead, lateral portions of the face, arms and back close to sacral region. Bilateral neprocalcinosis without renal disfunction was reported.
Pina-Neto et al., 1986	Brazil	Brazil	Case report	Ramon	M/F	9y-18y	4	3 Yes/1 No	Probable AR	NR	The series of cases reported four affected individuals, 3 belong to the same sibship and one is a second cousins. All individuals had a cherubism facial appearance showing and intense bilateral and symmetrical intense in volume in malar region and lower margin of mandible. All individuals had mild mental retardation and difficulties to walk in various degrees. Two individuals had moderate hypertrichosis on torso and limbs. In three of them juvenile rheumatoid arthritis was diagnosed.
Prasad et al., 2012	India	India	Case Report	Gingival fibromatosis with distinctive facies	F	13 y	1	Yes	Probable AR	NR	No medical history of seizures or metabolic disorder, bushy eyebrows with synophrys, flat nasal bridge, cupid bow mouth, bimaxillary protrusion, short stature and gingival enlargement.
Poulter et al., 2015	UK	Pakistan/ UK/ Costa Rica	Case report	Enamel renal.	2M	17y/ 15y	2	No	AR	*FAM20A*	Amelogenesis imperfecta was described in the two individuals evaluated.
Queiroz et al., 2005	Brazil	Brazil	Case Report	Ramon	F	25 y	1	NR	NR	NR	Cherubism, mild mental retardation, epilepsy, hypertrichosis, arthritis juvenile rheumatoid and gingival fibromatosis.

Ramon et al., 1967	Israel	Syria	Case Report	Gingival fibromatosis with cherubism	M	9/12y	2	Yes	Probable AR	NR	Two siblings were reported one of them had epilepsy, mental retardation, bilateral mandibular enlargement and the other only had mental retardation.
Roginsky et al., 2009	Russia	Russia	Case report	Ramon	M	6y	1	NR	NR	NR	Thirty individuals with descrition of cherubism were reported. However, only one had abnormal facial contours, multiple mandibular cystic angular radiolucencies and maxillary gingival fibromatosis compatible with Ramon syndrome.
Roopa et al., 2016	India	India	Case Report	Jones	M	15 y	1	NR	NR	NR	Mental retardation, hearing loss, and gingival overgrowth.
Roquebert et al., 2008	USA/ Panama	Panama	Case report	Amelogenesis imperfecta, rough hypoplastic type, dental follicular hamartomas and gingival hyperplasia	M	10y	1	NR	NR	NR	No evidence of any systemic disease. Enamel dysplasia, delayed eruption, pericoronal follicular hamartomas, malformed molar roots, globular enamel, calcifications, pulpal calcifications, interradicular irregular dentin, hypercementosis, generalized gingival hyperplasia, gingival dystrophic calcifications.
Shah et al., 2004	India	India	Case Report	Zimmermann-Laband	M	3 y	1	No	NR	NR	Large fleshy nose, malformed external ear lobes, developmental bilateral cataract, moderate learning disability, mild hearing loss and thick lips. Abnormalities of the hands and feet, short stubby fingers, some deformed, and absence of nails, no hepatosplenomegaly and normal joints. Massive maxillary gingival overgrowth with unerupted deciduous teeth.
Singer et al.,1993	Australia	Australia	Case Report	Autosomal recessive hereditary gingival fibromatosis	1F/1 M	11/ 15 y	2	No	Probable AR	NR	Two siblings were reported with similar orofacial features. Both of them had relative macrocephay with a distinctive facial appearance characterized by hypertelorism with anti-mongoloid slanting palpebral fissures and the eyebrows were bushy with synophry, flattening of nasal bridge and hypoplastic nares, lips were prominent and she had a cupid bow mouth and attached generalized gingival hyperplasia but more pronounced tuberosity region.
Stefanova et al., 2003	Germany	Germany	Case report	Zimmermann–Laband	2F	40y/ 5y	2	No	AD	Chromosomal translocation t(3;8)(p21.2;q24.3)	Both mother and daughter showed large fleshy nose, macrostomia, full lips, large tongue, large thick eyelashes. Additionally, the mother showed dystrophic finger-nails and aplasia of the toe-nails whereas her daughter had finger- and toe-nail aplasia, prominent ears, and generalized hirsutism.

Sunil et al., 2016	India	India	Case Report	Werewolf	M	9 y	1	No	NR	NR	Generalized hypertrichosis in all the body, no history of seizures, bushy eyebrows, thick lips, gingival hyperplasia.
Tang et al., 2014	China	China	Case Report	Syndromic gingival fibromatosis with ocular findings	M	21 y	1	No	NR	NR	Facial dysmorphism with poor vision in both eyes since his birth, bilateral congenital cataracts, esotropia, high myopia. His best-corrected visual acuity, was 0.12 in the right eye and 0.1 in left eye upon decimal visual acuity unit examination, cerebral and cerebellar anomalies, mild mental retardation, mixed hearing loss, osteofibrosis of maxillary alveolar bone, generalized gingival fibromatosis, multiple unerupted teeth.
Tomminska et al., 2017	Finland	2 Finland/1; Argentina	Case report	Pituitary hormone deficiency and maternally inherited gingival fibromatosis.	6M/ 5F	2-17y	11	No	AD	*KCNQ1*	Three unrelated families were reported. Eleven individuals were described with pituitary hormone deficiencies (growth hormone and/or gonatropin, ACTH, thryrotropin) gingival fibromatosis inherited maternally. Eight of them also had mild craniofacial features as a child not described in the report.
Torres et al., 2018	Brazil	Brazil	Case report	Enamel renal	M	11y	1	NR	NR	NR	Amelogenesis imperfecta, unerupted teeth with increased pericoronal hyperplasic follicles, nephrocalcinosis with low levels of calcium, phosphate and creatinine.
Wang et al., 2013	USA	3 Caribbean country not specified/ 1 Jordan/ 2 Iran	Case report	Enamel renal	3M/ 3F	NR	6	No/Yes/No	AR	*FAM20A*	Three families were reported only one was investigated for renal calcifications and the proband had nephrocalcinosis. All patients had hypoplastic amelogenesis imperfecta, delayed tooth eruption, pericoronal radiolucencies and pulpal calcifications. No other changes have been reported.
Winter & Simpkiss, 1974	UK	UK	Case report	Hypertrichosis with hereditary gingival hyperplasia	2F	8y/ 14y	2	No	NR	NR	Two unrelated cases of hypertrichosis. In one case generalized gingival hyperplasia was associated with retarded eruption of deciduous and permanent teeth and macrodontia. In the other case the mucosal hyperplasia was limited to the palatal surface. No other changes have been reported.
Yalçin et al., 1999	Turkey	Turkey	Case report	Cherubism	F	11 y	1	Yes	Probable AR	NR	Facial dysmorphism, bilateral fullness of cheeks with painless swelling of submandibular regions, mental retardation, generalized gingival overgrowth.

Abbreviations: UK: United Kingdom; USA: United States of America; AIFGS: Amelogenesis Imperfecta and Fibromatosis Gingival Syndrome; ND: Not defined; M: Male; F: Female; y: year; m: month; NR: Not reported; AR: Autosomal recessive; AD: Autosomal dominant; FAM20C:

Family with sequence similarity 20, member C; ABCC9: ATP Binding Cassette Subfamily C Member 9; KCNK4: Potassium Channel Subfamily K Member 4; FAM20A: Family with sequence similarity 20, member A; KCNH1: Potassium Channel Subfamily H Member 1; KCNQ1: Potassium Voltage-Gated Channel KQT-Like Subfamily Q Member 1.

Table S4. Gingival fibromatosis associated with oro-dental features

Author Year	Syndrome Name	Diagnostic Method (Clinical/ Radiographic/ Histopathological)	Extent of Fibromatosis (Generalized/ Localized)	Fibromatosis Severity (Mild/ Moderate/ Severe)	Gingival Calcifications	Dental anomalies	Mandible Maxilla Alterations
Abo-Dalo et al., 2008	Zimmermann–Laband	Clinical	Generalized	Mild	NR	NR	NR
Acevedo et al., 2015	Raine	Clinical Radiographic Histopathological	Generalized	Moderate-Severe	Y	Amelogenesis Imperfecta, incisal notch of central incisors, interglobular dentine, pulpal calcifications, incomplete root formation ectopic and delayed tooth eruption.	High palate, malocclusion
Afifi et al., 2015	Congenital generalized hypertrichosis terminalis with gingival hyperplasia	Clinical Histopathological	Generalized	Mild	N	NR	High palate with a narrow square-shaped vault, prominent premaxilla, deep overbite.
Afifi et al., 2016	Cantu	Clinical	Generalized	Moderate	NR	NR	High arched palate.
Anderson et al., 1969	Hereditary gingival fibromatosis	Clinical Radiographic Histopathological	Generalized	Severe	N	NR	NR
Ashkenazi et al., 2014	Enamel renal	Clinical Radiographic	Generalized	Mild	NR	Retained teeth, amelogenesis imperfecta, intrapulpal calcification, dilacerated roots.	NR
Bakaeen & Scully, 1991	Zimmermann-Laband	Clinical Radiographic	Generalized	Severe	NR	NR	NR
Balaji & Balaji, 2017	Gingival fibromatosis with hypertrichosis	Clinical	Generalized	Severe	NR	NR	NR
Bauer et al., 2018	Facial dysmorphism, hypertrichosis, epilepsy, intellectual/developmental delay, and gingival overgrowth	Clinical	Generalized	Severe	NR	NR	Micrognathia/receding chin
Benazza et al., 2005	Cherubism	Clinical Radiographic	Generalized	Severe	NR	NR	Anterior open bite, extreme labial protrusion of the anterior teeth.
Bhesania et al., 2015	Enamel renal	Clinical Radiographic Histopathological	Generalized	Moderate	Y	Agenesis, amelogenesis imperfecta, pulpal calcifications, impacted teeth.	Accessory mental foramen.

Cabral et al., 2013	Autosomal recessive gingival hyperplasia and dental anomalies	Clinical	Generalized	Moderate-Severe	NR	Amelogenesis imperfecta, delayed tooth eruption, failure of tooth development.	NR
Castori et al., 2013	Zimmermann-Laband	Clinical Radiographic	Generalized	Moderate	NR	NR	Anterior open bite
Chacon-Camacho et al., 2011	Zimmermann-Laband	Clinical Radiographic	Localized	Severe	NR	NR	NR
Chadwick et al., 1994	Zimmermann-Laband	Clinical	Localized	Mild	NR	NR	Anterior open bite.
Cho et al., 2011	AIFGS	Clinical Radiographic	Generalized	Moderate	NR	Amelogenesis imperfecta, intrapulpal calcification, delayed or failed eruption, impacted and root dilaceration, agenesis	NR
Cuestas-Carnero & Bornancini, 1988	Fibromatosis and hypertrichosis	Clinical Radiographic Histopathological	Generalized	Severe	N	NR	NR
Davalos et al., 2011	Zimmermann-Laband	Clinical Radiographic	Generalized	Severe	NR	Delay of eruption	High arched palate
Debnath et al., 2019	Enamel renal	Clinical Radiographic Histopathological	Generalized	Mild	Y	Enamel hypoplasia, dental agenesis, intrapulpal calcifications.	NR
de la Tranchade et al., 2003	Amelogenesis imperfecta and nephrocalcinosis	Clinical Radiographic Histopathological	Generalized	Moderate	N	Amelogenesis imperfecta, dental structure abnormality, delayed eruption.	ND
Dourado et al., 2019	Enamel renal	Clinical Radiographic Histopathological	Generalized	Mild	N	Microdontia, dental agenesis, retention of teeth, intrapulpal calcifications, unerupted teeth, dental resorption, shape abnormality.	NR
Elavarasu et al., 2017	Cherubism	Clinical Radiographic Histopathological	Generalized	Severe	N	Missing tooth bud.	Bilateral multilocular radiolucency involves the body and ramus of the mandible.
Feitosa et al., 2011	Cowden	Clinical Radiographic Histopathological	Generalized	Mild	N	NR	NR
Giansanti et al., 1973	Gingival fibromatosis, hypertelorism, anti-mongoloid obliquity, multiple telangiectases and café au lait pigmentation	Clinical Radiographic Histopathological	Localized	Mild	N	NR	Tori palatal and mandibular. Posterior cross-bite and anterior open bite.
Gita et al., 2014	Jones	Clinical Radiographic Histopathological	Generalized	Severe	N	Delayed dental eruption.	NR

Goldblatt & Singer, 1992	Autosomal recessive gingival fibromatosis with distinctive facies	Clinical Histopathological	Generalized	Moderate	N	Irregular dentition, incomplete eruption, reteined tooth.	NR
Goodwin et al., 2014	Costello	Clinical Radiographic	Localized	Mild	NR		
Guevara-Sanginés et al., 2002	Congenital generalized terminal hypertrichosis with gingival hyperplasia	Clinical Histopathological	Generalized	Severe	N	NR	NR
Hallett et al., 1995	Klippel-Trénaunay-Weber	Clinical Radiographic Histopathological	Localized	Severe	N	Delayed eruption, ectopic tooth, teeth fissure.	Tuberosity areas enlarged, mandibular retrognathism.
Harrison et al., 1998	Prune-belly	Clinical Histopathological	Generalized	Severe	N	NR	Tuberosity areas enlarged, mandibular retrognathism.
Hartsfield et al., 1985	Jones	Clinical	Generalized	Mild	NR	NR	Torus palatinus
Haytac & Ozcelik, 2007	ND	Clinical Radiographic	Generalized	Severe	NR	NR	Tuberosity areas enlarged, mandibular retrognathism.
He & Ping, 2012	ND	Clinical Radiographic Histopathological	Generalized	Severe	N	Impacted teeth, supernumerary tooth.	Maxillary alveolar bone enlarged.
Holzhausen et al., 2003	Zimmermann-Laband	Clinical Radiographic Histopathological	Generalized	Mild	N	Supernumerary teeth.	Anterior open bite, high arched palate.
Hungund et al., 2013	Cherubism	Clinical Radiographic Histopathological	Generalized	Severe	N	Impaction of erupted teeth.	Multiloculated soap bubble-like bone cavities.
Ishita et al., 2016	Ambras	Clinical Radiographic	Localized	Severe	NR	NR	NR
Jaouad et al., 2015	AIFGS	Clinical Radiographic	Generalized	Moderate	NR	Amelogenesis imperfecta, delayed tooth eruption, tooth retention.	NR
Johnson et al., 1986	ND	Clinical Histopathological	Generalized	Severe	N	NR	NR
Kaisare, 2007	Niemann-Pick	Clinical Radiographic Histopathological	Generalized	Moderate	N	NR	NR
Kanagotagi et al., 2015	Gingival fibromatosis with distinctive facies	Clinical Radiographic Histopathological	Generalized	Severe	N	NR	NR
Kang et al., 2018	Gingival fibromatosis with abnormal root development	Clinical Radiographic Histopathological	Generalized	Moderate	N	Delayed root development, supernumerary tooth.	Anterior open bite.
Kantaputra et al., 2014	Enamel renal	Clinical Radiographic Histopathological	Generalized	Severe	N	Amelogenesis imperfecta hyperplastic, prolonged retention of primary teeth, malposition of teeth, and multiple	NR

						unerupted maxillary and mandibular permanent incisors, premolars, and molars. Very large dental follicles, pulpar calcification, hypodontia.	
Kantaputra et al., 2017	Enamel renal	Clinical Radiographic Histopathological	Generalized	Moderate	Y	Amelogenesis imperfecta hypoplastic, prolonged retention, delayed eruption, pulpal calcifications, taurodontism, very large dental folicules.	Increased overbite and marked reduction in vertical dimension.
Kim et al., 2007	Zimmermann–Laband	Clinical Histopathological	Generalized	Severe	N	Absence of tooth eruption	ND
Kissi et al., 2006	Zimmermann-Laband	Clinical Radiographic Histopathological	Generalized	Moderate	N	ND	ND
Koch et al., 1992	Zimmermann–Laband	Clinical Radiographic Histopathological	Generalized	Severe	N	Teething delayed	ND
Kortüm et al., 2015	Zimmermann–Laband	Clinical Radiographic	Generalized	Moderate-Severe	NR	ND	ND
Kulkarni et al., 2011	ND	Clinical Radiographic Histopathological	Generalized	Severe	N	ND	High arched square palate.
Kundoor et al., 2016	Ambras	Clinical Radiographic	Generalized	Severe	NR	Impacted teeth	high arched palate
Laband et al., 1964	ND	Clinical Radiographic Histopathological	Generalized	Severe	N	ND	receding mandible
Landoulsi et al., 2012	ND	Clinical Radiographic Histopathological	Generalized	Severe	N	Delayed tooth eruption	ND
Laouina & Zupan, 2017	Enamel renal	Clinical Radiographic	Generalized	Mild	NR	Amelogenesis imperfecta hiperplastic, delayed teeth, taurodontism, dilaceration.	ND
Lee et al., 1993	Hypertrichosis universalis congenita	Clinical	Generalized	Moderate	NR	ND	ND
Lin et al., 2010	Zimmermann-Laband	Clinical Radiographic Histopathological	Generalized	Moderate	N	Supernumerary teeth	Anterior end- to-end bite
Mangino et al., 2003	Congenital generalized hypertrichosis terminalis with gingival hyperplasia	Clinical	Generalized	Mild	NR	ND	ND
Martelli-Júnior. et al., 2008	Gingival fibromatosis and dental abnormalities	Clinical Radiographic Histopathological	Generalized	Mild	Y	Microdontia, root dilaceration, dental agenesis, retention of teeth, intrapulpal calcifications, unerupted teeth..	ND

Martins et al., 2010	Barber-Say	Clinical Radiographic Histopathological	Generalized	Moderate	N	Abnormalities in tooth shape, taurodontism, increased enamel deposition, delayed tooth eruption,	Maxillary hyperdevelopment, anterior open bite
McIndoe & Smith, 1957	ND	Clinical Radiographic Histopathological	Generalized	Severe	N	NR	NR
Mégarbané et al., 2016	Temple-Baraitser	Clinical Radiographic	Generalized	Moderate	NR	NR	Narrow and high palate
Molano et al., 1996	Fibromatosis gingival, amelogenesis imperfecta, tooth eruption defects	Clinical Radiographic Histopathological	Generalized	Moderate	Y	Amelogenesis imperfecta hypoplastic, deep bite and lack of occlusal contact.	ND
O'Connell et al., 2013	Enamel renal	Clinical Radiographic Histopathological	Generalized	Mild	Y	Multiple impacted, ectopic and unerupted teeth, amelogenesis imperfecta hypoplastic, dilacered roots.	Bimaxillary proclination and an anterior open bite..
Oikarinen et al., 1990	Gingival fibromatosis and growth hormone deficiency	Clinical Radiographic Histopathological	Generalized	Moderate	N	ND	ND
Ooya et al., 1988	Autosomal recessive rough hypoplastic amelogenesis imperfecta	Clinical Radiographic Histopathological	Generalized	Severe	Y	Amelogenesis imperfecta hypoplastic, pulpal calcification, delayed eruption.	ND
Pachajoa et al., 2018	ND	Clinical Radiographic	ND	ND	NR	Wide-spaced teeth	Dental malocclusion
Paula et al, 2005	Enamel renal	Clinical Radiographic Histopathological	Generalized	Moderate	N	Amelogenesis imperfecta hypoplastic, pericoronal hamartoma, retention of deciduous teeth, delayed eruption, intrapulpal calcifications.	ND
Pêgo et al., 2016	Enamel renal	Clinical Radiographic Histopathological	Generalized	Moderate	Y	Amelogenesis imperfecta hypoplastic, pulpal calcification, delayed eruption.	Anterior and posterior cross-bite
Pina-Neto et al., 1986	Ramon	Clinical Radiographic Histopathological	Generalized	Severe	N	Buried teeth.	Fibrous dysplasia maxillar, multiloculated soap bubble-like bone cavities, narrow palate.
Poulter et al., 2015	Enamel renal	Clinical Radiographic	Generalized	Moderate	NR	Amelogenesis imperfecta hypoplastic.	ND
Prasad et al., 2012	Gingival fibromatosis with distinctive facies	Clinical Histopathological	Generalized	Moderate	N	ND	ND
Queiroz et al., 2005	Ramon	Clinical Radiographic	Generalized	Severe	NR	ND	Multiloculated soap bubble-like bone cavities.
Ramon et al., 1967	Gingival fibromatosis with cherubism	Clinical Radiographic Histopathological	Generalized	Severe	N	Displaced and impacted teeth.	Bilateral mandibular enlargement,Multiloculated soap bubble-like bone cavities.

Roginsky et al., 2009	Ramon	Clinical Radiographic Histopathological	Generalized	Severe	N	Hypodontia, anodontia, malpositions, crowding, dental loss, enamel hypoplasia.	Expansive bone remodeling, cortex thinning and disruption, multilocular radiolucencies with a coarse trabecular pattern.
Roopa et al., 2016	Joness	Clinical Radiographic Histopathological	Generalized	Severe	N	ND	ND
Roquebert et al., 2018	Amelogenesis imperfecta, rough hypoplastic type, dental follicular hamartomas and gingival hyperplasia	Clinical Radiographic Histopathological	Generalized	Severe	Y	Enamel dysplasia, delayed eruption, malformed molar roots, globular enamel calcifications, pulpal calcifications, interradicular irregular dentin, flat or slightly scalloped dentino-enamel junction, hypercementosis	ND
Shah et al., 2004	Zimmermann-Laband	Clinical Radiographic Histopathological	Generalized	Severe	N	ND	ND
Singer et al.,1993	Autosomal recessive hereditary gingival fibromatosis	Clinical Radiographic Histopathological	Generalized	Moderate	N	ND	ND
Stefanova et al., 2003	Zimmermann–Laband	Clinical	Generalized	Severe	NR	ND	ND
Sunil et al., 2016	Werewolf	Clinical Radiographic	Generalized	Severe	NR	ND	ND
Tang et al., 2014	Syndromic gingival fibromatosis with ocular findings	Clinical Histopathological	Generalized	Severe	N	Multiple unerupted teeth.	Osteofibrosis of maxillary alveolar bone.
Tommiska et al., 2017	Pituitary hormone deficiency and maternally inherited gingival fibromatosis.	Clinical Radiographic	Generalized	Severe	NR	ND	ND
Torres et al., 2018	Enamel renal	Clinical Radiographic Histopathological	Generalized	Moderate	Y	Amelogenesis imperfecta hypoplastic, delayed eruption.	Anterior crossbite
Wang et al., 2013	Enamel renal	Clinical Radiographic Histopathological	Generalized	Moderate	N	Unerupted teeth, amelogenesis impefecta hypoplastic, pulp calcifications, pericoronalradiolucencies, crown resorption.	ND

Winter & Simpkiss, 1974	Hypertrichosis with hereditary gingival hyperplasia	Clinical	Generalized	Moderate	NR	Unerupted teeth	Bilateral bony tori, high central palatal vault
Yalçin et al., 1999	Cherubism	Clinical Radiographic Histopathological	Generalized	Severe	N	Unerupted teeth.	Multiloculated soap bubble-like bone cavities.

Abbreviations: ND: Not determinated; NR: Not related; Y: Yes; N: No.

Figure S2. Ectopic calcification by study.

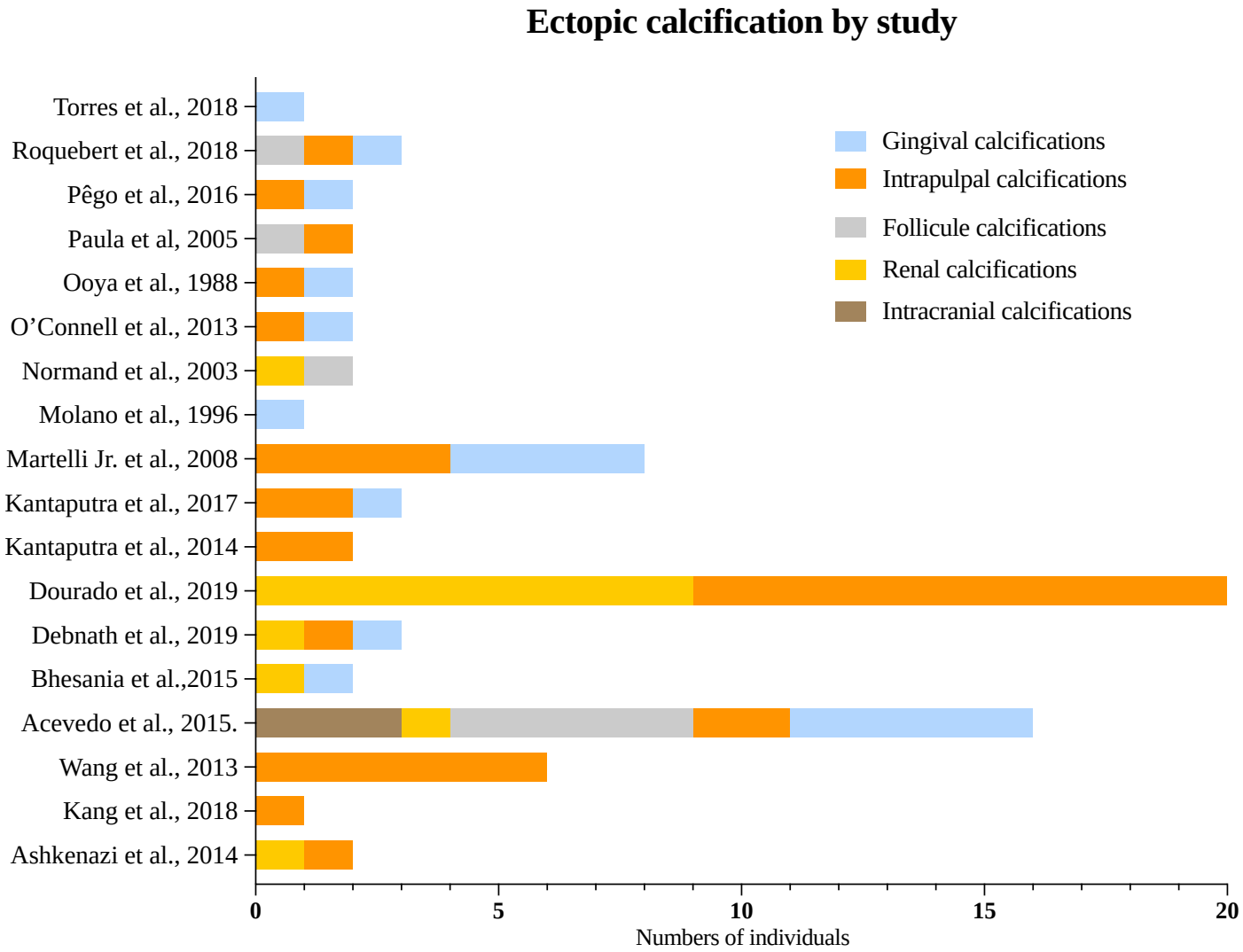


Table S5. Description of the syndrome name and OMIM associated with Gingival Fibromatosis

Syndrome name (OMIM)	Included studies	Number of individuals
Enamel renal syndrome (ERS; OMIM#204690)	19	54
Zimmermann-Laband syndrome (ZLS; OMIM #135500, OMIM#616455)	15	24
Gingival fibromatosis with hypertrichosis (HTC3; OMIM#135400)	6	14
Ramon syndrome (OMIM 266270)	4	8
Cherubism (CRBM; OMIM#118400)	4	4
Jones syndrome (GFD; OMIM%135550)	3	7
Fibromatosis gingival with distinctive faces (OMIM 228560)	3	7
Ambras syndrome (HTC1; OMIM%145701)	3	3
Cowden syndrome (CWS1; OMIM#158350)	2	2
Costello syndrome (CSTLO; OMIM#218040)	1	41
Pituitary hormone deficiency and maternally inherited gingival fibromatosis	1	11
Raine syndrome (RNS; OMIM#259775)	1	5
Fibromatosis and hypertrichosis	1	5

Facial dysmorphism, hypertrichosis, epilepsy, intellectual / developmental delay, and gingival overgrowth syndrome (FHEIG; OMIM#618381)	1	3
Autosomal recessive hereditary gingival fibromatosis	1	2
Barber-Say syndrome (BBRSAY; OMIM#209885)	1	1
Cantu syndrome (OMIM#239850)	1	1
Niemann-Pick syndrome (NPC; OMIM#257220, OMIM#607616)	1	1
Prune-Belly syndrome (PBS; OMIM#100100)	1	1
Temple-Baraitser syndrome (TMBTS; OMIM#611816)	1	1
Klippel-Trénaunay-Weber syndrome (KTW; OMIM%149000)	1	1
Amelogenesis imperfecta, rough hypoplastic type, dental follicular hamartomas and gingival hyperplasia	1	1
Fibromatosis gingival, amelogenesis imperfecta, tooth eruption defects, gingival fibromatosis and growth hormone deficiency	1	1
Gingival fibromatosis with abnormal root development, syndromic gingival fibromatosis with ocular findings	1	1
Werewolf	1	1
ND	7	16

Legend: Phenotype description, molecular basis known (#); Phenotype description or locus, molecular basis unknown (%); No determinate (ND).

Table S6. The Joanna Briggs Institute critical appraisal modified checklist for case report (n=82).

Questions	Abo-Dalo et al., 2008	Acevedo et al., 2015.	Afifi et al., 2015	Afifi et al., 2016	Anderson et al., 1969	Ashkenazi et al., 2014	Bakaeen & Scully, 1991	Balaji & Balaji, 2017	Bauer et al, 2018	Benazza et al., 2005	Bhesania et al., 2015	Cabral et al., 2013
Were patient's demographic characteristics clearly described?	U	Y	Y	Y	U	Y	Y	U	U	U	U	Y
Was the patient's history clearly described and presented as a timeline?	U	Y	Y	Y	Y	Y	Y	U	Y	Y	U	U
Was the current clinical condition of the patient on presentation clearly described?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were diagnostic tests or methods and the results clearly described?	U	Y	Y	U	Y	U	U	U	U	U	Y	U
Was the intervention(s) or treatment procedure(s) clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Was the post-intervention clinical condition clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Were adverse events (harms) or unanticipated events identified and described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Does the case report provide takeaway lessons?	Y	Y	Y	Y	Y	U	Y	U	Y	U	Y	Y

Questions	Castori et al., 2013	Chacon-Camacho et al., 2011	Chadwick et al., 1994	Cuestas-Carnero & Bornancini, 1988	Davalos et al., 2011	Debnath et al., 2019	de la Tranchade et al., 2003	Dourado et al., 2019	Elavarasu et al., 2017	Feitosa et al., 2011	Giansanti et al., 1973	Gita et al., 2014
Were patient's demographic characteristics clearly described?	U	U	Y	Y	U	U	U	U	U	N	U	U
Was the patient's history clearly described and presented as a timeline?	Y	Y	Y	Y	Y	N	Y	Y	N	N	U	Y
Was the current clinical condition of the patient on presentation clearly described?	Y	Y	Y	Y	Y	U	Y	Y	U	U	Y	Y
Were diagnostic tests or methods and the results clearly described?	U	U	U	U	U	U	Y	Y	U	U	U	U
Was the intervention(s) or treatment procedure(s) clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Was the post-intervention clinical condition clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Were adverse events (harms) or unanticipated events identified and described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Does the case report provide takeaway lessons?	Y	Y	Y	U	Y	U	Y	Y	U	U	U	Y

Questions	Goldblatt & Singer, 1992	Guevara-Sanginés et al., 2002	Hallett et al., 1995	Harrison et al., 1998	Hartsfield et al., 1985	Haytac & Ozcelik, 2007	He & Ping, 2012	Holzhausen et al., 2003	Hungund et al., 2013
Were patient's demographic characteristics clearly described?	U	U	Y	U	U	U	U	U	U
Was the patient's history clearly described and presented as a timeline?	Y	Y	Y	Y	Y	U	U	Y	U
Was the current clinical condition of the patient on presentation clearly described?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were diagnostic tests or methods and the results clearly described?	U	U	Y	Y	U	U	U	Y	Y
Was the intervention(s) or treatment procedure(s) clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA
Was the post-intervention clinical condition clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA
Were adverse events (harms) or unanticipated events identified and described?	NA	NA	NA	NA	NA	NA	NA	NA	NA
Does the case report provide takeaway lessons?	U	Y	Y	Y	U	U	U	Y	U

Questions	Ishita et al., 2016	Jaouad et al., 2015	Johnson et al., 1986	Kaisare, 2007	Kanagotagi et al., 2015	Kang et al., 2018	Kantaputra et al., 2014	Kantaputra et al., 2017	Kim et al., 2007	Kissi et al., 2006	Koch et al., 1992
Were patient's demographic characteristics clearly described?	U	Y	U	U	U	U	Y	Y	U	U	U
Was the patient's history clearly described and presented as a timeline?	Y	Y	U	Y	Y	U	Y	Y	Y	U	Y
Was the current clinical condition of the patient on presentation clearly described?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were diagnostic tests or methods and the results clearly described?	U	U	Y	U	Y	Y	Y	Y	U	Y	U
Was the intervention(s) or treatment procedure(s) clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Was the post-intervention clinical condition clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Were adverse events (harms) or unanticipated events identified and described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Does the case report provide takeaway lessons?	Y	Y	U	U	Y	U	Y	Y	Y	Y	Y

Questions	Kortüm et al., 2015	Kulkarni et al., 2011	Kundoor et al., 2016	Laband et al., 1964	Landoulsi et al., 2012	Laouina & Zupan, 2017	Lee et al., 1993	Lin et al., 2010	Mangino et al., 2003	Martelli-Júnior. et al., 2008	Martins et al., 2010
Were patient's demographic characteristics clearly described?	Y	Y	U	Y	U	Y	U	U	U	U	U
Was the patient's history clearly described and presented as a timeline?	Y	Y	U	Y	U	Y	Y	Y	U	Y	Y
Was the current clinical condition of the patient on presentation clearly described?	Y	Y	Y	Y	U	Y	Y	Y	Y	Y	Y
Were diagnostic tests or methods and the results clearly described?	Y	Y	U	U	U	U	U	Y	U	Y	U
Was the intervention(s) or treatment procedure(s) clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Was the post-intervention clinical condition clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Were adverse events (harms) or unanticipated events identified and described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Does the case report provide takeaway lessons?	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y

Questions	McIndoe & Smith, 1957	Mégarbané et al., 2016	Molano et al., 1996	O'Connell et al., 2013	Oikarinen et al., 1990	Ooya et al., 1988	Pachajoa et al., 2018	Paula et al, 2005	Pêgo et al., 2016	Pina-Neto et al., 1986
Were patient's demographic characteristics clearly described?	Y	Y	Y	Y	U	Y	Y	U	U	U
Was the patient's history clearly described and presented as a timeline?	Y	Y	U	Y	U	U	U	Y	Y	Y
Was the current clinical condition of the patient on presentation clearly described?	Y	U	Y	Y	Y	Y	U	Y	Y	Y
Were diagnostic tests or methods and the results clearly described?	Y	U	Y	Y	Y	Y	U	Y	Y	Y
Was the intervention(s) or treatment procedure(s) clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Was the post-intervention clinical condition clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Were adverse events (harms) or unanticipated events identified and described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Does the case report provide takeaway lessons?	U	Y	U	Y	U	U	Y	Y	Y	Y

Questions	Poulter et al., 2015	Prasad et al., 2012	Queiroz et al., 2005	Ramon et al., 1967	Roginsky et al., 2009	Roopa et al., 2016	Roquebert et al., 2008	Shah et al., 2004	Singer et al.,1993	Stefanova et al., 2003
Were patient's demographic characteristics clearly described?	Y	U	U	Y	U	U	Y	U	U	U
Was the patient's history clearly described and presented as a timeline?	N	U	U	Y	U	U	U	Y	U	U
Was the current clinical condition of the patient on presentation clearly described?	U	Y	U	Y	Y	Y	Y	Y	Y	Y
Were diagnostic tests or methods and the results clearly described?	U	U	U	Y	U	Y	Y	U	Y	U
Was the intervention(s) or treatment procedure(s) clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Was the post-intervention clinical condition clearly described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Were adverse events (harms) or unanticipated events identified and described?	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Does the case report provide takeaway lessons?	Y	U	U	Y	Y	Y	U	Y	U	Y

Questions	Sunil et al., 2016	Tang et al., 2014	Tomminska et al., 2017	Torres et al., 2018	Wang et al., 2013	Winter & Simpkiss, 1974	Yalçin et al., 1999
Were patient's demographic characteristics clearly described?	U	Y	Y	U	Y	U	U
Was the patient's history clearly described and presented as a timeline?	Y	U	Y	U	Y	Y	U
Was the current clinical condition of the patient on presentation clearly described?	Y	Y	Y	Y	Y	Y	Y
Were diagnostic tests or methods and the results clearly described?	U	U	U	Y	U	U	U
Was the intervention(s) or treatment procedure(s) clearly described?	NA	NA	NA	NA	NA	NA	NA
Was the post-intervention clinical condition clearly described?	NA	NA	NA	NA	NA	NA	NA
Were adverse events (harms) or unanticipated events identified and described?	NA	NA	NA	NA	NA	NA	NA
Does the case report provide takeaway lessons?	Y	U	Y	Y	Y	NA	U

Abbreviation: Yes (Y); No (N); Unclear (U); Not applicable (NA).

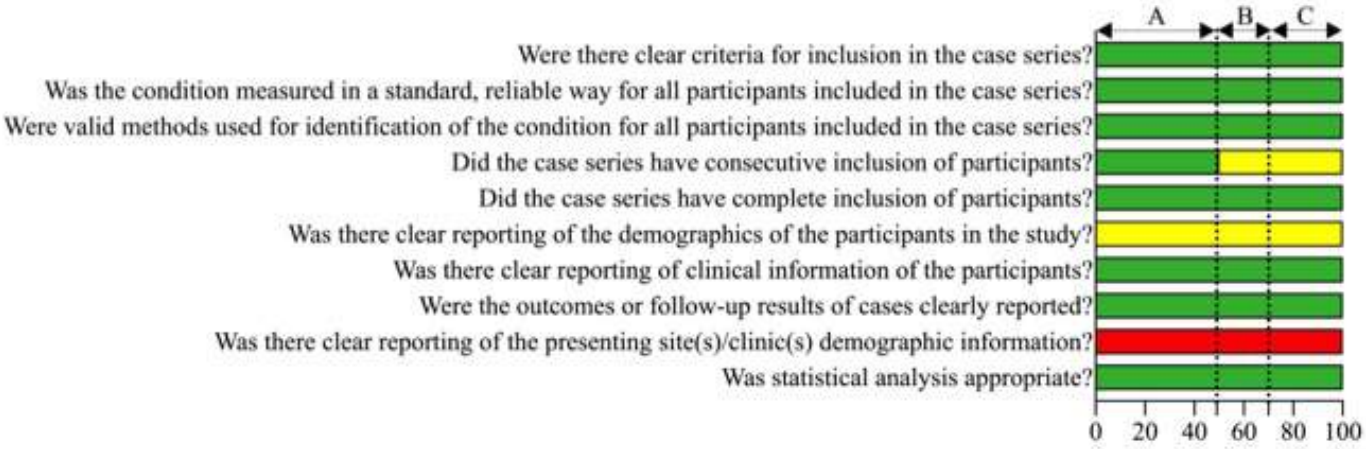
Table S7. The Joanna Briggs Institute critical appraisal modified checklist for case series (n=2).

Questions	Cho et al., 2011	Goodwin et al., 2014
Were there clear criteria for inclusion in the case series?	Y	Y
Was the condition measured in a standard, reliable way for all participants included in the case series?	Y	Y
Were valid methods used for identification of the condition for all participants included in the case series?	Y	Y
Did the case series have consecutive inclusion of participants?	U	Y
Did the case series have complete inclusion of participants?	Y	Y
Was there clear reporting of the demographics of the participants in the study?	U	U
Was there clear reporting of clinical information of the participants?	Y	Y
Were the outcomes or follow-up results of cases clearly reported?	Y	Y
Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	N	N
Was statistical analysis appropriate?	NA	Y

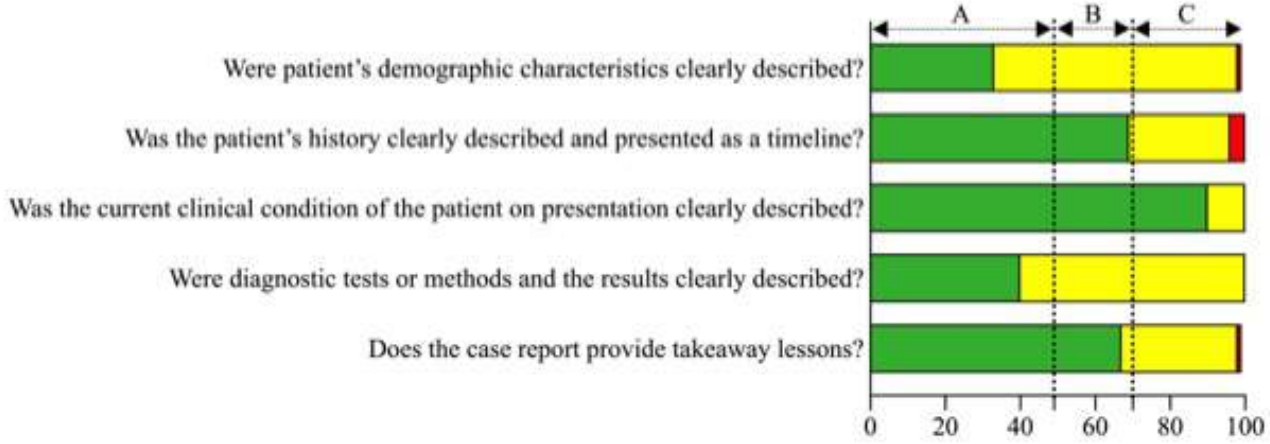
Abbreviation: Yes (Y); No (N); Unclear (U); Not applicable (NA).

Figure S3. Risk of bias for cases series graph (A). Risk of bias for case reports graph (B). The graphs A and B show the authors' judgments about each item of risk of bias established as percentages in all included studies. This risk of bias analysis was carried out using the Joanna Briggs Institute's critical assessment tools.

A


Risk of bias for case series
A
B
C
Were there clear criteria for inclusion in the case series?
Was the condition measured in a standard, reliable way for all participants included in the case series?
Were valid methods used for identification of the condition for all participants included in the case series?
Did the case series have consecutive inclusion of participants?
Did the case series have complete inclusion of participants?
Was there clear reporting of the demographics of the participants in the study?
Was there clear reporting of clinical information of the participants?
Were the outcomes or follow-up results of cases clearly reported?
Was there clear reporting of the presenting site(s)/clinic(s) demographic information?
Was statistical analysis appropriate?
0 20 40 60 80 100

B


Risk of bias for case reports
A
B
C
Were patient's demographic characteristics clearly described?
Was the patient's history clearly described and presented as a timeline?
Was the current clinical condition of the patient on presentation clearly described?
Were diagnostic tests or methods and the results clearly described?
Does the case report provide takeaway lessons?
0 20 40 60 80 100

REFERENCES (for Article 1)

Abo-Dalo, B., Roes, M., Canún, S., Delatycki, M., Gillessen-Kaesbach, G., Hrytsiuk, I., ... & Steiner, C. E. (2008). No mutation in genes of the WNT signaling pathway in patients with Zimmermann–Laband syndrome. *Clinical Dysmorphology*, 17(3), 181-185.

Acevedo, A. C., Poulter, J. A., Alves, P. G., de Lima, C. L., Castro, L. C., Yamaguti, P. M., ... & Johnson, C. A. (2015). Variability of systemic and oro-dental phenotype in two families with non-lethal Raine syndrome with FAM20C mutations. *BMC Medical Genetics*, *16*(1), 8.

Afifi, H. H., Abdel-Hamid, M. S., Eid, M. M., Mostafa, I. S., & Abdel-Salam, G. M. (2016). De Novo Mutation in ABCC 9 Causes Hypertrichosis Acromegaloid Facial Features Disorder. *Pediatric Dermatology*, 33(2), e109-e113.

Afifi, H. H., Fukai, R., Miyake, N., Gamal el Din, A. A., Eid, M. M., Eid, O. M., ... & Matsumoto, N. (2015). De Novo 17q24. 2–q24. 3 microdeletion presenting with generalized hypertrichosis terminalis, gingival fibromatous hyperplasia, and distinctive facial features. *American Journal of Medical Genetics Part A*, 167(10), 2418-2424.

Anderson, J., Cunliffe, W. J., Roberts, D. F., & Close, H. (1969). Hereditary gingival fibromatosis. *British Medical Journal*, 3(5664), 218.

Ashkenazi, M., Rafe, Z., Sarnat, H., & Levin, L. (2014). Nephrocalcinosis associated with continuous enamel hypoplasia and severe alveolar bone loss: a case report and literature review. *Pediatric Dentistry*, 36(3), 250-253.

Bakaeen, G., & Scully, C. (1991). Hereditary gingival fibromatosis in a family with the Zimmermann Laband syndrome. *Journal of Oral Pathology & Medicine*, 20(9), 457-459.

Balaji, P., & Balaji, S. M. (2017). Gingival fibromatosis with hypertrichosis syndrome: Case series of rare syndrome. *Indian Journal of Dental Research*, 28(4), 457.

Bauer, C. K., Calligari, P., Radio, F. C., Caputo, V., Dentici, M. L., Falah, N., ... & Pizzi, S. (2018). Mutations in KCNK4 that affect gating cause a recognizable neurodevelopmental syndrome. *The American Journal of Human Genetics*, 103(4), 621-630.

Benazza, D., Elmouadden, M., Benjalloun, A., Jiddane, M., Elatiaoui, F., & Benzarti, N. (2005). Gingival fibromatosis associated with cherubism. *Revue de Stomatologie et de Chirurgie Maxillo-faciale*, 106(3), 174-176.

Bhesania, D., Arora, A., & Kapoor, S. (2015). Enamel renal syndrome with associated amelogenesis imperfecta, nephrolithiasis, and hypocitraturia: A case report. *Imaging Science in Dentistry*, 45(3), 181-185.

Booth, A., Clarke, M., Ghersi, D., Moher, D., Petticrew, M., & Stewart, L. (2011). An international registry of systematic-review protocols. *The Lancet*, *377*(9760), 108-109.

Cabral, R. M., Kurban, M., Rothman, L., Wajid, M., Shimomura, Y., Petukhova, L., & Christiano, A. M. (2013). Autosomal recessive gingival hyperplasia and dental anomalies caused by a 29-base pair duplication in the FAM20A gene. *Journal of Human Genetics*, 58(8), 566.

Casas-Alba, D., Martínez-Monseny, A., Pino-Ramírez, R. M., Alsina, L., Castejón, E., Navarro-Vilarrubí, S., ... & García-Alix, A. (2018). Hyaline fibromatosis syndrome: Clinical update and phenotype–genotype correlations. *Human Mutation*, 39(12), 1752-1763.

Castori, M., Valiante, M., Pascolini, G., Leuzzi, V., Pizzuti, A., & Grammatico, P. (2013). Clinical and genetic study of two patients with Zimmermann–Laband syndrome and literature review. *European Journal of Medical Genetics*, 56(10), 570-576.

Chacon-Camacho, O. F., Vázquez, J., & Zenteno, J. C. (2011). Expanding the phenotype of gingival fibromatosis–mental retardation–hypertrichosis (Zimmermann–Laband) syndrome. *American Journal of Medical Genetics Part A*, 155(7), 1716-1720.

Chadwick, B., Hunter, B., Hunter, L., Aldred, M., & Wilkie, A. (1994). Laband syndrome: Report of two cases, review of the literature, and identification of additional manifestations. *Oral Surgery, Oral Medicine, Oral Pathology*, 78(1), 57-63.

Cho, S. H., Seymen, F., Lee, K. E., Lee, S. K., Kweon, Y. S., Kim, K. J., ... & Tuna, E. B. (2012). Novel FAM20A mutations in hypoplastic amelogenesis imperfecta. *Human Mutation*, 33(1), 91-94.

Coletta, R. D., & Graner, E. (2006). Hereditary gingival fibromatosis: a systematic review. *Journal of Periodontology*, *77*(5), 753-764.

Cuestas-Carnero, R., & Bornancini, C. A. (1988). Hereditary generalized gingival fibromatosis associated with hypertrichosis: report of five cases in one family. *Journal of Oral and Maxillofacial Surgery*, *46*(5), 415-420.

Davalos, I. P., Brambila-Tapia, A. J. L., Davalos, N. O., Durán-González, J., Gonzalez-Mercado, M. G., Cruz-Ramos, J. A., ... & Garcia-Cruz, D. (2011). Wide clinical spectrum in Zimmermann-Laband syndrome. *Genetic Counseling*, 22(1), 1.

de La Dure-Molla, M., Quentric, M., Yamaguti, P. M., Acevedo, A. C., Mighell, A. J., Vikkula, M., ... & Bloch-Zupan, A. (2014). Pathognomonic oral profile of Enamel Renal Syndrome (ERS) caused by recessive FAM20A mutations. *Orphanet Journal of Rare Diseases*, 9(1), 84.

de la Tranchade, I., Bonarek, H., Marteau, J. M., Boileau, M. J., & Nancy, J. (2003). Amelogenesis imperfecta and nephrocalcinosis: a new case of this rare syndrome. *Journal of Clinical Pediatric Dentistry*, 27(2), 171-175.

Debnath, K., Couthino, A., Chatterjee, A., & Shenoy, S. (2019). Enamel renal gingival syndrome: A rare case report. *Journal of Indian Society of Periodontology*, 23(1), 69.

Dhadse, P. V., Yeltiwar, R. K., Pandilwar, P. K., Gosavi, S. R. (2012). Hereditary gingival fibromatosis. *Journal of Indian Society of Periodontology*, 16(4), 606-609.

Dourado, M. R., dos Santos, C. R. R., Dumitriu, S., Iancu, D., Albanyan, S., Kleta, R., ... & Mesquita, A. T. M. (2019). Enamel renal syndrome: A novel homozygous FAM20A founder mutation in 5 new Brazilian families. *European Journal of Medical Genetics*, 62(11), 103561.

Elavarasu, S., Thangavelu, A., Naveen, D., & Selvaraj, S. (2017). Cherubism with idiopathic gingival enlargement: A rare case report. *Journal of Indian Society of Periodontology*, 21(6), 503.

Feitosa, D. S., Santamaria, M. P., Casati, M. Z., Sallum, E. A., Júnior, F. H. N., & de Toledo, S. (2011). Surgical management of gingival overgrowth associated with Cowden sydrome: A case report and current understanding. *Quintessence International*, *42*(5).

Gawron, K., Łazarz-Bartyzel, K., Potempa, J., & Chomyszyn-Gajewska, M. (2016). Gingival fibromatosis: clinical, molecular and therapeutic issues. *Orphanet Journal of Rare Diseases*, *11*(1), 9.

Giansanti, J. S., McKenzie, W. T., & Owens, F. C. (1973). Gingival Fibromatosis, Hypertelorism, Anti-Mongoloid Obliquity, Multiple Telangiectases and Cafe Au Lait Pigmentation; A Unique Combination of Developmental Anomalies. *Journal of Periodontology*, *44*(5), 299-302.

Gita, B., Chandrasekaran, S., Manoharan, P., & Dembla, G. (2014). Idiopathic gingival fibromatosis associated with progressive hearing loss: A nonfamilial variant of Jones syndrome. *Contemporary Clinical Dentistry*, 5(2), 260.

Goldblatt, J., & Singer, S. L. (1992). Autosomal recessive gingival fibromatosis with distinctive facies. *Clinical Genetics*, 42(6), 306-308.

Goodwin, A. F., Oberoi, S., Landan, M., Charles, C., Massie, J. C., Fairley, C., ... & Klein, O. D. (2014). Craniofacial and dental development in Costello syndrome. American *Journal of Medical Genetics Part A*, 164(6), 1425-1430.

Guevara-Sanginés, E., Villalobos, A., Vega-Memije, M. E., Mosqueda-Taylor, A., Canún-Serrano, S., & Lacy-Niebla, R. M. (2002). Congenital generalized terminal hypertrichosis with gingival hyperplasia. *Pediatric Dermatology*, 19(2), 114-118.

Hallett, K. B., Bankier, A., Chow, C. W., Bateman, J., & Hall, R. K. (1995). Gingival fibromatosis and Klippel-Trenaunay-Weber syndrome: case report. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *79*(5), 578-582.

Harrison, M., Odell, E. W., Agrawal, M., Saravanamuttu, R., & Longhurst, P. (1998). Gingival fibromatosis with prune-belly syndrome. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 86(3), 304-307.

Hart, T. C., & Hart, P. S. (2009). Genetic studies of craniofacial anomalies: clinical implications and applications. *Orthodontics & Craniofacial Research*, 12(3), 212-220.

Hart, T. C., Zhang, Y., Gorry, M. C., Hart, P. S., Cooper, M., Marazita, M. L., ... & Pallos, D. (2002). A mutation in the SOS1 gene causes hereditary gingival fibromatosis type 1. *The American Journal of Human Genetics*, 70(4), 943-954.

Hartsfield Jr, J. K., Bixler, D., Hazen, R. H., Opitz, J. M., & Reynolds, J. F. (1985). Gingival fibromatosis with sensorineural hearing loss: an autosomal dominant trait. *American Journal of Medical Genetics*, 22(3), 623-627.

Haytac, M. C., & Ozcelik, O. (2007). The phenotypic overlap of syndromes associated with hereditary gingival fibromatosis: follow-up of a family for five years. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 103(4), 521-527.

He, L., & Ping, F. Y. (2012). Gingival fibromatosis with multiple unusual findings: report of a rare case. *International Journal of Oral Science*, 4(4), 221.

Holzhausen, M., Gonçalves, D., Corrêa, F. D. O. B., Spolidorio, L. C., Rodrigues, V. C., & Orrico, S. R. P. (2003). A case of Zimmermann-Laband syndrome with supernumerary teeth. *Journal of Periodontology*, 74(8), 1225-1230.

Hungund, S. A., Singh, V., & Nagaraja, C. (2013). Non-familial cherubism: A case report with its surgical management. *Journal of Indian Society of Periodontology*, 17(6), 816.

Ishita, A., Sujatha, G. P., Pramod, G. V., & Ashok, L. (2016). Ambras syndrome: A rare case report. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, 34(2), 189.

Jaouad, I. C., El Alloussi, M., Laarabi, F. Z., Lyahyai, J., & Sefiani, A. (2015). Further evidence for causal FAM20A mutations and first case of amelogenesis imperfecta and gingival hyperplasia syndrome in Morocco: a case report. *BMC Oral Health*, 15(1), 14.

Jaureguiberry, G., De la Dure-Molla, M., Parry, D., Quentric, M., Himmerkus, N., Koike, T., ... & Patel, V. (2012). Nephrocalcinosis (enamel renal syndrome) caused by autosomal recessive FAM20A mutations. *Nephron Physiology*, 122(1-2), 1-6.

Jenicek, M. (2008). Clinical case reports and case series research in evaluating surgery. Part II. The content and form: uses of single clinical case reports and case series research in surgical specialties. *Medical Science Monitor*, 14(10), RA149-RA162.

Johnson, B. D., El-Guindy, M., Ammons, W. F., Sampath Narayanan, A., & Page, R. C. (1986). A defect in fibroblasts from an unidentified syndrome with gingival hyperplasia as the predominant feature. *Journal of Periodontal Research*, 21(4), 403-413.

Kaisare, S. (2007). Gingival enlargement in Niemann-Pick disease: a coincidence or link? Report of a unique case. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 104(5), e35-e39.

Kanagotagi, S., Sidana, S., Rajguru, S., & Padhye, A. (2015). Gingival Fibromatosis with Distinctive Facies–A Three Generation Case Report. *Journal of Clinical and Diagnostic Research: JCDR*, 9(5), ZD05.

Kang, C. M., Lee, J. H., Jeon, M., Song, J. S., & Kim, S. O. (2018). The effect of MMP-13, MMP-12, and AMBN on gingival enlargement and root deformation in a new type of gingival fibromatosis. *Journal of Clinical Pediatric Dentistry*, 42(1), 50-54.

Kantaputra, P. N., Bongkochwilawan, C., Kaewgahya, M., Ohazama, A., Kayserili, H., Erdem, A. P., ... & Guven, Y. (2014). Enamel–Renal–Gingival syndrome, hypodontia, and a novel FAM20A mutation. *American Journal of Medical Genetics Part A*, 164(8), 2124-2128.

Kantaputra, P. N., Bongkochwilawan, C., Lubinsky, M., Pata, S., Kaewgahya, M., Tong, H. J., ... & Chaisrisookumporn, N. (2017). Periodontal disease and FAM20A mutations. *Journal of Human Genetics*, 62(7), 679.

Kim, H. G., Higgins, A. W., Herrick, S. R., Kishikawa, S., Nicholson, L., Kutsche, K., ... & Morton, C. C. (2007). Candidate loci for Zimmermann–Laband syndrome at 3p14. 3. *American Journal of Medical Genetics Part A*, 143(2), 107-111.

Kissi, L., Benyahya, I., & Rifki, A. (2006). Fibromatose gingivale dans le cadre d'une atteinte syndromique. *Revue de Stomatologie et de Chirurgie Maxillo-faciale*, 107(6), 477-480.

Koch, P., Wettstein, A., Knauber, J., & Zaun, H. (1992). A new case of Zimmermann-Laband syndrome with atypical retinitis pigmentosa. *Acta Dermato-venereologica*, 72(5), 376-379.

Kortüm, F., Caputo, V., Bauer, C. K., Stella, L., Ciolfi, A., Alawi, M., ... & Grammatico, P. (2015). Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome. *Nature Genetics*, 47(6), 661.

Kulkarni, P., Agrawal, N., Tyagi, S., & Kambalimath, H. (2011). A rare case of gingival fibromatosis associated with hypertrichosis and a dysmorphic face. *Journal of Clinical Pediatric Dentistry*, 35(3), 305-308.

Kundoor, V. K. R., Maloth, K. N., Kesidi, S., & Moni, T. (2016). Ambras syndrome with gingival hyperplasia: A rare entity. *International Journal of Trichology*, 8(2), 81.

Laband, P. F., Habib, G., & Humphreys, G. S. (1964). Hereditary gingival fibromatosis: Report of an affected family with associated splenomegaly and skeletal and soft-tissue abnormalities. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, 17(3), 339-351.

Landoulsi, A., Ben, R. S., Bayouth, W., Fodha, H., Zairi, I., Adouani, A., & Nefzi, A. (2012). Hypertrichosis, gingival hypertrophia, epilepsy and mental retardation: a separate entity? *La Tunisie Medicale*, 90(10), 746-748.

Laouina, S., & Zupan, A. B. (2017). Enamel–renal syndrome with congenital heart defects and asthma: a rare association in a Moroccan child. *Clinical Dysmorphology*, 26(2), 114-116.

Lee, I. J., Im, S. B., & Kim, D. K. (1993). Hypertrichosis universalis congenita: a separate entity, or the same disease as gingival fibromatosis? *Pediatric Dermatology*, 10(3), 263-266.

Lin, Z., Wang, T., Sun, G., & Huang, X. (2010). Report of a case of Zimmermann–Laband syndrome with new manifestations. *International Journal of Oral and Maxillofacial Surgery*, 39(9), 937-941.

Mangino, M., Pizzuti, A., Dallapiccola, B., Bonfante, A., Saccilotto, D., & Cucchiara, E. (2003). Hereditary gingival fibromatosis (HGF) with hypertrichosis is unlinked to the HGF1 and HGF2 loci. *American Journal of Medical Genetics Part A*, 116(3), 312-314.

Martelli-Júnior, H., Bonan, P. R. F., dos Santos, L. A. N., Santos, S. M. C., Cavalcanti, M. G., & Coletta, R. D. (2008). Case reports of a new syndrome associating gingival fibromatosis and dental abnormalities in a consanguineous family. *Journal of Periodontology*, 79(7), 1287-1296.

Martins, F., Ortega, K. L., Hiraoka, C., Ricardo, P., & Magalhaes, M. (2010). Oral and dental abnormalities in Barber–Say syndrome. *American Journal of Medical Genetics Part A*, 152(10), 2569-2573.

McIndoe, A., & Smith, B. O. (1958). Congenital familial fibromatosis of the gums with the teeth as a probable etiological factor: Report of an affected family. *British Journal of Plastic Surgery*, 11, 62-71.

Mégarbané, A., Al-Ali, R., Choucair, N., Lek, M., Wang, E., Ladjimi, M., ... & Jithesh, P. V. (2016). Temple-baraitser syndrome and zimmermann-laband syndrome: one clinical entity? *BMC Medical Genetics*, 17(1), 42.

Mehawej, C., Hoischen, A., Farah, R. A., Marey, I., David, M., Stora, S., ... & Mégarbané, A. (2018). Homozygous mutation in *ELMO2* may cause Ramon syndrome. *Clinical Genetics*, *93*(3), 703-706.

Meng, L., Huang, M., Ye, X., Fan, M., & Bian, Z. (2007). Increased expression of collagen prolyl 4-hydroxylases in Chinese patients with hereditary gingival fibromatosis. *Archives of Oral Biology*, *52*(12), 1209-1214.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of internal medicine*, *151*(4), 264-269.

Molano, P., Blank, A., Tamayo, O., & Isaza, C. (1996). Fibromatosis gingival, amelogénesis imperfecta, retardo en la erupción dental y retardo en el crecimiento y desarrollo.¿ Un nuevo síndrome? *Colombia Médica*, 27(3, 4), 150-155.

Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, Currie M, Lisy K, Qureshi R, Mattis P, Mu P. (2017) Chapter 7: Systematic reviews of etiology and risk. In: Aromataris E, Munn Z (Editors). *Joanna Briggs Institute Reviewer's Manual*.

Munn, Z., Moola, S., Lisy, K., Riitano, D., & Tufanaru, C. (2015). Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. *International Journal of Evidence-based Healthcare*, *13*(3), 147-153.

Murad, M. H., Sultan, S., Haffar, S., & Bazerbachi, F. (2018). Methodological quality and synthesis of case series and case reports. *BMJ Evidence-based Medicine*, *23*(2), 60-63.

Nalbant, D., Youn, H., Nalbant, S. I., Sharma, S., Cobos, E., Beale, E. G., ... & Williams, S. C. (2005). FAM20: an evolutionarily conserved family of secreted proteins expressed in hematopoietic cells. *BMC genomics*, *6*(1), 11.

O'Sullivan, J., Bitu, C. C., Daly, S. B., Urquhart, J. E., Barron, M. J., Bhaskar, S. S., ... & Coletta, R. D. (2011). Whole-Exome sequencing identifies FAM20A mutations as a cause of amelogenesis imperfecta and gingival hyperplasia syndrome. *The American Journal of Human Genetics*, 88(5), 616-620.

O'Connell, S., Davies, J., Smallridge, J., & Vaidyanathan, M. (2014). Amelogenesis imperfecta associated with dental follicular-like hamartomas and generalised gingival enlargement. *European Archives of Paediatric Dentistry*, 15(5), 361-368.

Oikarinen, K., Salo, T., Ka, M. L., Lahtela, P., & Altonen, M. (1990). Hereditary gingival fibromatosis associated with growth hormone deficiency. *British Journal of Oral and Maxillofacial Surgery*, 28(5), 335-339.

Ooya, K., Nalbandian, J., & Noikura, T. (1988). Autosomal recessive rough hypoplastic amelogenesis imperfecta: A case report with clinical, light microscopic, radiographic, and electron microscopic observations. *Oral Surgery, Oral Medicine, Oral Pathology*, 65(4), 449-458.

Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan—a web and mobile app for systematic reviews. *Systematic Reviews*, *5*(1), 210.

Pachajoa, H., López-Quintero, W., Vanegas, S., Montoya, C. L., & Ramírez-Montaño, D. (2018). Novel mutation in ABBC9 gene associated with congenital hypertrichosis and acromegaloid facial features, without cardiac or skeletal anomalies: a new phenotype. *The Application of Clinical Genetics*, 11, 15.

Paula, L. M., Melo, N. S., Guerra, E. S., Mestrinho, D. H., & Acevedo, A. C. (2005). Case report of a rare syndrome associating amelogenesis imperfecta and nephrocalcinosis in a consanguineous family. *Archives of Oral Biology*, 50(2), 237-242.

Pêgo, S. P. B., Coletta, R. D., Dumitriu, S., Iancu, D., Albanyan, S., Kleta, R., ... & Martelli-Júnior, H. (2017). Enamel-renal syndrome in 2 patients with a mutation in FAM20 A and

atypical hypertrichosis and hearing loss phenotypes. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, 123(2), 229-234.

Pehlivan, D., Abe, S., Ozturk, S., Kayhan, K. B., Gunduz, E., Cefle, K., ... & Palanduz, S. (2009). Cytogenetic analysis and examination of SOS1 gene mutation in a Turkish family with hereditary gingival fibromatosis. *Journal of Hard Tissue Biology*, 18(3), 131-134.

Pina-Neto, J. M., Moreno, A. F. C., Silva, L. R., Velludo, M. A. S., Petean, E. B. L., Ribeiro, M. V. M., ... & Reynolds, J. F. (1986). Cherubism, gingival fibromatosis, epilepsy, and mental deficiency (Ramon syndrome) with juvenile rheumatoid arthritis. *American Journal of Medical Genetics*, 25(3), 433-441.

Poonia, K., Gogia, P., & Bhalla, M. (2018). Localized hypertrichosis at vaccination site. *International Journal of Trichology*, *10*(3), 138.

Poulter, J. A., Smith, C. E., Murrillo, G., Silva, S., Feather, S., Howell, M., ... & Inglehearn, C. F. (2015). A distinctive oral phenotype points to FAM 20A mutations not identified by S anger sequencing. *Molecular Genetics & Genomic Medicine*, 3(6), 543-549.

Prasad, S. S. R., Radharani, C., Sinha, S., & Kumar, S. K. (2012). Hereditary gingival fibromatosis with distinctive facies. *The Journal of Contemporary Dental Practice*, 13(6), 892-6.

Queiroz, A. M., Nelson-Filho, P., de Freitas, A. C., Assed, S., Soares, U. N., & Faria, J. F. R. (2005). Querubismo-revisão de literatura, relato de caso e tratamento odontológico-cirúrgico em paciente portador da síndrome de Ramon. *Revista da Faculdade de Odontologia-UPF*, 10(1).

Ramon, Y., Berman, W., & Bubis, J. J. (1967). Gingival fibromatosis combined with cherubism. *Oral Surgery, Oral Medicine, Oral Pathology*, 24(4), 435-448.

Rodríguez-Vázquez, M., Carrascosa-Romero, M. C., Pardal-Fernández, J. M., & Iniesta, I. (2007). Congenital gingival hyperplasia in a neonate with foetal valproate syndrome. *Neuropediatrics*, *38*(05), 251-252.

Roginsky, V. V., Ivanov, A. L., Ovtchinnikov, I. A., & Khonsari, R. H. (2009). Familial cherubism: the experience of the Moscow Central Institute for Stomatology and Maxillo-Facial Surgery. *International Journal of Oral and Maxillofacial Surgery*, 38(3), 218-223.

Roopa, D. A., Singh, S., Gupta, I., & Gopal, S. (2016). Gingival Enlargement in a Case of Variant Jones Syndrome: a Case Report. *Journal of Dentistry*, 17(1), 62.

Roquebert, D., Champsaur, A., del Real, P. G., Prasad, H., Rohrer, M. D., Pintado, M., ... & Koutlas, I. G. (2008). Amelogenesis imperfecta, rough hypoplastic type, dental follicular hamartomas and gingival hyperplasia: report of a case from Central America and review of the literature. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 106(1), 92-98.

Saleh, D., & Cook, C. (2018). Hypertrichosis. In *StatPearls [Internet]*. StatPearls Publishing.

Sayre, J. W., Toklu, H. Z., Ye, F., Mazza, J., & Yale, S. (2017). Case reports, case series–from clinical practice to evidence-based medicine in graduate medical education. *Cureus*, *9*(8).

Shah, N., Gupta, Y. K., & Ghose, S. (2004). Zimmermann–Laband syndrome with bilateral developmental cataract–a new association?. *International Journal of Paediatric Dentistry*, 14(1), 78-85.

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., ... & Stewart, L. A. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *Bmj*, *349*, g7647.

Singer, S. L., Goldblatt, J., Hallam, L. A., & Winters, J. C. (1993). Hereditary gingival fibromatosis with a recessive mode of inheritance. Case reports. *Australian Dental Journal*, *38*(6), 427-432.

Stefanova, M., Atanassov, D., Krastev, T., Fuchs, S., & Kutsche, K. (2003). Zimmermann–Laband syndrome associated with a balanced reciprocal translocation t (3; 8)(p21. 2; q24. 3) in mother and daughter: Molecular cytogenetic characterization of the breakpoint regions. *American Journal of Medical Genetics Part A*, 117(3), 289-294.

Sunil, M. K., Trivedi, A., Arora, S., & Gupta, S. (2016). Werewolf syndrome associated with gingival fibromatosis: A rare case report. *Journal of Indian Academy of Oral Medicine and Radiology*, 28(2), 219.

Tagliabracci, V. S., Engel, J. L., Wen, J., Wiley, S. E., Worby, C. A., Kinch, L. N., ... & Dixon, J. E. (2012). Secreted kinase phosphorylates extracellular proteins that regulate biomineralization. *Science*, *336*(6085), 1150-1153.

Tang, Y. L., Shentu, X. C., Zhao, S. J., Tang, X. J., He, L., & Ping, F. Y. (2014). Ocular findings in syndromic gingival fibromatosis: a case study and electronic microscopic investigation of lens. *International Journal of Ophthalmology*, 7(3), 574.

Teoh, L., Moses, G., & McCullough, M. J. (2019). A review and guide to drug-associated oral adverse effects-dental, salivary and neurosensory reactions. Part 1. *Journal of Oral Pathology & Medicine*, 48(7), 626-636.

Tommiska, J., Känsäkoski, J., Skibsbye, L., Vaaralahti, K., Liu, X., Lodge, E. J., ... & Lahermo, P. (2017). Two missense mutations in KCNQ1 cause pituitary hormone deficiency and maternally inherited gingival fibromatosis. *Nature Communications*, 8(1), 1289.

Torres, L. H. S., de-Azevedo-Vaz, S. L., Barroso, D. R. C., Silva, D. N., Velloso, T. R. G., & de Barros, L. A. P. (2018). Enamel-Renal-Syndrome: case report. *Special Care in Dentistry*, 38(3), 172-175.

Urrego, D., Tomczak, A. P., Zahed, F., Stühmer, W., & Pardo, L. A. (2014). Potassium channels in cell cycle and cell proliferation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1638), 20130094.

Wang, S. K., Aref, P., Hu, Y., Milkovich, R. N., Simmer, J. P., El-Khateeb, M., ... & Hu, J. C. (2013). FAM20A mutations can cause enamel-renal syndrome (ERS). *PLoS Genetics*, 9(2), e1003302.

Winter, G. B., & Simpkiss, M. J. (1974). Hypertrichosis with hereditary gingival hyperplasia. *Archives of Disease in Childhood*, 49(5), 394-399.

Yalçin, S., Yalçin, F., Soydinç, M., Palandüz, S., & Günhan, Ö. (1999). Gingival fibromatosis combined with cherubism and psychomotor retardation: a rare syndrome. *Journal of Periodontology*, 70(2), 201-204.

Zhang, H., Zhu, Q., Cui, J., Wang, Y., Chen, M. J., Guo, X., ... & Xiao, J. (2018). Structure and evolution of the Fam20 kinases. *Nature communications*, *9*(1), 1218.

ARTIGO 2

Title: Characterization of gingival tissues and fibroblasts in patients with Enamel Renal syndrome and Raine syndrome.

**This article will be submitted to the Journal of Periodontology.*

INTRODUCTION

The *FAM20A* and *FAM20C* genes encode secreted proteins which comprise the "family with sequence similarity 20" (FAM20). The FAM20 family includes FAM20A, FAM20B and FAM20C proteins which have potential roles in regulating cellular functions, differentiation, and mineralization in several tissues (Nalbant et al, 2005; Ishikawa et al., 2012). The FAM20A protein is a pseudokinase that phosphorylates proteins involved in tooth enamel, in pulp ectopic calcifications, in root formation and eruption of teeth, and in the homeostasis of calcium and phosphate in the kidneys (Nalbant et al, 2005; Wang, S. K. et al., 2013, Cui et al., 2015). The FAM20C protein is a casein kinase located inside the Golgi complex that has a broad spectrum of phosphorylation including more than 100 phosphoproteins, such as FGF23 and the SIBLING family ("Small Integrin-Binding Ligand, N-linked Glycoprotein") (Fisher and Fedarko, 2003; Xiao et al., 2013; Tagliabracci et al., 2015; Tagliabracci et al., 2012). Although its most well-known and studied function is the control of biomineralization, its involvement in processes unrelated to mineral metabolism, such as healing, migration, cell adhesion and lipid homeostasis has been suggested (Tagliabracci et al., 2015). More recently, it was shown that the FAM20A protein functions as a pseudokinase in a protein complex with FAM20C, suggesting that FAM20A regulates the function and secretion of FAM20C into the extracellular environment (Cui et al, 2015; Ohyama et al. 2016).

In humans, pathogenic variants in *FAM20A* and *FAM20C* genes cause the Enamel Renal syndrome (ERS, OMIM #204690) and the Raine syndrome (RNS, OMIM #259775), respectively. These syndromes have similar oro-dental features that include ectopic

calcifications in connective tissue, dental abnormalities, and gingival fibromatosis (GF) (Dure-Molla et al., 2014 Faundes et al., 2014; Acevedo et al., 2015).

The ERS has been described as an autosomal recessive syndrome characterized by renal and oro-dental features. Initially, ERS was described as two distinct conditions: Amelogenesis Imperfecta and Neprocalcinosis, and Amelogenesis Imperfecta and Gingival Fibromatosis Syndrome (AIGFS, MIM#614253) (MacGibbon, 1972; Martelli-Júnior et al., 2008). More recently, molecular genetic studies identified *FAM20A* recessive pathogenic variants in both conditions suggesting a common etiology (O'Sullivan et al., 2011; Wang, X. et al, 2013; Jaureguiberry et al. 2013; Nitayavardhana et al., 2020). At present, both conditions are considered the same syndrome with variable expressivity. ERS patients have a distinctive oro-dental phenotype that includes generalized hypoplastic amelogenesis, pulp stones, delayed tooth eruption, hyperplastic dental follicles, and gingival fibromatosis with variable severity and calcified laminated nodules. Gingival fibromatosis is pathognomonic in ERS and histopathological analyses show a well-structured gingival epithelial layer with elongated papillae and fibrous connective tissue as well as the presence of myofibroblasts as revealed by α-SMA labelling (Paula et al., 2005; Martelli-Júnior et al., 2008; Dure-Molla et al., 2014; Costa et al., 2020).

The RNS is a rare bone dysplasia characterized by osteosclerosis and craniofacial anomalies with two different phenotypes, one lethal, in which there is perinatal death, probably due to respiratory failure consequent to thoracic malformation, and the other non-lethal, where adult individuals also present hypophosphatemic rickets and dental abnormalities (Raine et al., 1989; Fradin et al., 2011; Rafaelsen et al., 2013; Sheth et al., 2018). In 2015, Acevedo et al. reported two unrelated Brazilian non-lethal RNS families with recessive pathogenic variants in *FAM20C*. In addition to alterations in bone metabolism, the affected patients also presented with hypoplastic AI and dentine abnormalities with a variable degree of severity as well as gingival overgrowth. Histopathological analysis of patients' gingiva revealed inflammatory infiltrate, epithelial acanthosis, and fibrous connective tissue suggesting that GF behavior is similar to that described in ERS. However, histochemical and immunohistochemical analysis of the fibrous and cellular compartments were not performed.

In order to further characterize gingival fibromatosis in ERS and RNS patients, the aim of the present study was to analyze morphologically the gingival tissue of ERS and RNS

patients as well as to establish and characterize the biological properties of primary human gingival cell cultures obtained from affected patients.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Faculty of Medicine of the University of Brasília (CEP/FM-UnB). All tissues used in this study were obtained from teeth extractions with clinical indication and donated by patients after informed consent was obtained from all participants or responsible when under 18 years old..

The individuals participating in the study were five healthy individuals as a control group between 18 and 24 years old; one 15-years-old female individual as ERS that we will describe a new case carrying a homozygous mutation (ERS; c.1112G>A; p.Trp371*); and two (10 and 26-years-old) male individuals as RNS (RNS1, c.1487C>T, p.Pro496Leu; and RNS2, c.784+5G>C, p.Trp202Cys*37), these being from unrelated families as described in Acevedo et al (2015). The patients were seen at the Clinic of Patients with Dental Anomalies, in the Dentistry Division of the University Hospital of Brasília, Brazil.

Histopathological analyses:

The gingival fragments collected for therapeutic reasons were fixed in paraformaldehyde (4%) for 48 hours. After rising in PBS for 24 hours, the fragments were dehydrated in increasing concentrations of ethanol and cleared in toluene, and finally embedded in Paraplast®. Serial sections (7 μm) were cut with a microtome (RM2125RT, Leica, Germany), deparaffinized, rehydrated, and stained with Hematoxylin and Eosin, Masson's Trichrome, PicroSirius Red, Golder's Trichrome, Alizarin Red and Verhoeff, and observed under an Axiophot light microscope (Zeiss, Germany). The stained sections were photographed with a digital camera (Zeiss ERC 5s, Germany), with the ZEN Blue Edition software (Zeiss, Germany). The sections stained with PicroSirius Red were photographed using a microscope with a photodocumentation system and polarized light (DM200, Leica, Germany).

Immunohistochemistry

Immunohistochemistry was performed on 3 μm tissue sections using the avidin-biotin-peroxidase complex method. In essence, sections were deparaffinized and dehydrated using a graded series of ethanol. Sections were then subjected to antigen retrieval with 0.01 M citrate buffer pH 6.0 and incubation with 3% aqueous hydrogen peroxide for 15 min to quench endogenous peroxidase. The sections were labelled with the primary goat polyclonal anti-FAM20A (1/200, D-17, Santa Cruz Biotechnology, CA, USA), rabbit polyclonal anti-FAM20C (1/200, DMP4, Aviva Systems Biology, CA, USA), and mouse monoclonal anti-Alpha Smooth Muscle Actin (1/200, α-SMA, Abcam, Cambridge, UK) antibodies. Sections were then blocked overnight at 4ºC with ready-to-use (2.5%) normal horse blocking (ImmPRESS reagent kit, Vector Laboratories, Burlingame, CA, USA). After washing in PBS, immuno cross-reactivity was visualized using peroxidase substrate (Novared, Vector Laboratories, Burlingame, CA, USA) and counterstained with Harris' hematoxylin.

For the immunofluorescent staining, the method was performed according to Escorcia et al (2020) and the sections were labelled with rabbit polyclonal anti-cytokeratin 14 antibody (1/100, CK14, Abcam, Cambridge, UK) as primary antibody and incubated with fluorescein secondary antibody Alexa Fluor 594 (Life Technologies, Carlsbad, CA, USA). The nuclei were labeled with diamidino-phenyl-indole (DAPI, Invitrogen, Carlsbad, CA, USA) for 5 minutes and the coverslips were mounted onto microscope slides using Fluoromount-G® (SouthernBiotech, USA). The sections were analyzed using a Axio Imager 2 (Zeiss, Germany) and processed using ZEN (Zeiss) and ImageJ softwares (LOCI, Madison, USA).

Establishment of primary culture of hGFCs:

Cell cultures was established by the explant outgrowth method according to Amorim et al., 2019. Cells at passage 3 to 5 were used for experiments and maintained in high-glucose Dulbecco modified Eagle medium (DMEM; Sigma-Aldrich, St Louis, MO, USA) containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 50 U/mL penicillin, and 50 μg/mL streptomycin (Sigma-Aldrich, St Louis, MO, USA).

Cell morphology by nuclear diameter analysis

The nuclei were previously stained with DAPI (Invitrogen, Carlsbad, CA, USA) and the images were obtained by fluorescence. The particle analysis was performed using the ImageJ software (LOCI, Madison, USA), calculating the cell nuclei diameter present in each image.

Cell metabolic activity Assay

hGFCs **(**$5x10^3$ cell/well**)** were seeded in 96-well plates with the DMEM with 10% FBS. After 24 hours, cell metabolic activity was assessed by adding 0.5 mg/mL MTT solution (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (Sigma-Aldrich, St. Louis, MO, USA). The formazan crystals produced were solubilized in 100 µL of acidified isopropanol, and optical density was measured at a wavelength of 570 nm.

Wound healing (Scratch) assay

Cells ($1x10^6$/well) were seeded to 6-well plates. These were further cultured until a monolayer was formed. Then, a wound was produced on the formed monolayer by scratching the monolayer using the tip of a sterile 200 µL pipette and further washed with PBS. The images were captured at 0, 12, and 24 hr. The migrated cells were imaged by an inverted light microscope (Zeiss Primo Vert, Germany) at a magnification of 10x. The distance between both the edges of scratched monolayers was determined and the percentage (%) wound closure was calculated.

Immunofluorescence in cell culture

Cells seeded (1 x 10^6/well) on Nunc™ Lab-Tek™ II Chamber Slide™ System slides (ThermoFisher Scientific™, Waltham, MA, USA) were rinsed with PBS, fixed with PBS plus 4% PFA and 5% sucrose, and permeabilized with 0.4% Triton in PBS for 10 min. The cells were incubated overnight (4°C with humidity) with mouse anti-Golgin-97 (1/200, CDF-4, ThermoFisher Scientific™, Waltham, MA, USA), rabbit anti-ERK 1/2 (1/200, p44/42 MAPK,

Cell Signalling Technology, MA, USA), rabbit anti-p-ERK 1/2 (1/200, phospho-p44/42 MAPK, Cell Signalling Technology, MA, USA), goat anti-FAM20A (1/200, D-17, Santa Cruz Biotechnology, CA, USA), mouse anti-FAM20C (1/200, DMP4, Aviva Systems Biology, CA, USA), and mouse anti-Smooth Muscle Actin (1/200, α-SMA, Sigma-Aldrich, St Louis, MO, USA). The secondary antibodies used were Alexa Fluor® 488 and Alexa Fluor® 594 (1/500, Life Technologies, Carlsbad, CA, USA). The nuclei were labeled with diamidino-phenyl-indole (DAPI, Invitrogen, Carlsbad, CA, USA) for 5 minutes and the coverslips were mounted onto microscope slides using Fluoromount-G® (SouthernBiotech, USA). Microphotographs were performed using the confocal microscopy (Zeiss LSM8, Germany) and processed using ZEN (Zeiss, Germany) and ImageJ softwares (LOCI, Madison, USA).

Quantitative Real Time Polymerase Chain Reaction

Total RNA was isolated using TRI Reagent (Sigma-Aldrich, St Louis, MO, USA), followed by DNase I (Sigma-Aldrich, St Louis, MO, USA), and cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA). Quantitative real-time polymerase chain reaction (qPCR) was performed in triplicate in 10 µL reactions by using PowerUp SYBR®Green Master Mix (Applied Biosystems, CA, USA). Relative quantification was calculated using the $2^{-\Delta Ct}$ method (Livak et al, 2001). Genes analyzed were *FAM20A* and *FAM20C*. Beta-actin was used as a housekeeping gene. The primer sequences are listed in Supplementary Table 1 and the validation of the primers can be seen in Supplementary Figure 1.

Mineralization assay

Cells were seeded ($1x10^5$ /well) in a 6-well plate and maintained for 7, 14 and 21 days in proliferative medium (PM; DMEM 10% SFB), mineralizing medium (MM; α-MEM 10% SFB, 10mM β-glycerophosphate and 50 µg/mL ascorbic acid) and osteogenic medium (OM; α-MEM 10% SFB, 10mM β-glycerophosphate, 50 µg/mL ascorbic acid and 10 nM of dexamethasone). The Alizarin Red was used to visualize the calcified structures. Finally, the cells were washed with PBS, fixed 20 min with 10% formalin in PBS, and incubated with 2% Alizarin S Red

(Sigma-Aldrich, St Louis, MO, USA), pH 4.2, for 1h at RT. Afterward, the wells were washed with distilled water, dried and the images were observed under a light inverted microscope.

Statistical analyses

All experiments were performed in triplicate. Data are presented as the mean standard error of the mean (M SEM). All statistical analyzes were performed on Prism 8 statistical software (GraphPad Software Inc, San Diego, CA, USA). Statistical differences were determined by using the Student-Newman-Keuls multiple comparison test after one-way analysis of variance (viability and proliferation assays), one-way analysis of variance (nuclear diameter area), or a one-way analysis of variance and Dunnett's Multiple Comparison postTest (gene expression assay). $p<0.05$ was accepted as statistically significant.

RESULTS

Histopathological analysis confirmed fibromatosis gingival and ectopic calcifications in ERS and RNS patients.

To better characterize GF in patients with ERS and RNS in this study, histopathological analysis of the gingival tissues was performed, showing differences in the organization of the epithelium and connective tissue, when compared to gingiva of control patients. The ERS and RNS gingiva had stratified epithelium with an increase in the spinous layer suggestive of epithelial acanthosis (Figure 1, B to D). Extensions of the epithelial ridges that invading the subjacent lamina propria connective tissue in the RNS2 gingival tissues were observed, creating at the base a structure of concentric epithelial cells with the formation structure suggesting a cornea pearl (Figure 1, E and F). The immunofluorescence labelling with anti-CK14 showed the presence of CK-14 can be seen in the epithelium both in the control and RNS2 tissues (Figure 1, G and H).

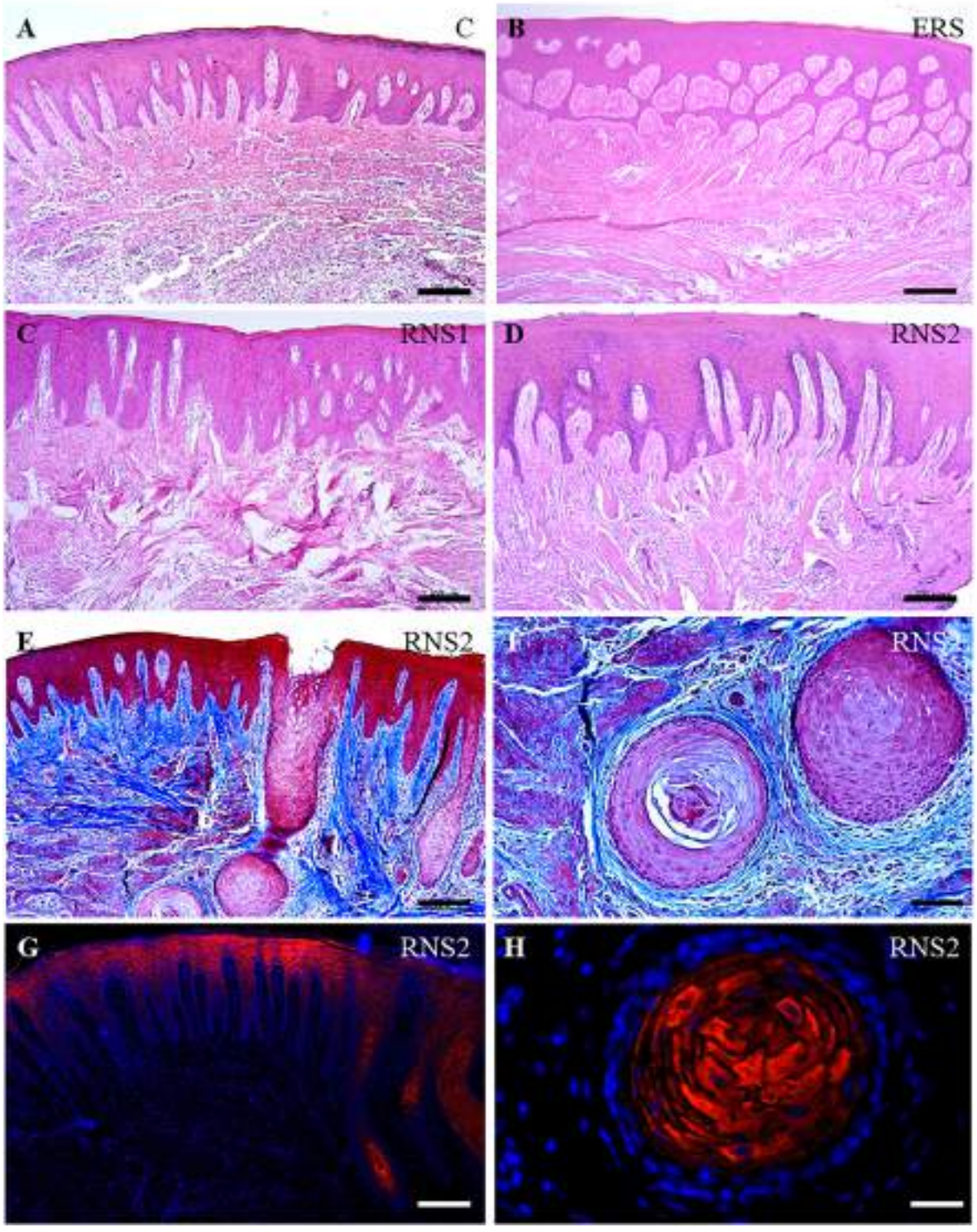


Figure 1. Gingival fibromatosis in patients with ERS and RNS. Representative histological characteristics of normal gingiva (A), and with gingival fibromatosis in ERS (B), RNS1 (C) and RNS2 (D) patients. Presence of epithelial extensions invading the gingival tissue of patient RNS2 (E). A spherical structure is observed with concentric cells forming an accumulation of keratin inside (F). Hematoxylin & Eosin stain (A, B, C, D); Masson's trichrome stain (E, F); Immunofluorescence for detection of CK-14 (G, H). Scale bar: 200 μm (A-E, G), 100 μm (F), 50 μm (H).

When stained with Masson's Trichrome a greater amount of collagen fibers in the connective tissue of ERS RNS compared to control (Figure 2, A to D) was observed. Finally, the sections stained with Picrosirius Red confirmed fibrosis of the connective tissue in patients with RNS and ERS. It was possible to observe thicker and reddish-orange fibers, suggestive of type I collagen fibers (Figure 2, E to H). The results confirmed the presence of GF in both patients with ERS and RNS. Additionally, Verhoff staining revealed the absence of elastic fibers in the gingival tissues (data not shown).

In addition, ectopic calcifications in the connective tissue of patients with ERS and RNS was confirmed. Goldner's trichrome showed the presence of mineralized nodules evidenced by the turquoise color. The mineral component was confirmed by staining with Alizarin Red, in which the calcifications are intensely stained red (Figure 2, I to L). These calcified structures were observed in deeper areas of the lamina propria of the RNS specimens, while in the ERS gingiva the distribution occurred both in superficial and deeper regions of the lamina propria.

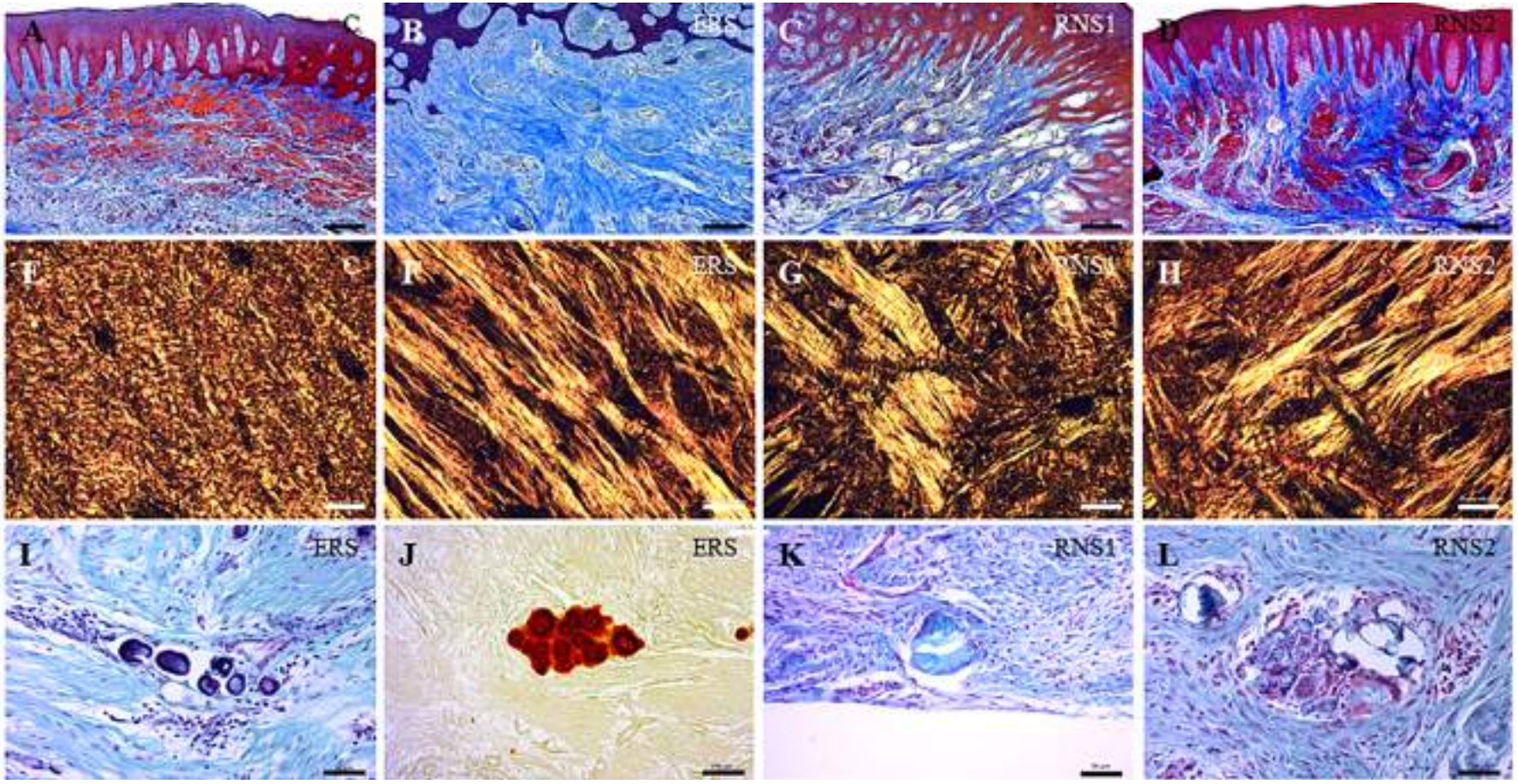


Figure 2. Increase in collagen fibers and presence of ectopic gingival calcifications of ERS and RNS patients. Increased thickness of collagen fiber bundles in ERS and RNS patients. Unlike healthy gingiva (A, E), ERS samples (B, F) shows collagen fibers in a more orderly manner, while the RNS sample (RNS1, C, G; and RNS2, D, H) the fibers appear in several directions. Masson's Trichrome stain (A - D) and Picro Sirius Red (E - L). Goldner's Trichrome (I, K, L) and Alizarin Red (J) stains showed the presence of ectopic calcifications in gingival tissue of ERS (I, J), RNS1 (K) and RNS2 (L). Scale bar: 200 μm (A-D), 50 μm (E-L).

FAM20A and FAM20C are expressed in gingival tissue of ERS and RNS

Then, we investigated the expression of the FAM20A and FAM20C proteins in the gingival tissue of control patients, ERS, and RNS. We evaluated the FAM20A and FAM20C to see whether proteins could be present in healthy gingival tissues and with GF. The labeling was observed in epithelial tissue, blood vessels (Figure 3), and fibroblasts in the gingival tissues, both in the control tissue and in the ERS and RNS tissues (Figure 4).

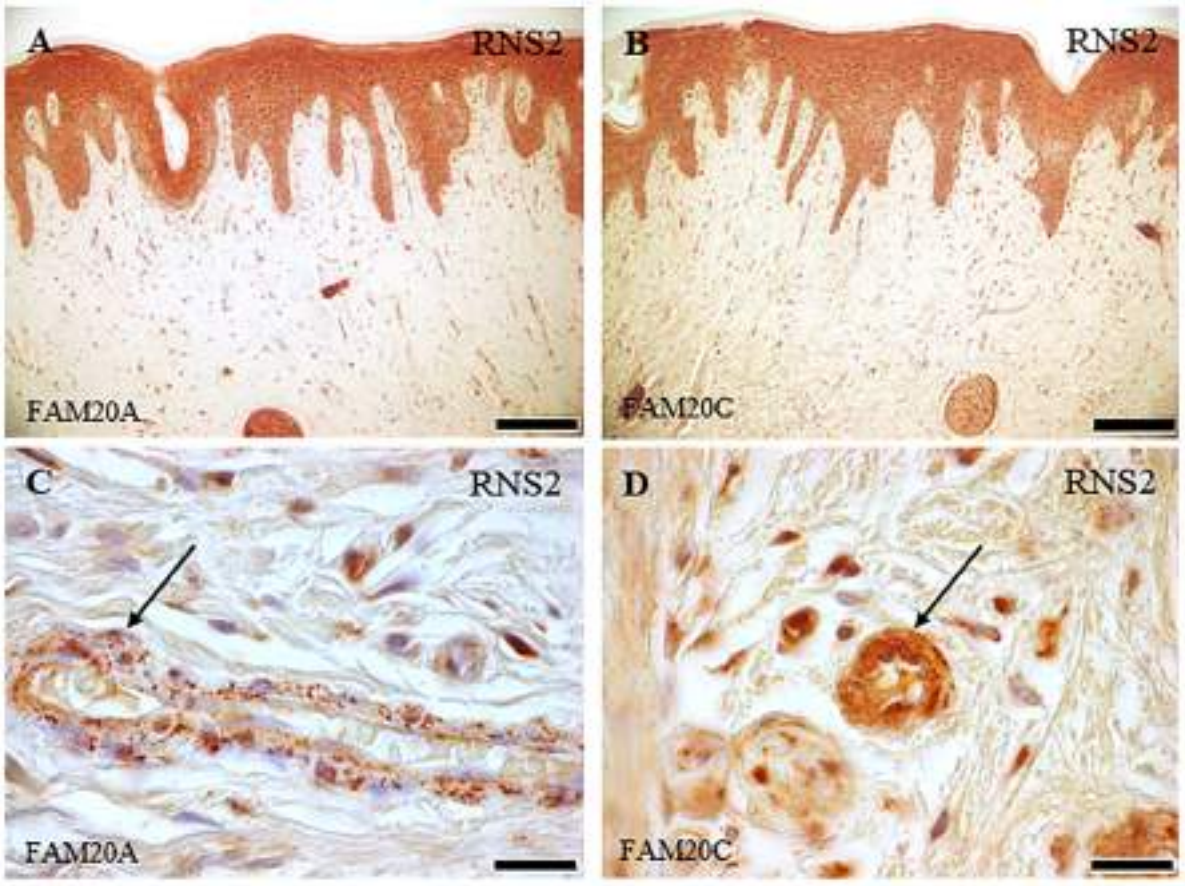


Figure 3. FAM20A and FAM20C expression in epithelial and vascular tissue of RNS2 patient. The expressions of FAM20A and FAM20C can be seen in epithelial tissue (A, B) and in vascular epithelial cells (C, D). Scale bar: 200 μm (A, B), 25 μm (C-D).

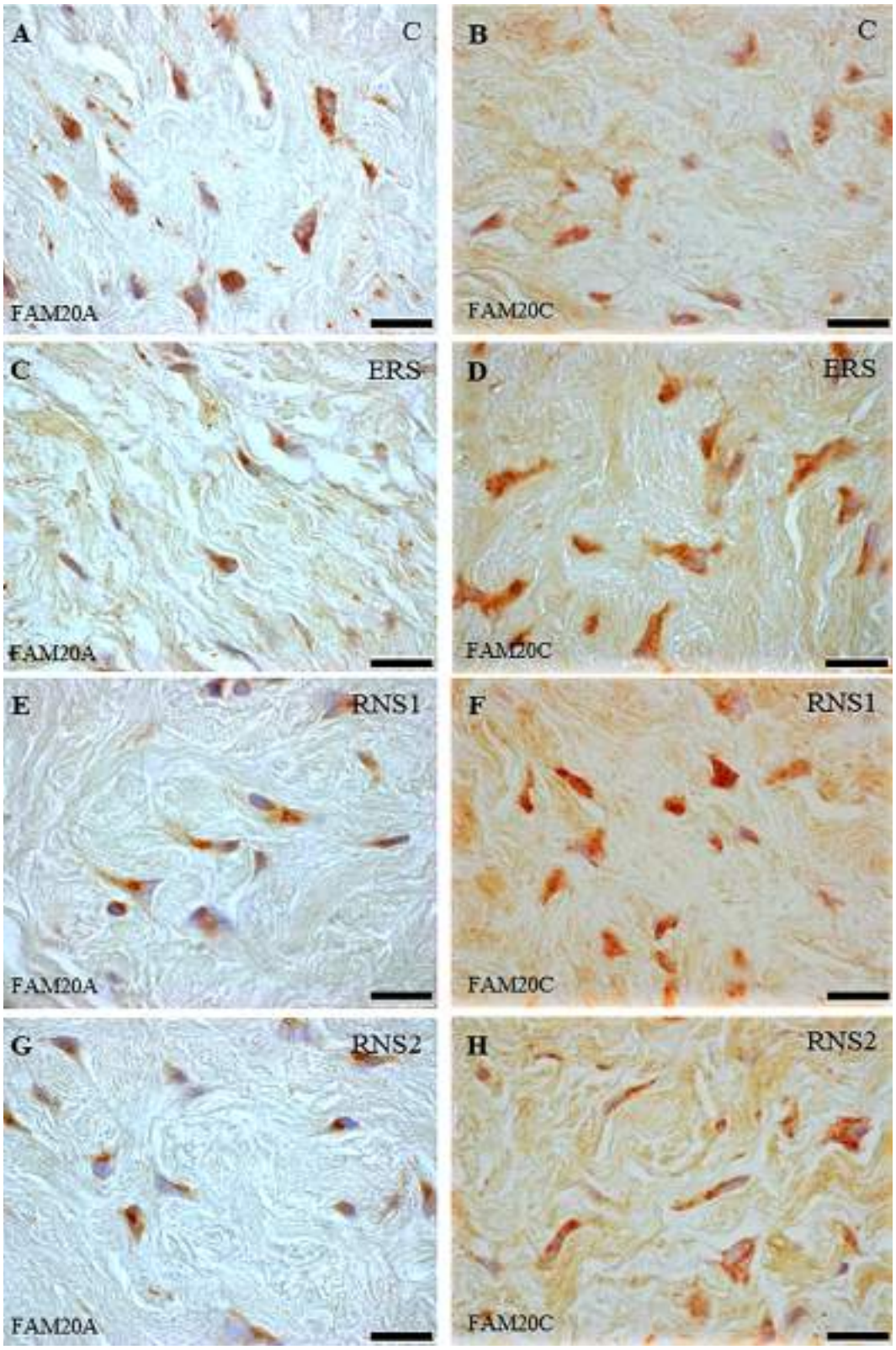


Figure 4. Fibroblasts from the lamina propria of the control, ERS and RNS express FAM20A and FAM20C. FAM20A and FAM20C expression can be seen with more intense immunostaining in the control fibroblasts (A, B), ERS (C, D), RNS1 (E, F), and RNS2 (G, H). Scale bar: 25 μm.

ERS and RNS gingival tissue expresses α-SMA

To observe the possiblepresence of myofibroblasts involved in GF, immunohistochemistry was performed to evaluate the expression of α-SMA. The results showed no labeling on fibroblasts in the lamina propria of control specimens, showing only labeling of blood vessels. The expression of α-SMA in fibroblasts is suggested in gingival tissues with GF from ERS and RNS, mainly in RNS1 (Figure 5).

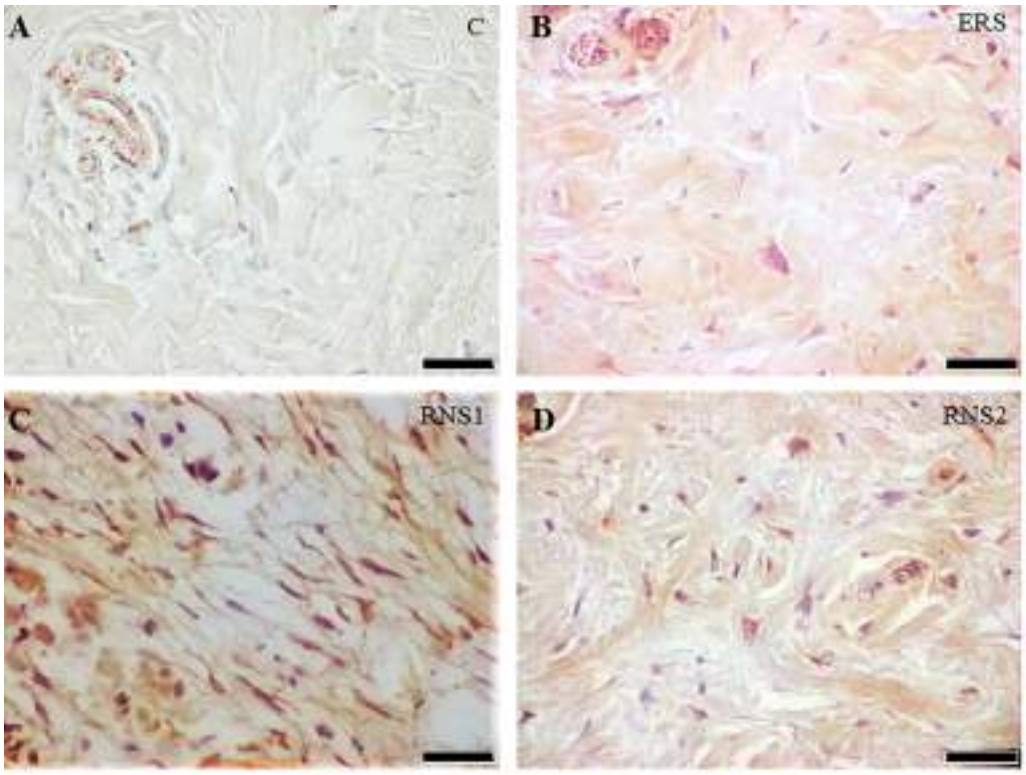


Figure 5. Gingival fibromatosis of ERS and RNS express α-SMA. Immunoreactivity of α-SMA can be observed in the control group only in the vascular endothelium (A), while in the gingival lamina propria of patients ERS (B), RNS1 (C), and RNS2 (D). Scale bar: 50 μm.

Establishment and characterization of primary culture of hGFCs

The primary culture of hGFCs has been successfully established for five healthy patients (controls), one patient with ERS, and two RNS patients (RNS1 and RNS2). We found that the cell morphology showed an elongated or starry phenotype, with long cytoplasmic extensions, with no relevant changes between groups, typical characteristics of fibroblast cultures *in vitro* (Figure 6A, 6E and 6I).

hGFCs RNS have increase cell size

We hypothesized the difference in size would be possible between the cultured cells of control patients and patients with RNS. Thus, we found that RNS hGFCs show an increase in size compared to control cells, with a statistically significant result ($p<0.0001$). The control cell nuclei had an average area of 5.84 μm^2, and the cell nuclei of the RNS hGFCs showed areas of 7.03 μm^2 (RNS1) and 7.37 μm^2 (RNS2) (Figure 6M).

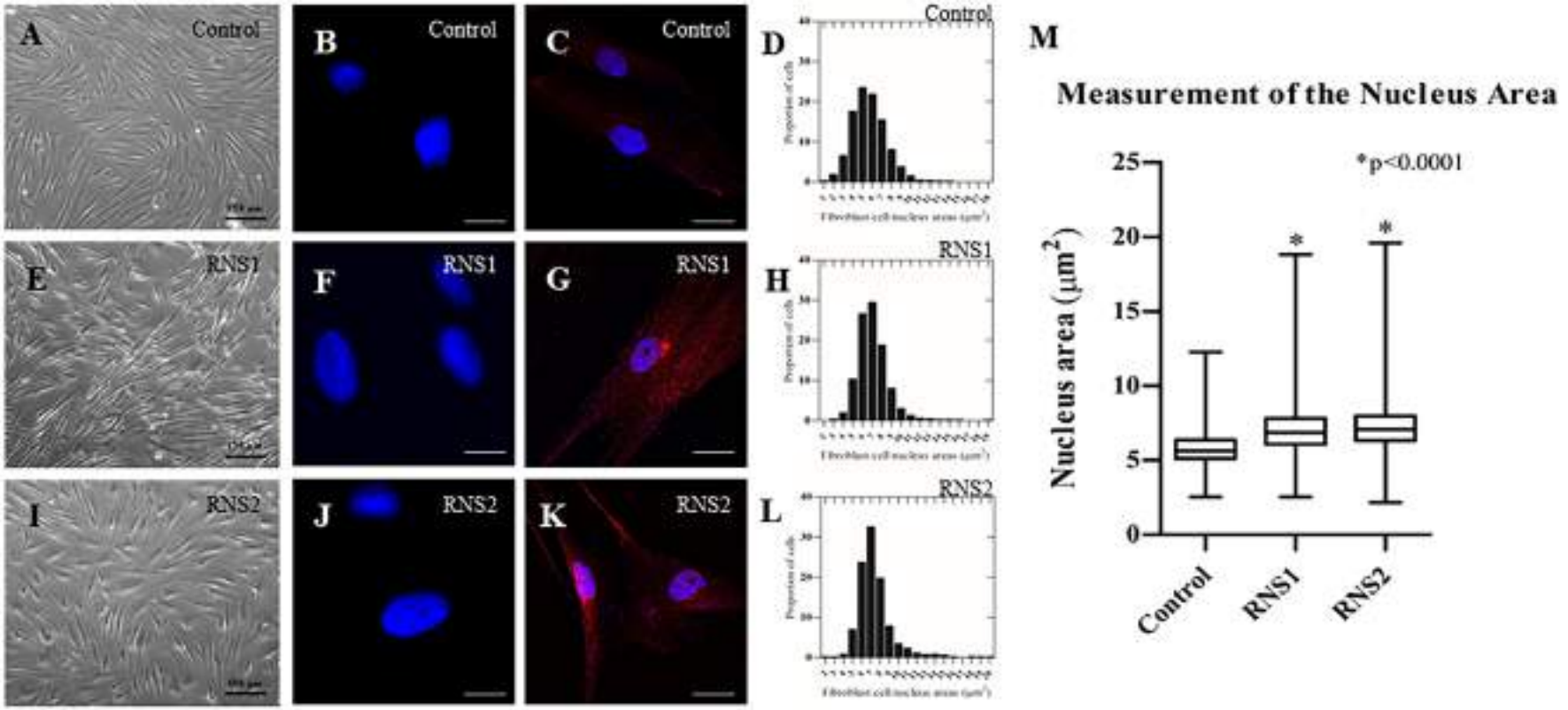


Figure 6. Morphological difference and measurement of the hGFCs nucleus area of RNS compared to control. Primary cell culture of hGFCs from control (A), RNS1 (E) and RNS2 (I) without evidence of morphological difference. Cell nuclei stained with (DAPI) from hGFCs control (B), RNS1 (F) and RNS2 (J). α-SMA labeling hGFCs control (C), RNS1 (G), and RNS2 (K). The proportion of RNS1 (H) and RNS2 (L) fibroblasts displaced to the right of the graph compared to those of the control group (D) shows a greater number of nuclei with a larger diameter. The cell nuclei of the control had an area of 5.84 μm^2 and the cell nuclei of RNS had areas of 7.03 μm^2 (RNS1) and 7.37 μm^2 (RNS2). The cell nucleus areas of RNS are 1.19 μm^2 and 1.53 μm^2 larger, respectively, when compared to the nuclei of the control (M). Statistically significant result (* $p < 0.0001$) by the one-way ANOVA test. Scale bar: 154 μm (A, E, I), 20 μm (B, C, F, G, J, K).

Metabolic activity and migration profile of ERS and RNS hGFCs

The ERS hGFCs showed a reduction in cell metabolic activity, but without a statistical difference in any of the evaluated periods (24 and 48 hours) when compared to the control. The behavior of the RNS1 hGFCs showed an increase in metabolic activity after 24 hours, but showed a considerable decrease after 48 hours, with a statistical difference in the second time when compared to the control group (Figure 7A).

In addition, ERS and RNS hGFCs showed a decreasing trend of migration when compared to control. In the evaluated periods (12 and 24 hours) the ERS hGFCs showed less proliferation capacity when compared to the control, and the RNS1 and RNS2 hGFCs showed greater proliferation difficulties than the ERS hGFCs (Figure 7B and Figure 7C).

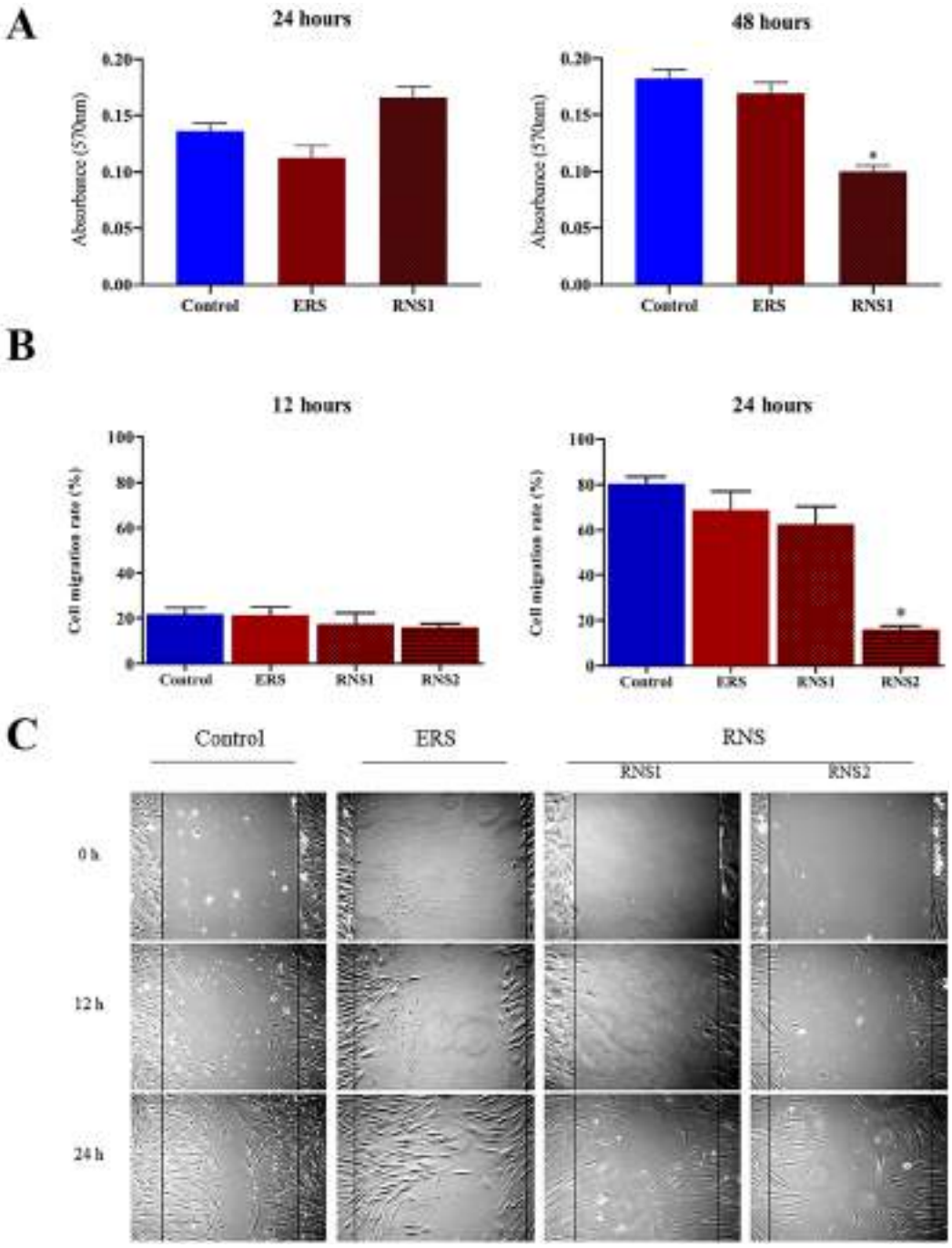


Figure 7. ERS and RNS hGFCs show alterations in mitochondrial activity and cell proliferation. Evaluation of mitochondrial activity of gingival fibroblasts in ERS and RNS1 after 24 and 48 hours and compared with control cells (A). In 24 hours, the RNS1 gingival fibroblasts showed greater cell viability while the ERS decreased this capacity compared to the control. After 48 hours, syndromic fibroblasts showed a reduction in viability. Note the statistical difference in the decrease in cell viability of fibroblasts from an RNS1 after 48 hours (* $p < 0.05$) (A). Migration capacity of gingival fibroblasts from ERS, RNS1, and RNS2 (B, C). The data at 12 and 24 hours demonstrated that the cell migration capacity is reduced for the fibroblasts ERS, RNS1 and RNS2 (B, C). Significant results can be seen for the RNS2 when compared to the control (B) (* $p < 0.05$).

ERS and RNS hGFCs express FAM20A, FAM20C and α-SMA

The hGFC gene expression revealed a significant reduction in *FAM20A* expression in ERS and RNS1 cells ($p < 0.05$) (Figure 8c). In addition, the *FAM20C* gene expression in ERS and RNS1 hGFCs was shown, with no statistical difference (Figure 8d).

In addition, hGFCs shown FAM20A, FAM20C and α-SMA expression in the cell cytoplasm. In addition, it was observed that the localization of FAM20C expression in RNS hGFCs accumulated on the intracellular periphery of hGFCs (Figure 8a, G and H). The interaction between the two proteins was not possible to assess by intracellular co-localization using immunofluorescence in the hGFCs of this study, both controls, and syndromes (Figure 8a, I to L). However, the immunofluorescence perinuclear co-localization observed with Golgin-97 suggested that FAM20A is located in the region corresponding to the Golgi apparatus (Figure 8b, I to L).

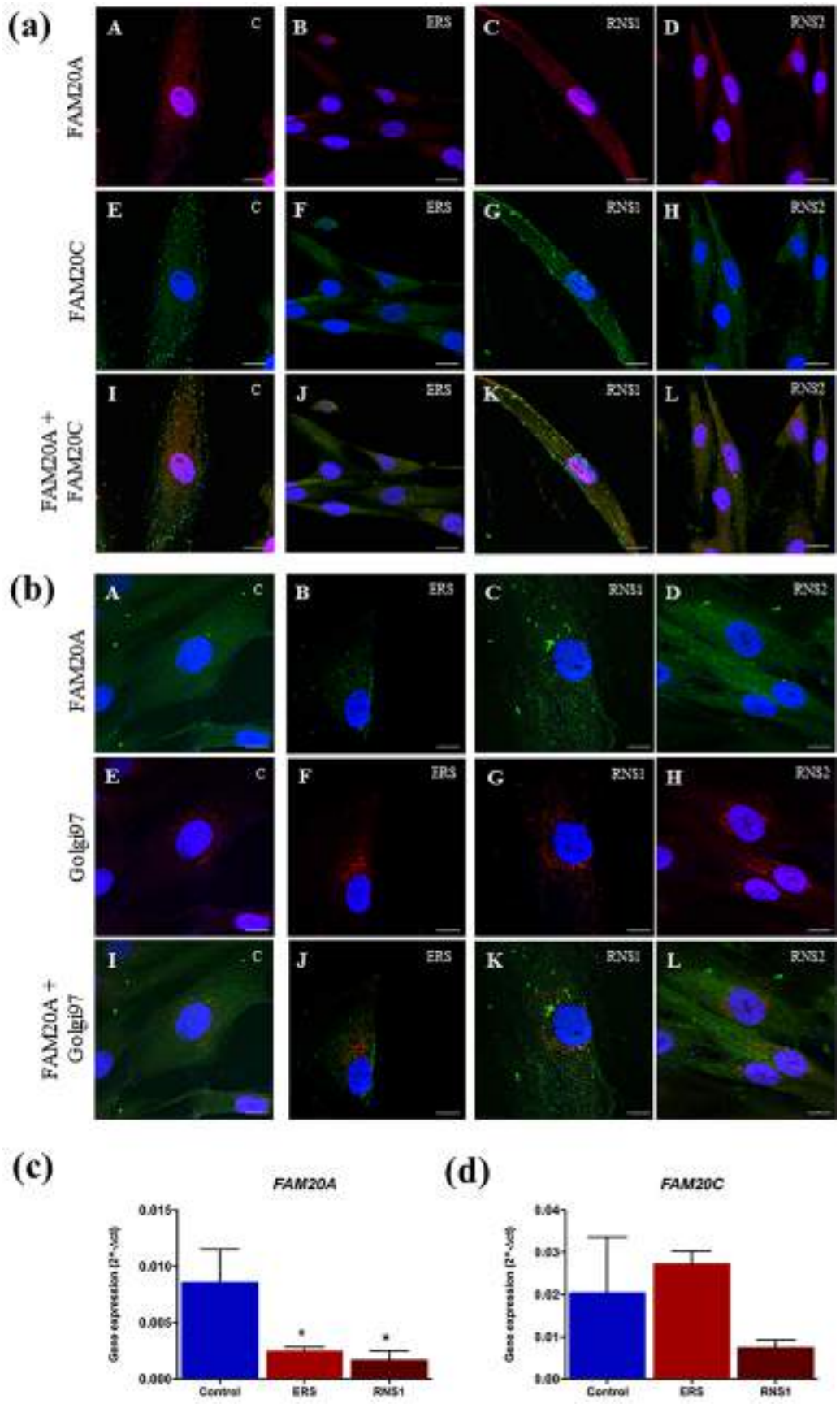


Figure 8. FAM20A and FAM20C are expressed in hGFCs. (a) FAM20A can be seen in control (A), ERS (B), RNS1 (C), and RNS2 (D) hGFCs. FAM20C can be seen in control (E), ERS (F), RNS1 (G),

and RNS2 (H) hGFCs. It was not possible to observe the colocalization of FAM20A and FAM20C in the evaluated cells (I-L). **(b)** The Golgi97 protein residing in the Golgi complex is shown (E-H). The colocalization of FAM20A in the Golgi can be seen by the orange staining in the control (I), ERS (J), RNS1 (K), and RNS2 (L) hGFCs. **(c)** The *FAM20A* gene is under expressed in ERS and RNS1, when compared to the control (* $p < 0.05$). **(d)** The *FAM20C* gene showed a tendency to overexpression in ERS and under-expression in RNS1 when compared to control. Scale bar: 20 μm.

ERS and RNS hGFCs show decreased expression of ERK 1/2 and p-ERK 1/2

Considering that ERK 1/2 phosphorylation has been reported in various cell types as an important contributor to cell proliferation, migration, and extracellular matrix remodelling we investigated the of ERK 1/2 AND P-ERK 1/2 expression. ERK 1/2 labelling in the cytoplasm of control hGFCs and reduced expression in ERS and RNS fibroblasts was observed. Control cells expressed p-ERK 1/2 in the cytoplasm, with the usual location distributed in the proximity of the cell nucleus, while ERS and RNS hGFCs shown reduction of p-ERK 1/2 expression (Figure 9).

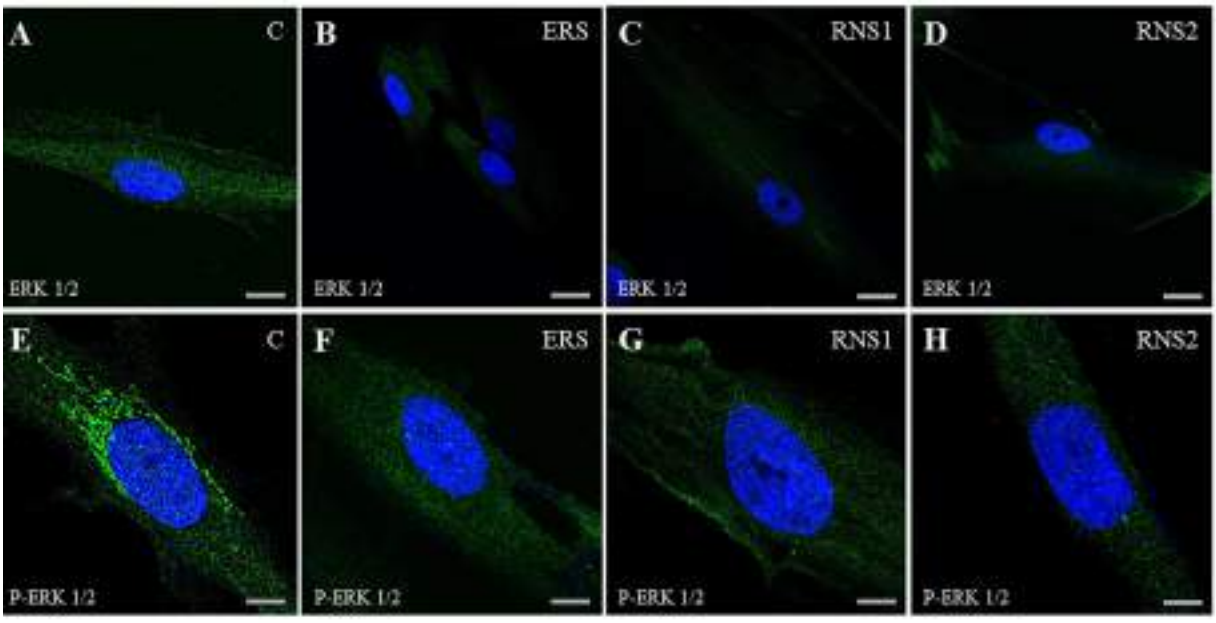


Figure 9. ERS and RNS hGFCs show reduced expression of ERK 1/2 and p-ERK 1/2 when compared to control. ERK can be suggested by the slight expression in the control (A), and slightly reduced in the hGFCs of ERS (B), RNS1 (C) and RNS2 (D). P-ERK can be seen expressed in control (E), but inhibited in ERS (F), RNS1 (G), and RNS2 (H) hGFCs. Scale bar: 20 μm (A-D), 10 μm (E-F).

hGFCs of ERS and RNS can produce mineral nodules when stimulated.

The mineralization assay showed that ERS and RNS hGFCs can form mineral nodules when stimulated. At 7 days of induction, the beginning of the formation of mineralized nodules was shown, different from the control group that maintained without these characteristics (Figure 10). The primary culture of RNS had the capacity to form mineral nodules, but not as much as ERS, which in 7 days already showed a greater amount of mineral formation. In 21 days, considerable mineral deposition occurred, and it was not possible to distinguish between the culture of ERS and RNS fibroblasts. We also showed that, regardless of the use of dexamethasone in the inducing medium, hGFCs were able to produce calcifications.

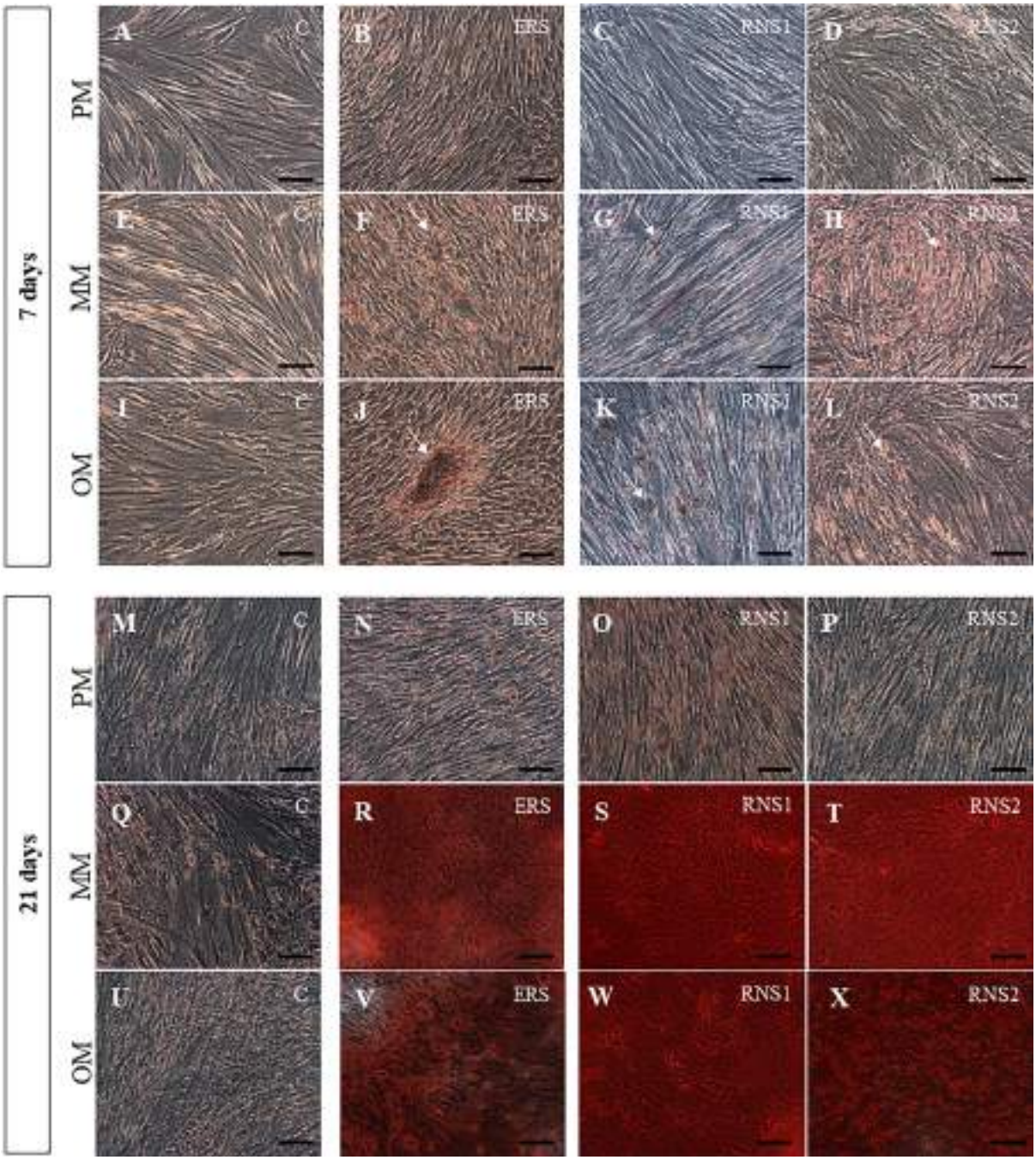


Figure 10. hGFCs from ERS and RNS show potential for mineralization when stimulated. The formation of mineral nodules begins at seven days of stimulation (white arrows) using both mineralizing (MM) and osteogenic (OM) media when compared to the proliferative media (PM). At 21 days, it is possible to observe the intense red color indicating an increased deposition of mineralized nodules. Scale bar: 150 μm.

DISCUSSION

This study characterized the gingival tissues of patients with ERS and RNS, the first time being described the establishment of primary cell culture and the cell morphological spectra of RNS hGFCs.

We confirmed that the ERS patient showed GF according to histopathological analysis, indicated as a pathognomonic factor of this disease, as suggested by Dure-Molla et al. (2014). Currently, the GF is considered to belong to both the variable expressiveness of ERS and AIGFS, diseases caused by sequence variations in the *FAM20A* gene. The two RNS families also presented GF and their oral findings are similar to those of ERS (Acevedo et al., 2015). Thus, this similarity between these can be indicated because the diseases showed changes in FAM20A and FAM20C, proteins that have a dimeric relationship and affect the phosphorylation of other target proteins in the biomineralization process (Cui et al., 2015; Cui et al., 2017). Therefore, pathogenic variants affect the enamel development, inducing GF and ectopic calcifications in the gingival tissue.

Also, in gingival tissue, epithelial changes were observed mainly in the patient RNS2. In his histopathological analysis, numerous epithelial invasions were observed, and the same can be seen more discreetly in the tissue analysis of the other two affected patients in his family (data not shown in this study). However, little is known about epithelial changes in GF and keratinocytes. Studies have shown that drug-induced gingival overgrowth shows these same aspects assessed in our studies, showing epithelial extensions that extend deeply to the connective tissue and collagen accumulation in the lamina propria (Dill and Iacopino, 1997; Castro et al., 2010). Still, a recent study suggests the participation of SPOCK1, TGF- ß1, and MMP-9 in gingival fibrosis and the epithelial to mesenchymal transition (Alshargabi et al., 2020). The role of FAM20A, in turn, can be suggested as an important factor in epithelial proliferation (Li et al., 2016; Wang, S. K. et al., 2019; Simancas Escorcia et al., 2020), however, it is still necessary that studies of these mechanisms be evaluated in the gingiva of syndromic patients.

In this study, the gingival tissues from patients with ERS and RNS showed immunostaining for α-SMA, mainly in the gingiva of RNS1 patient. Myofibroblasts have been described in pathological tissues related to hypertrophic scars, fibromatoses, fibrocontractive diseases and in epithelial tumor reactions (Desmoulière et al., 2003; Desmoulière et al., 2005;

Smith et al, 2019). In gingival tissue, the presence of this cell type has been reported in medication-induced GF (Chung et al., 2015; Arora et al., 2016), in hereditary GF (Bitu et al., 2006; Coletta and Graner, 2006) and in GF related to the syndrome (Martelli-Júnior et al., 2008). Further investigation to validate the present results and to verify the presence of myofibroblasts in gingiva of RNS patients are necessary.

Our study also successfully established primary culture of human ERS and RNS cells. This obtaining of cells was important to understand morphological aspects involved with the gingival fibroblasts of syndromic patients. Our results showed that the cells of RNS patients have changes in cell size. This is due to the fact that these cultured cells are likely to be myofibroblasts or perhaps they may also be senescent cells, either by reaching the maximum number of cell divisions or induced by oxidative or inflammatory stress (Dierick et al., 2002). Our results suggest the senescence characteristics, such as proliferative interruption, alteration of expression and secretion of proteins, and the increase in cell size (Ren et al., 2009; Rodier et al., 2011). In addition, a reduction in mitochondrial metabolic activity and in the cellular migration capacity of RNS hGFCs was observed, agreeing with other reports that suggested the involvement of FAM20C in the processes of cell adhesion and migration (Tagliabracci et al., 2015; Liu et al., 2018).

Still with cell culture, it was possible to verify the expression of the FAM20A and FAM20C genes in hGFCs, both from control fibroblasts and ERS and RNS cells. Also, to verify whether hGFCs expressed proteins FAM20A and FAM20C, immunocytochemistry was performed, and the results showed that hGFCs show cytoplasmic expression of these proteins. In addition, we sought to verify the possible interaction between FAM20A and FAM20C as already reported in some studies (Cui et al., 2015; Cui et al., 2017). However, it was not possible to visualize colocalization by the technique used in our study. The interactions between proteins within the cell can depend on the cell type itself, be short-lived and establish very quickly, which should coincide between the moment of cell fixation and the interaction between these proteins for possible visualization (Cui et al., 2015; Ohyama et al., 2016; Cui et al., 2017). Although we have not achieved this demonstration, studies indicate that FAM20A can potentiate FAM20C activity by controlling its location and extracellular phosphorylation in the secretory pathway, acting dimerically (Cui et al., 2015; Ohyama et al., 2016; Cui et al., 2017; Zhang et al., 2018).

However, in the primary culture of hGFCs from ERS and RNS patients, we were able to observe the co-location between FAM20A and the Golgi apparatus, as also proposed in other studies (Tagliabracci et al., 2012; Cui et al., 2015). It was demonstrated the possibility of Fam20a to associate with the Golgi apparatus and retain Fam20c in this organelle, regulating phosphorylation of the secretory pathway (Tagliabracci et al., 2012). Still, the studies of Cui et al. (2015) demonstrate that both Fam20a and Fam20c are located on the Golgi apparatus, but suggest that Fam20a does not serve as a primary mechanism for redistributing Fam20c activity subcellular and as retention for the Fam20a/Fam20c complex. The present study did not verify the location of FAM20C in the Golgi apparatus because of the lack of available antibodies of different animal species to perform the experiment, however we suggest a further investigation of a more precise cellular localization of hGFCs in ERS and RNS patients.

In addition, this study showed the difference in expression of ERK and p-ERK between control hGFCs and those of ERS and RNS, which may indicate an alteration in the signal transduction of gingival cells. Also, recent studies with hGFCs have associated changes in the ERK signaling pathway with cell proliferation and collagen synthesis, adherence and remodeling (Imai et al., 2019; Rajshankar et al., 2020). The p-ERK signaling pathway was also related to gingival growth in hereditary GF (Jang et al., 2007), besides participating in the inhibition of periodontal inflammatory processes and interfering in the human periodontal ligament cells proliferation (Zhu et al., 2020). Also, ERK signaling pathway has also been related to the regulation of matrix metalloproteinases involved in gingival fibromatosis (Moulik et al., 2014; Rajshankar et al., 2020).

Finally, our study also indicates that ERS and RNS hGFCs have the capability under mineralizing conditions to form mineral nodules. Concerning the underlying mechanisms of ectopic calcification in gingival mucosa in both syndromes scarse evidence has been reported in the literature. Recently, Simancas Escorcia et al., 2020 suggested that ectopic calcifications found in the gingival tissue of ERS patient could be associated with epithelial degeneration and transdifferentiation of the gingival fibroblasts to chondro/osteoblastic cells (Simancas Escorcia et al., 2020). Further studies are necessary in order to investigate if the suggested mechanisms for ERS are also observed in the patients analyzed in this study.

CONCLUSION

This study confirms the presence of GF and mineral nodules in the gingival tissue of ERS and RNS patients, also demonstrating the expression of FAM20A, FAM20C and α-SMA proteins in fibroblasts in these tissues.

In addition, we have successfully established, for the first time, the primary culture of gingival cells obtained from two Brazilian families with non-lethal RNS. It was also possible to establish a cell culture of control patients and an ERS patient. The characterization indicated that the hGFCs showed morphological alterations, with reduced proliferation capacity, nuclei increased in size, and capacity to produce calcification nodules. We also demonstrated the colocalization of FAM20A in the Golgi apparatus in human hGFCs from ERS and RNS. Further studies and post-transcriptional analyzes with human cell samples are essential to elucidate the mechanisms that result in gingival abnormalities and to better understand the activity of FAM20A and FAM20C involved in ERS and RNS. The ERK pathway also needs to be further studied since the hGFCs of ERS and RNS show a reduction in the expression of the proteins involved.

REFERENCES (for article 2)

ACEVEDO, A. C. et al. Variability of systemic and oro-dental phenotype in two families with non-lethal Raine syndrome with FAM20C mutations. **BMC Med Genet,** v. 16, p. 8, Feb 21 2015. ISSN 1471-2350.

AMORIM, B. R. et al. Protocols for Genetic and Epigenetic Studies of Rare Diseases Affecting Dental Tissues. **Methods Mol Biol,** v.1922, p. 453-492, 2019.

ALSHARGABI, R. et al. SPOCK1 is a novel inducer of epithelial to mesenchymal transition in drug-induced gingival overgrowth. **Sci Rep**, v. 10, n. 1, p. 1-15, 2020.

BITU, C. C. et al. Heterogeneous presence of myofibroblasts in hereditary gingival fibromatosis. **J Clin Periodontol**, v. 33, n. 6, p. 393-400, 2006.

CASTRO, L. A. et al. Long-term effects of nifedipine on human gingival epithelium: a histopathological and immunohistochemical study. **J Oral Science**, v. 52, n. 1, p. 55-62, 2010.

COSTA, C. R. R. et al. Syndromes with gingival fibromatosis: A systematic review. **Oral Dis**, Apr 2020.

CUI, J. et al. A secretory kinase complex regulates extracellular protein phosphorylation. **Elife,** v. 4, p. e06120, Mar 19 2015.

CUI, J. Structure of Fam20A reveals a pseudokinase featuring a unique disulfide pattern and inverted ATP-binding. **Elife,** v. 6, Apr 2017.

DIERICK, J. et al. Stress-induced premature senescence and replicative senescence are different phenotypes, proteomic evidence. **Biochem Pharmacol**, v. 64, n. 5, p. 1011-17, 2002.

DILL, R. E.; IACOPINO, A. M. Myofibroblasts in phenytoin-induced hyperplastic connective tissue in the rat and in human gingival overgrowth. **J Periodontol**, v. 68, n. 4, p. 375-380, 1997.

DURE-MOLLA, M. D. L. et al. Pathognomonic oral profile of Enamel Renal Syndrome (ERS) caused by recessive FAM20A mutations. **Orphanet J Rare Dis,** v. 9, n. 1, p. 84, 2014.

FAUNDES, V. et al. Raine syndrome: an overview. **Eur J Med Genet,** v. 57, n. 9, p. 536-42, Sep 2014. ISSN 1769-7212.

FISHER, L. W.; FEDARKO, N. S. Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. **Connect Tissue Res,** v. 44 Suppl 1, p. 33-40, 2003.

FRADIN, M. et al. Osteosclerotic bone dysplasia in siblings with a Fam20C mutation. **Clin Genet**, v. 80, n. 2, p. 177-183, 2011.

HART, T. C.; HART, P. S. Genetic studies of craniofacial anomalies: clinical implications and applications. **Orthod Craniofac Res,** v. 12, n. 3, p. 212-220, 2009.

IMAI, K. et al. Biological Effects of Shikonin in Human Gingival Fibroblasts via ERK 1/2 Signaling Pathway. **Molecules**, v. 24, n.19, p. 3542, 2019.

ISHIKAWA, H. O. et al. The Raine syndrome protein FAM20C is a Golgi kinase that phosphorylates bio-mineralization proteins. **PLoS One,** v. 7, n. 8, p. e42988, 2012.

JANG, S. et al. Germ Line Gain of Function with SOS1 Mutation in Hereditary Gingival Fibromatosis. **J Biol Chem**, v. 282, n. 28, p. 20245-20255, 2007.

JAUREGUIBERRY, G. et al. Nephrocalcinosis (enamel renal syndrome) caused by autosomal recessive FAM20A mutations. **Nephron Physiol,** v. 122, n. 1-2, p. 1-6, 2012.

LI, L. et al. Loss of epithelial FAM20A in mice causes amelogenesis imperfecta, tooth eruption delay and gingival overgrowth. **Int J Oral Science,** v. 8, n. 2, p. 98-109, 2016-06-01 2016.

LIU, C. et al. Abrogation of Fam20c altered cell behaviors and BMP signaling of immortalized dental mesenchymal cells. **Exp Cell Res,** v. 363, n. 2, p. 188-195, 2018.

MACGIBBON, D. Generalized enamel hypoplasia and renal dysfunction. **Aust Dent J,** v. 17, n. 1, p. 61-3, 1972.

MARTELLI-JÚNIOR, H. et al. Case reports of a new syndrome associating gingival fibromatosis and dental abnormalities in a consanguineous family. **J Periodontol,** v. 79, n. 7, p. 1287-96, Jul 2008.

MOULIK, S. et al. Role of ERK in modulating MMP 2 and MMP 9 with respect to tumour invasiveness in human cancer cell line MCF-7 and MDA-MB-231. **J Tumor**, v. 2, n. 2, 2014.

NALBANT, D. et al. FAM20: an evolutionarily conserved family of secreted proteins expressed in hematopoietic cells. **BMC Genomics**, v. 6, n. 1, p. 11, 2005.

NITAYAVARDHANA, I. et al. Four novel mutations of FAM20A in amelogenesis imperfecta type IG and review of literature for its genotype and phenotype spectra. **Mol Genet Genomics,** v. 295, n. 4, p. 923-931, 2020.

O'SULLIVAN, J. et al. Whole-Exome sequencing identifies FAM20A mutations as a cause of amelogenesis imperfecta and gingival hyperplasia syndrome. **Am J Hum Genet,** v. 88, n. 5, p. 616-20, 2011.

OHYAMA, Y. et al. FAM20A binds to and regulates FAM20C localization. **Sci Rep,** v. 6, p. 27784, 2016.

PROUDFOOT, D. Calcium signaling and tissue calcification. **Cold Spring Harb Perspec Biol**, v. 11, n. 10, p. a035303, 2019.

RAFAELSEN, S. H. et al. Exome sequencing reveals FAM20C mutations associated with fibroblast growth factor 23–related hypophosphatemia, dental anomalies, and ectopic calcification. **J Bone Miner Res**, v. 28, n. 6, p. 1378-1385, 2013.

RAINE, J. et al. Unknown syndrome: microcephaly, hypoplastic nose, exophthalmos, gum hyperplasia, cleft palate, low set ears, and osteosclerosis. **J Med Genet,** v. 26, n. 12, p. 786-8, 1989.

RAJSHANKAR, D. et al. Focal adhesion kinase regulates tractional collagen remodeling, matrix metalloproteinase expression, and collagen structure, which in turn affects matrix-induced signaling. **J Cell Physiol,** v. 235, n. 3, p. 3096-3111, 2020.

REN, J. L. et al. Inflammatory signaling and cellular senescence. **Cell Signal**, v. 21, n. 3, p. 378-383, 2009.

RODIER, F.; CAMPISI, J. Four faces of cellular senescence. **J Cell Biol**, v. 192, n. 4, p. 547-556, 2011.

SHETH, J. et al. A case of Raine syndrome presenting with facial dysmorphy and review of literature. **BMC Med Genet**, v. 19, n. 1, p. 76, 2018.

SIMANCAS ESCORCIA V., et al. Lack of FAM20A, Ectopic Gingival Mineralization and Chondro/Osteogenic Modifications in Enamel Renal Syndrome. **Front Cell Dev Biol**, v. 8, p. 1503, 2020.

SOBRAL L. M., MONTAN P. F., MARTELLI-JUNIOR H., GRANER E., COLETTA R. D. Opposite effects of TGF-beta1 and IFN-gamma on transdifferentiation of myofibroblast in human gingival cell cultures. **J Clin Periodontol**, v. 34, n. 5, p. 397-406, 2007.

TAGLIABRACCI, V. S. et al. Secreted kinase phosphorylates extracellular proteins that regulate biomineralization. **Science,** v. 336, n. 6085, p. 1150-3, Jun 1 2012.

TAGLIABRACCI, V. S. et al. A Single Kinase Generates the Majority of the Secreted Phosphoproteome. **Cell,** v. 161, n. 7, p. 1619-1632, 2015.

WANG, S. K. et al. FAM20A mutations can cause enamel-renal syndrome (ERS). **PLoS Genet,** v. 9, n. 2, p. e1003302, 2013.

WANG, X. et al. The specific role of FAM20C in amelogenesis. **J Dent Res,** v. 92, n. 11, p. 995-9, 2013.

WANG, Y. P. et al. Transcriptome analysis of gingival tissues of enamel-renal syndrome. **J Periodontal Res,** v. 54, n. 6, p. 653-661, 2019.

XIAO, J. et al. Crystal structure of the Golgi casein kinase. **Proc Natl Acad Sci USA,** v. 110, n. 26, p. 10574-9, 2013.

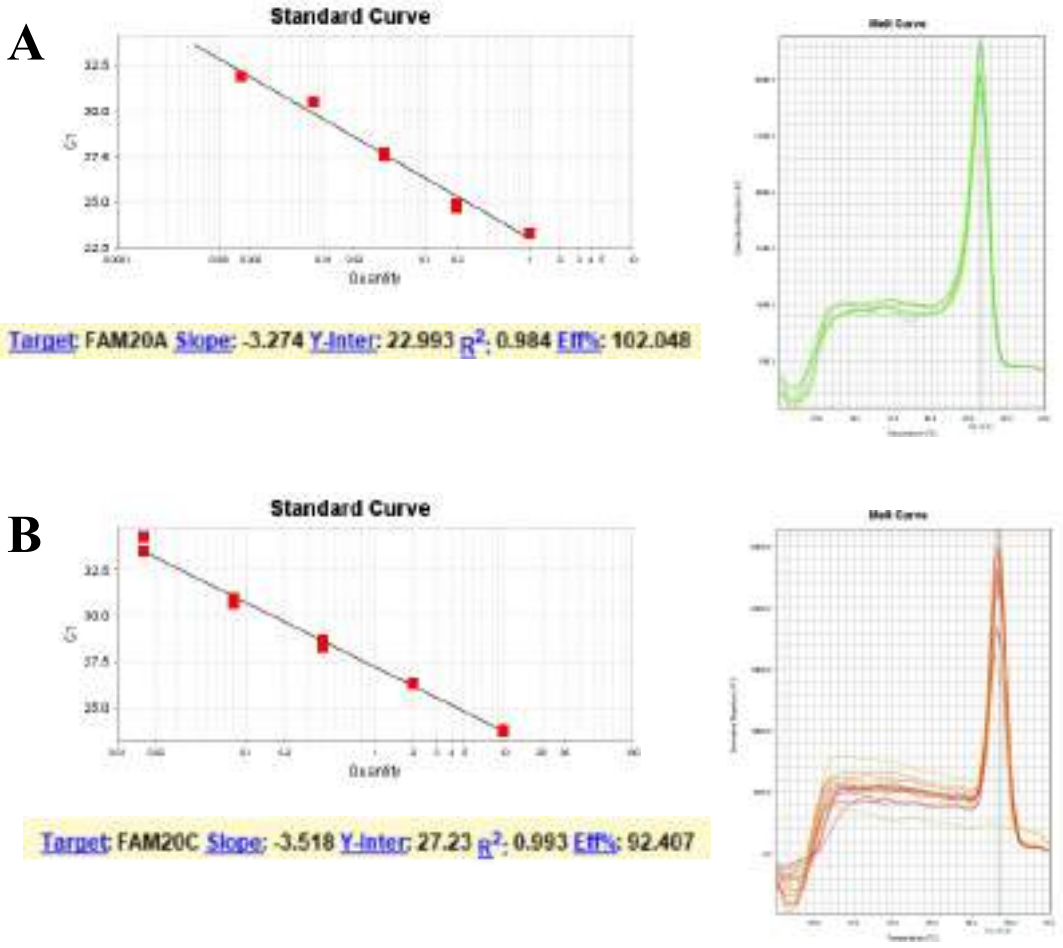
ZHANG, H. et al. Structure and evolution of the Fam20 kinases. **Nat Commun,** v. 9, n. 1, p. 1218, 2018.

ZHU, Y. et al. LncRNA-01126 inhibits the migration of human periodontal ligament cells through MEK/ERK signaling pathway. **J Periodontal Res**, Apr 2020.

SUPPLEMENTARY INFORMATIONS

Supplementary table 1. Primers sequence of Polymerase Chain Reaction (PCR) used in this study.

Name	Primers	Sequence	Amplicon BP
ß-ACTIN	Forward primer	TCACCCACACTGTGCCCATCTACG	295
	Reverse primer	CAGCGGAACCGCTCATTGCCAATG	
FAM20A	Forward primer	TGAAAGGAAGAGGGGGAGTCT	154
	Reverse primer	AAGCACCTCCCAGAAGTTCAT	
FAM20C	Forward primer	CTGTTCGAGCACCCGCTTTA	126
	Reverse primer	CGCATGCGGCCAGTCC	



Supplementary figure 1. Validation of primers for Polymerase Chain Reaction (PCR). Validation standard and melt curves of primers for *FAM20A* (A) and *FAM20C* (B).

3. DISCUSSÃO

Conforme apresentadas nos artigos deste trabalho, as discussões foram detalhadas em cada estudo individualmente e utilizaremos este espaço final para associarmos os resultados e propor perspectivas futuras envolvidas com a fibromatose gengival em pacientes ERS e RNS.

A FG é considerada uma característica orodental de importância clínica para pacientes que usam medicamentos que induzem o crescimento gengival e em FGH e FG relacionadas à síndrome (Coletta e Graner, 2006; Gawron et al., 2016; Alshargabi et al., 2020). Em primeiro lugar, nós identificamos as síndromes com FG enfatizando o fenótipo e a etiologia molecular dos indivíduos envolvidos na RS, atualizando os dados anteriormente descritos (Coletta e Graner, 2006; Gawron et al., 2016). Na sequência, nós incluímos um segundo manuscrito neste trabalho e avaliamos a FG em duas síndromes distintas, incluindo a ERS e a RNS. Desta forma, foi possível confirmar a FG nos pacientes atendidos pela equipe, como pode ser identificado em outros estudos incluídos na RS realizada (Acevedo et al., 2015; Poulter et al., 2015). Ainda, foi possível caracterizar tecido e cultura celular destes pacientes.

A revisão sistemática (Costa et al., 2020) mostrou a importância da caracterização fenotípica e identificação das bases moleculares para um melhor diagnóstico e acompanhamento de pacientes sindrômicos. Ainda, as bases moleculares de diversas síndromes não têm sido totalmente esclarecidas. A revisão sistemática também atualizou os dados de revisões precedentes (Coletta e Graner, 2006), confirmou a presença de FG e diversas síndromes previamente descritas dentre elas a ERS. Na última década um aumento na identificação das variantes patogênicas na ERS foi evidenciado. Em geral, independente da síndrome, este estudo de RS mostrou que existe pouca caracterização detalhada e poucas descrições que investigam a etiopatogenia da FG nas síndromes. Portanto, esses resultados nos encorajam a maiores investigações com tecidos e cultura primária de células gengivais de pacientes com ERS e RNS.

Assim, foi proposto o estudo experimental em que foram analisadas mais detalhadamente as alterações gengivais de paciente ERS e principalmente dos pacientes RNS, já com diagnóstico clínico e molecular realizados pela equipe, apresentados no artigo 2. Em nosso estudo, foi possível observar alterações morfológicas semelhantes aos resultados recentes de Simancas Escorcia et al. (2020), que aborda a FG em ERS. Simancas Escorcia et al. (2020)

hipotetizaram que alterações da FAM20A no epitélio podem contribuir para a degeneração e transdiferenciação das células epiteliais gengivais que levam à formação de calcificações ectópicas. Portanto, é ainda preciso investigar mais profundamente as alterações epiteliais observadas nos pacientes com RNS para melhor compreender o aparecimento de prolongamentos epiteliais e suas possíveis contribuições para as características da FG destes pacientes.

Além da avaliação epitelial, o artigo experimental detalhou a FG abordando os achados na lâmina-própria e em células de fibroblastos gengivais humanos demonstrando a marcação para FAM20A, FAM20C e α-SMA em ERS e RNS. Como perspectiva futura, ressaltamos a necessidade de se realizar estudos quantitativos para avaliar a marcação dessas proteínas. Sugerimos aqui o possível potencial dessas proteínas na homeostasia do tecido gengival dos pacientes sindrômicos, bem como acreditamos que possam influenciar nas alterações morfológicas observadas.

Ainda, nós levantamos a discussão de que, além do acúmulo de colágeno, há a sugestão de que os componentes não colagênicos também participam da FG e portanto devem ser melhor investigados para avaliar o desequilíbrio entre a síntese e a degradação da MEC. Uma vez que alterações em MMPs e TIMPs já foram relatadas em hiperplasia gengival induzida por drogas (Brown e Arany, 2017; Alshargabi et al, 2020) e em FG hereditária (Coletta et al., 1999; Martelli-Júnior et al., 2003; Gawron et al., 2016), nossos estudos sugeriram a avaliação da expressão gênica de MMPs em cultura primária de células de pacientes ERS e RNS, avaliando as possíveis alterações desses componentes em pacientes sindrômicos. Além disso, a transdução de sinais da via ERK também pode ser mais estudada nos fibroblastos gengivais de ERS e RNS, uma vez que a redução da expressão de proteínas ERK e p-ERK observadas neste estudo podem contribuir para a regulação da proliferação celular e expressão das MMPs envolvidas na FG (Moulik et al., 2014; Rajshankar et al., 2020).

As células deste estudo apresentaram alterações que podem ser usadas como ferramentas para futuros estudos e, sendo assim, sugerimos aprofundar com enzimografia para avaliar a expressão das MMPs. A expressão de proteínas envolvidas na mineralização de tecidos conjuntivos frouxos, tais como Fetuína-A, Periostina e RUNX2, poderá também ser avaliada. Além disso, há a necessidade de se realizar fosfosecretoma das culturas primárias dos nossos pacientes ERS e RNS com a finalidade de identificar as possíveis fosfoproteínas alvos da FAM20A e FAM20C. Nesse contexto, foi iniciada a extração de proteínas de tecidos

gengivas e de células e meio de cultura primária de células gengivais de pacientes com ERS e RNS. Os experimentos encontram-se em execução por equipes colaboradoras e aguardamos os resultados para análise e interpretação. Para isso, já foram preparadas as amostras para avaliação da proteômica de tecidos gengivais de ERS e RNS por meio de *Laser Capture Microdissection* (LCM), assim como a coleta de células e meio de cultura para a avaliação proteômica e fosfoproteômica das culturas primárias de fibroblastos gengivais.

4. CONCLUSÕES

1. A revisão sistemática mostrou que a FG é uma característica orodental de diversas síndromes incluindo as síndromes de Zimmermann-Laband e Costello, assim como a ERS e RNS incluídas nesta pesquisa. A FG é uma manifestação orodental da ERS e a identificação das variantes patogênicas no gene *FAM20A* tem aumentado nos últimos anos. Além disso, a RS destacou a necessidade da fenotipagem sistemática orodental para as futuras descrições a fim de auxiliar os profissionais da odontologia a melhor diagnosticar as síndromes, uma vez que o diagnóstico inicial pode se basear nas manifestações orais dos pacientes.

2. A análise histopatológica de tecidos gengivais confirmou a presença de FG e de calcificações ectópicas nos pacientes ERS e RNS estudados.

3. Análise imuno-histoquímica demonstrou a expressão de FAM20A, FAM20C e α-SMA nos tecidos gengivais controle, ERS e RNS.

4. Os ensaios em culturas primárias de fibroblastos gengivais revelaram que nos pacientes RNS avaliados houve tendência à diminuição da viabilidade e proliferação celular e apresentam alteração morfológica com aumento de tamanho dos núcleos celulares quando comparados aos fibroblastos controles.

5. Os fibroblastos gengivais controle, ERS e RNS expressaram FAM20A e FAM20C. Foi também possível demonstrar a localização de FAM20A no complexo de Golgi, mas não foi possível visualizar a dualidade FAM20A-FAM20C utilizando a metodologia empregada em nossos estudos. As células de ERS e RNS apresentaram uma redução da expressão de ERK 1/2 e p-ERK 1/2 quando comparadas ao controle, sugerindo alterações na via de sinalização ERK.

6. Os fibroblastos gengivais de pacientes ERS e RNS apresentam potencial de mineralização quando estimulados por meio mineralizante.

REFERÊNCIAS

ACEVEDO, A. C. et al. Variability of systemic and oro-dental phenotype in two families with non-lethal Raine syndrome with FAM20C mutations. **BMC Med Genet,** v. 16, p. 8, 2015.

ACOSTA, A. X. et al. Raine dysplasia: a Brazilian case with a mild radiological involvement. **Clin Dysmorphol**, v. 9, n. 2, p. 99-101, 2000.

AL MANE, K. A.; COATES, R. K.; MCDONALD, P. Intracranial calcification in Raine syndrome. **Pediatr Radiol,** v. 26, n. 1, p. 55-8, 1996.

ALVES, R.D. et al. Calcifying vascular smooth muscle cells and osteoblasts: independent cell types exhibiting extracellular matrix and biomineralization-related mimicries. **BMC Genomics,** v. 15, n. 1, p. 1-14, 2014.

ARAUJO, C. S. A. et al. Histomorphometric characteristics and expression of epidermal growth factor and its receptor by epithelial cells of normal gingiva and hereditary gingival fibromatosis. **J Periodont Res**, v. 38, n. 3, p. 237-241, 2003.

ALSHARGABI, R. et al. SPOCK1 is a novel inducer of epithelial to mesenchymal transition in drug-induced gingival overgrowth. **Sci Rep**, v. 10, n. 1, p. 1-15, 2020.

BAUER, C. K. et al. Mutations in KCNK4 that affect gating cause a recognizable neurodevelopmental syndrome. **Am J Human Genet**, v. 103, n. 4, p. 621-630, 2018.

BAYRAM, Yavuz et al. REST final-exon-truncating mutations cause hereditary gingival fibromatosis. **Am J Human Genet**, v. 101, n. 1, p. 149-156, 2017.

BITU, C. C. et al. Heterogeneous presence of myofibroblasts in hereditary gingival fibromatosis. **J Clin Periodontol**, v. 33, n. 6, p. 393-400, 2006.

BROWN, R. S.; ARANY, P. R. Mechanism of drug-induced gingival overgrowth revisited: a unifying hypothesis. **Oral Dis**, v. 21, n. 1, p. e51-e61, 2015.

CHEN NX, O'NEILL KD, CHEN X, MOE SM: Annexin-mediated matrix vesicle calcification in vascular smooth muscle cells. **J Bone Miner Res,** v. 23, p. 1798-1805, 2008.

COLA, C. et al. Regulatory role of endothelium in the expression of genes affecting arterial calcification. **Biochem Biophys Res Commun,** v. 320, n. 2, p. 424-7, 2004.

COLETTA, R. D. et al. Alteration in expression of MMP-1 and MMP-2 but not TIMP-1 and TIMP-2 in hereditary gingival fibromatosis is mediated by TGF-beta 1 autocrine stimulation. **J Periodontal Res,** v. 34, n. 8, p. 457-63, 1999.

COLETTA, R. D.; GRANER, E. Hereditary gingival fibromatosis: a systematic review. **J Periodontol,** v. 77, n. 5, p. 753-64, 2006.

COSTA, C. R. R. et al. Syndromes with gingival fibromatosis: A systematic review. **Oral Dis**, Apr 2020.

CUI, J. et al. A secretory kinase complex regulates extracellular protein phosphorylation. **Elife,** v. 4, p. e06120, 2015.

CUI, J. et al. Structure of Fam20A reveals a pseudokinase featuring a unique disulfide pattern and inverted ATP-binding. **Elife,** v. 6, p. e23990, 2017.

DELLOW, E. L. et al. Amelogenesis imperfecta, nephrocalcinosis, and hypocalciuria syndrome in two siblings from a large family with consanguineous parents. **Nephrol Dial Transplant**, v. 13, n. 12, p. 3193-3196, 1998.

DILL, R. E.; IACOPINO, A. M. Myofibroblasts in phenytoin-induced hyperplastic connective tissue in the rat and in human gingival overgrowth. **J Periodontol**, v. 68, n. 4, p. 375-380, 1997.

DOURADO, M. R. et al. Enamel renal syndrome: A novel homozygous *FAM20A* founder mutation in 5 new Brazilian families. **Eur J Med Genet**, v. 62, n. 11, p. 103561, 2019.

DURE-MOLLA, MURIEL D. L. et al. Pathognomonic oral profile of Enamel Renal Syndrome (ERS) caused by recessive FAM20A mutations. **Orphanet J Rare Dis,** v. 9, n. 1, p. 84, 2014.

ELALAOUI, S. C. et al. Non lethal Raine syndrome and differential diagnosis. **Eur J Med Genet,** v. 59, n. 11, p. 577-583, 2016.

ELTAN, M. et al. A Rare Cause of Hypophosphatemia: Raine Syndrome Changing Clinical Features with Age. **Calcif Tissue Int**, v. 107, n. 1, p. 96-103, 2020.

FAUNDES, V. et al. Raine syndrome: an overview. **Eur J Med Genet,** v. 57, n. 9, p. 536-42, 2014.

FISHER, L. W.; FEDARKO, N. S. Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. **Connect Tissue Res,** v. 44 Suppl 1, p. 33-40, 2003.

FRADIN, M. et al. Osteosclerotic bone dysplasia in siblings with a Fam20C mutation. **Clin Genet,** v. 80, n. 2, p. 177-83, 2011.

GAWRON, K. et al. Gingival fibromatosis with significant de novo formation of fibrotic tissue and a high rate of recurrence. **Am J Case Reports,** v. 17, p. 671-675, 2016.

GOLDBLATT, J.; SINGER, L. Autosomal recessive gingival fibromatosis with distinctive facies. **Clin Genet**, v. 42, n. 6, p. 306-308, 1992.

GONZÁLEZ, E. A.; MARTIN, K. J. Renal osteodystrophy. **Rev Endocr Metab Disord,** v. 2, n. 2, p. 187-93, 2001.

HALL, R. K. et al. Amelogenesis imperfecta and nephrocalcinosis syndrome: Case studies of clinical features and ultrastructure of tooth enamel in two siblings. **Oral Surg Oral Med Oral Pathol Oral Radiol Endod**, v. 79, n. 5, p. 583-592, 1995.

HAO, J. et al. Dentin matrix protein 4, a novel secretory calcium-binding protein that modulates odontoblast differentiation. **J Biol Chem,** v. 282, n. 21, p. 15357-65, 2007.

HART, T. C. et al. A Mutation in the SOS1 Gene Causes Hereditary Gingival Fibromatosis Type 1. **American J Human Genet,** v. 70, n. 4, p. 943-954, 2002.

JAUREGUIBERRY, G. et al. Nephrocalcinosis (enamel renal syndrome) caused by autosomal recessive FAM20A mutations. **Nephron Physiol,** v. 122, n. 1-2, p. 1-6, 2012.

KATHER, J. et al. Clinical and histomorphometric characteristics of three different families with hereditary gingival fibromatosis. **Oral Surg Oral Med Oral Pathol Oral Radiol Endod,** v. 105, n. 3, p. 348-52, 2008.

KINGSTON, H. M.; FREEMAN, J. S.; HALL, C. M. A new lethal sclerosing bone dysplasia. **Skeletal Radiol**, v. 20, n. 2, p. 117-119, 1991.

KORUYUCU, M. et al. Nephrocalcinosis in Amelogenesis Imperfecta Caused by the FAM20A Mutation. **Nephron,** v. 139, n. 2, p. 189-196, 2018.

LI, L. et al. Loss of epithelial FAM20A in mice causes amelogenesis imperfecta, tooth eruption delay and gingival overgrowth. **Int J Oral Sci,** v. 8, n. 2, p. 98-109, 2016.

LIGNON, G. et al. FAM20A Gene Mutation: Amelogenesis or Ectopic Mineralization? **Front Physiol,** v. 8, p. 267, 2017.

LIU, C. et al. Abrogation of Fam20c altered cell behaviors and BMP signaling of immortalized dental mesenchymal cells. **Exp Cell Res,** v. 363, n. 2, p. 188-195, 2018.

KORTÜM, F. et al. Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome. **Nat Genet**, v. 47, n. 6, p. 661-667, 2015.

MACGIBBON, D. Generalized enamel hypoplasia and renal dysfunction. **Aust Dent J,** v. 17, n. 1, p. 61-3, 1972.

MAMEDOVA, E. et al. Non-lethal Raine syndrome in a middle-aged woman caused by a novel FAM20C mutation. **Calci Tissue Int**, v. 105, n. 5, p. 567-572, 2019.

MAMELI, C. et al. Natural history of non-lethal Raine syndrome during childhood. **Orphanet J Rare Dis**, v. 15, p. 1-9, 2020.

MARTELLI-JUNIOR, H. et al. Microscopic and proliferative comparison of gingival fibroblasts from patients with normal gingiva and with hereditary gingival fibromatosis. **Pesq Odontol Bras**, v. 14, n. 2, p. 123-129, 2000.

MARTELLI-JUNIOR, H. et al. Effect of Transforming Growth Factor-β1, Interleukin-6, and Interferon-γ on the Expression of Type I Collagen, Heat Shock Protein 47, Matrix Metalloproteinase (MMP)-1 and MMP-2 by Fibroblasts from Normal Gingiva and Hereditary Gingival Fibromatosis. **J Periodontol**, v. 74, n. 3, p. 296-306, 2003.

MARTELLI-JÚNIOR, H. et al. Case reports of a new syndrome associating gingival fibromatosis and dental abnormalities in a consanguineous family. **J Periodontol,** v. 79, n. 7, p. 1287-96, 2008.

MARTELLI-JÚNIOR, H. et al. Amelogenesis imperfecta and nephrocalcinosis syndrome: a case report and review of the literature. **Nephron Physiol**, v. 118, n. 3, p. p62-p65, 2011.

MEJÍA, N. et al. Chronic kidney disease--mineral and bone disorder: a complex scenario. **Nefrologia,** v. 31, n. 5, p. 514-9, 2011.

MENG, L. et al. Increased expression of collagen prolyl 4-hydroxylases in Chinese patients with hereditary gingival fibromatosis. **Arch Oral Biol,** v. 52, n. 12, p. 1209-14, 2007.

MOE, S. M. et al. Uremia induces the osteoblast differentiation factor Cbfa1 in human blood vessels. **Kidney Int,** v. 63, n. 3, p. 1003-11, 2003.

MOE, S. M. et al. Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). **Kidney Int,** v. 67, n. 6, p. 2295-2304, 2005.

MOULIK, S. et al. Role of ERK in modulating MMP 2 and MMP 9 with respect to tumour invasiveness in human cancer cell line MCF-7 and MDA-MB-231. **J Tumor**, v. 2, n. 2, 2014.

NALBANT, D. et al. FAM20: an evolutionarily conserved family of secreted proteins expressed in hematopoietic cells. **BMC Genomics,** v. 6, p. 11, 2005.

NEVIN, N. C. Hereditary gingival fibromatosis. **J Mental Defic Res**, v. 15, n. 2, p. 130-5, 1971.

NITAYAVARDHANA, I. et al. Four novel mutations of FAM20A in amelogenesis imperfecta type IG and review of literature for its genotype and phenotype spectra. **Mol Genet Genomics,** v. 295, n. 4, p. 923-931, 2020.

O'SULLIVAN, J. et al. Whole-Exome sequencing identifies FAM20A mutations as a cause of amelogenesis imperfecta and gingival hyperplasia syndrome. **Am J Hum Genet,** v. 88, n. 5, p. 616-20, 2011.

OSSAREH, S. Vascular calcification in chronic kidney disease: mechanisms and clinical implications. **Iran J Kidney Dis,** v. 5, n. 5, p. 285-99, 2011.

PAULA, L. M. et al. Case report of a rare syndrome associating amelogenesis imperfecta and nephrocalcinosis in a consanguineous family. **Arch Oral Biol,** v. 50, n. 2, p. 237-42, 2005.

PEHLIVAN, D. et al. Cytogenetic analysis and examination of SOS1 gene mutation in a Turkish family with hereditary gingival fibromatosis. **J Hard Tissue Biol**, v. 18, n. 3, p. 131-134, 2009.

PROUDFOOT, D. Calcium signaling and tissue calcification. **Cold Spring Harb Perspec Biol**, v. 11, n. 10, p. a035303, 2019.

RAESTE, A.; COLLAN, Y.; KILPINEN, E. Hereditary fibrous hyperplasia of the gingiva with varying penetrance and expressivity. **Eur J Oral Sci**, v. 86, n. 5, p. 357-365, 1978.

RAFAELSEN, S. H. et al. Exome sequencing reveals FAM20c mutations associated with fibroblast growth factor 23-related hypophosphatemia, dental anomalies, and ectopic calcification. **J Bone Miner Res,** v. 28, n. 6, p. 1378-85, 2013.

RAINE, J. et al. Unknown syndrome: microcephaly, hypoplastic nose, exophthalmos, gum hyperplasia, cleft palate, low set ears, and osteosclerosis. **J Med Genet,** v. 26, n. 12, p. 786-8, 1989.

RAJSHANKAR, D. et al. Focal adhesion kinase regulates tractional collagen remodeling, matrix metalloproteinase expression, and collagen structure, which in turn affects matrix-induced signaling. **J Cell Physiol,** v. 235, n. 3, p. 3096-3111, 2020.

REYNOLDS, J. L. et al. Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. **J Am Soc Nephrol,** v. 16, n. 10, p. 2920-30, 2005.

SIMANCAS ESCORCIA, V. et al. Lack of FAM20A, Ectopic Gingival Mineralization and Chondro/Osteogenic Modifications in Enamel Renal Syndrome. **Front Cell Dev Biol**, v. 8, p. 1503, 2020.

SIMPSON, M. A. et al. Mutations in FAM20C are associated with lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. **Am J Hum Genet,** v. 81, n. 5, p. 906-12, 2007.

SIMPSON, M. A. et al. Mutations in FAM20C also identified in non-lethal osteosclerotic bone dysplasia. **Clin Genet,** v. 75, n. 3, p. 271-6, 2009.

SPEER, M. Y.; GIACHELLI, C. M. Regulation of cardiovascular calcification. **Cardiovasc Pathol,** v. 13, n. 2, p. 63-70, 2004.

TAGLIABRACCI, V. S. et al. Secreted kinase phosphorylates extracellular proteins that regulate biomineralization. **Science,** v. 336, n. 6085, p. 1150-3, 2012.

TAGLIABRACCI, V. S. et al. A Single Kinase Generates the Majority of the Secreted Phosphoproteome. **Cell,** v. 161, n. 7, p. 1619-1632, 2015.

VANAKKER, O. M. et al. Low serum vitamin K in PXE results in defective carboxylation of mineralization inhibitors similar to the GGCX mutations in the PXE-like syndrome. **Lab Invest**, v. 90, n. 6, p. 895-905, 2010.

VOGEL, P. et al. Amelogenesis imperfecta and other biomineralization defects in Fam20a and Fam20c null mice. **Vet Pathol,** v. 49, n. 6, p. 998-1017, 2012.

WANG, X. et al. Expression of FAM20C in the osteogenesis and odontogenesis of mouse. **J Histochem Cytochem,** v. 58, n. 11, p. 957-67, 2010.

WANG, X. et al. Inactivation of a novel FGF23 regulator, FAM20C, leads to hypophosphatemic rickets in mice. **PLoS Genet,** v. 8, n. 5, p. e1002708, 2012a.

WANG, X. et al. FAM20C plays an essential role in the formation of murine teeth. **J Biol Chem,** v. 287, n. 43, p. 35934-42, 2012b.

WANG, S. K. et al. FAM20A mutations can cause enamel-renal syndrome (ERS). **PLoS Genet,** v. 9, n. 2, p. e1003302, 2013.

WANG, Y. P. et al. Transcriptome analysis of gingival tissues of enamel-renal syndrome. **J Periodontal Res,** v. 54, n. 6, p. 653-661, 2019.

XIAO, J. et al. Crystal structure of the Golgi casein kinase. **Proc Natl Acad Sci U S A,** v. 110, n. 26, p. 10574-9, 2013.

ZHANG, H. et al. Structure and evolution of the Fam20 kinases. **Nat Commun,** v. 9, n. 1, p. 1218, 2018.

Tabela Suplementar 1. Características genotípicas e fenotípicas de relatos de casos de síndrome de Raine.

Autor, ano	Total	Eltan et al., 2020	Mameli et al., 2020			Hernández-Zavala et al., 2020		Mamedova et al., 2019	Hung et al., 2019	Rolvien et al., 2018
Informações gerais										
Nº famílias relatadas	43	1	2			1		1	1	1
Famílias consanguíneas	29 sim 12 não 2 sem relato	Não	Sim		Sim	Sim		-	Sim	-
Nº indivíduos afetados	58	1	2		1	2		1	1	1
Especificação dos sujeitos		-	I	II	III	II.2	II.3	-	-	-
Sexo	M = 36 F = 21	M	F	M	M	M	M	F	M	M
Idade		9 meses	12 anos	5 meses	4 meses	Pré-natal		36 anos	Pós-natal	72 anos
Origem geográfica		Turquia	Paquistão		Paquistão	México		Armênia	Equador	-
Tempo de sobrevida		17 meses	-		-	3 dias	22 semanas de gestação	-	Pós-natal	-
Letalidade	34 letais 18 não letais	Letal	Não letal		Não letal	Letal		Não letal	Letal	Não letal
Causa da morte		Insuficiência respiratória	-		-	-	Aborto induzido	-	Insuficiência respiratória	-
Características genotípicas de variantes patogênicas em *FAM20C*										
Zigosidade		HET COMP	HOM		HOM	HET COMP		HET COMP	HOM	HET COMP
Tipo de variante		Substituição; Inserção	Substituição		Substituição	Deleção; duplicação		Inserção; substituição	Substituição	Substituição (duplicação)
Efeito da variante		Missense; Splice site	Missense		Missense	Frameshift		Frameshift; missense	Missense	Missense (Splice site)
Alteração no DNA		c.1645C>T; c.863+5G>C*	c.1351G>A		c.496G>T	c.474delC; c.456dupC		c.1107_1108insTACTG; c.1375C>G	c.1007T> G	c.906C>A (c.952_956+30dup)
Exons/Introns		-	7		1	1		6; 8	5	4
Alteração de aminoácidos		p.Arg549Trp	p.Asp451Asn		p.Glu166X	p.Ser159ProfsTer28; p.Gly153ArgfsTer56		p.Tyr369fs; p.Arg459Gly	p. Met336Arg	p.Phe302Leu
Alterações clínicas	**Ocorrências descritas**									
Microcefalia	30	+	+	+	+	-	-	-	+	-
Craniostenose	19	+	+	+	-	+	+	-	+	-
Hidrocefalia	5	+	-	-	-	-	-	-	-	-
Exoftalmia	48	+	-	+	+	+	+	+	+	-
Deficiência visual	7	+	+	-	-	-	-	-	-	-
Hipoplasia do terço médio da face	53	+	-	+	+	+	+	+	+	-

Depressão da ponte nasal	52	+	-	+	+	+	+	+	+	-
Atresia coanal	30	-	-	+	+	+	-	-	-	-
Dificuldades respiratórias ao nascimento	31	-	-	+	+	+	-	-	+	-
Orelhas baixas	36	-	-	-	+	+	+	+	-	-
Orelhas displásicas	7	-	-	-	-	+	+	-	-	-
Perda de audição	11	-	+	-	-	-	-	-	-	-
Estatura baixa	15	-	+	-	+	-	-	-	+	-
Malformação de membros	23	+	+	-	-	+	+	-	-	-
Ossos longos submineralizados	8	+	-	-	-	-	-	-	+	-
Dedos curtos	22	+	-	-	-	+	+	+	+	-
Clinodactilia	9	-	-	-	-	-	-	-	-	-
Fraturas ósseas	5	+	-	-	-	-	-	+	+	-
Convulsões	8	-	-	-	-	+	-	-	-	-
Atraso no desenvolvimento	22	+	+	+	+	-	-	-	+	-
Osteoesclerose	48	+	-	+	+	+	+	+	+	+
Calcificações ectópicas										
Calcificação renal	5	-	-	-	-	-	-	+	-	-
Calcificação gengival	5	-	-	-	-	-	-	-	-	-
Calcificação folicular	6	-	-	-	-	-	-	-	-	-
Calcificação pulpar	2	-	-	-	-	-	-	-	-	-
Calcificação intracraniana	39	+	+	+	-	+	+	+	+	-
Alterações orodentais										
Micrognatia	32	+	-	-	-	+	+	-	+	-
Defeitos no palato	33	+	-	-	-	+	+	-	+	-
Mal oclusão	7	-	-	-	-	-	-	-	-	-
Gengiva aumentada	25	+	-	-	+	-	-	-	-	-
Malformação dentária	18	-	+	-	+	-	-	+	-	-
Erupção ectópica	4	-	-	-	-	-	-	-	-	-
Dente permanente não irrompido	5	-	-	-	-	-	-	-	-	-
Formação incompleta das raízes	4	-	-	-	-	-	-	-	-	-
Lesão periapical	7	-	-	-	-	-	-	-	-	-
Língua saliente	8	+	-	-	-	-	-	-	-	-
Análises bioquímicas										
Hipocalcemia	8	+	-	+	+	-	-	-	+	-
Hipofosfatemia	18	+	+	+	+	-	-	+	-	+
Fosfatase alcalina elevada	5	-	-	-	-	-	-	-	-	-
Elevado TPH	7	+	-	+	-	-	-	+	-	-
Deficiência de vit. D	6	+	-	+	-	-	-	+	-	-

Sheth et al., 2018	Tamai et al., 2018	Whyte et al., 2017		Elalaoui et al., 2016		Seidahmed et al., 2015	Acevedo et al., 2015					Takeyari et al., 2014
1	1	1		1		1	2					1
Sim	Não	Não		Sim		Sim	Sim			Sim		Sim
1	1	2		2		1	3			2		1
-	-	II.3	II.4	III.7	III.10	-	IV:4	IV:5	IV:6	VI-1	VI-2	-
F	F	M	F	F	M	M	M	M	F	M	M	M
6 anos	2 anos	Pós-natal	Pré-natal	18 anos	15 anos	Pré-natal	16 anos	12 anos	10 anos	13 anos	12 anos	61 anos
India	Japão	-		Marrocos		Arábia Saudita	Brasil					Japão
-	-	2 anos	12 dias	-	-	1 dia	-					-
Não letal	Não letal	Letal		Não letal		Letal	Não letal			Não letal		Não letal
-	-	Parada respiratória	Parada respiratória	-	-	Insuficiência respiratória	-					-
HOM	HOM	HET		HOM		-	HOM					HOM
Substituição	Substituição	Substituição		Substituição		Substituição	Inserção			Substituição		Substituição
Missense	Missense	Missense		Missense		Missense	Splice site			Missense		Missense
c1228T>A	c.1219T>G	c.1094>A		c.676T>A		c.1225C> T	c.784+5G>C*			c.1487C>T		c.1222C>T
10	6	6		2		-	2			9		6
p.Ser410Thr	p.Try407Gly	p.Gly365Asp		p.Trp226Arg		p.Arg409Cys	p.Trp202Cysfs*37			p.Pro496Lys		p.Arg408Trp
+	-	+	+	-	+	-	+	+	-	+	+	-
-	-	+	+	-	-	-	-	-	-	+	+	-
-	-	+	-	-	-	-	-	-	-	-	-	-
-	+	+	+	+	+	+	+	+	+	+	+	-
-	-	-	+	-	-	-	-	-	-	+	-	-
+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	-	-	-	+	+	-
-	+	-	-	-	-	+	+	-	-	+	-	-
-	+	+	+	-	-	+	+	-	-	+	+	-
+	-	+	+	+	+	+	-	-	-	+	+	-
-	-	-	-	-	-	-	+	+	+	-	-	-
-	+	-	-	+	+	-	+	+	+	-	-	-
-	+	+	+	-	-	-	-	-	-	+	+	+
+	+	-	-	+	+	+	-	-	-	+	+	-
-	-	+	+	-	-	+	-	-	-	+	+	-
-	+	-	-	-	+	+	-	+	-	+	+	-
+	-	-	-	-	+	+	-	-	-	+	+	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	+	-	-	+	-	-	+	-	-

+	+	+	+	+	+	+	-	-	-	+	+	-
+	+	+	+	-	-	+	-	-	-	-	-	+
-	-	-	-	-	-	-	-	-	-	+	-	-
-	-	-	-	-	-	-	+	+	+	+	+	-
-	-	-	-	-	-	-	+	+	+	+	+	-
-	-	-	-	-	-	-	-	+	+	-	-	-
-	+	+	+	+	-	+	-	-	+	+	+	+
-	+	+	+	-	-	+	-	-	-	-	-	-
-	-	-	-	+	+	+	+	+	+	+	+	-
-	-	-	-	+	+	-	+	+	+	+	+	-
-	-	-	-	+	+	+	+	+	+	+	+	-
-	+	+	-	+	+	-	+	+	+	+	+	+
-	-	-	-	-	-	-	-	+	+	+	-	-
-	-	-	-	+	+	-	-	-	-	+	+	-
-	-	-	-	+	-	-	-	-	-	+	+	-
-	-	-	-	-	-	-	+	+	+	+	+	-
-	-	-	-	+	-	+	-	-	-	-	-	-
-	+	+	-	-	-	-	-	-	-	-	-	-
-	-	-	+	+	-	-	+	-	+	+	+	+
-	-	-	-	-	-	-	+	+	+	+	+	-
-	-	-	-	+	-	-	-	-	-	+	+	-
-	-	-	-	-	-	-	-	-	-	-	-	+

Mahmood et al., 2014	Vishwanath et al., 2014	Ababneh et al., 2013	Rafaelsen et al, 2013		Gaigi et al., 2011				Fradin et al, 2011		Koob et al., 2011[a]	Michael et al., 2011	Kochar et al., 2010
1	1	1	1		1				1			1	1
Sim	Sim	Sim	Não		Sim				Sim			Sim	Sim
1	1	1	2		4				3			1	1
-	-	-	II.2	II.3	1	2	3	4	1	-	2	3	-
M	F	M	M	M	M	M	F	F	M	F	F	F	M
2 meses	Prós-natal	Pós-natal	18	16	Pós-natal	Pós-natal	Pós-natal	Pós-natal	Pós-natal	1 ano	4 anos	1 ano	Pré-natal
Paquistão	Índia	Arábia Saudita	Noruega		-				Algéria			Sudão	Índia
-	1 mês e 20 dias	8 meses	-	-	1 dia	4 horas	2 dias	4 dias	38 dias	-	-	-	Morte uterina
Não letal	Letal	Letal	Não letal		Letal				Não letal			Letal	Letal
-	-	Pneumonia por *Klebsiella pneumoniae*	-	-	Insuficiência respiratória		-		Pneumonia e sepse	-	-	-	Morte uterina
HOM	-	HOM	HET COMP		-				HOM			-	HOM
Substituição	-	Rearranjo complexo	Substituição		-				Substituição			-	Substituição
Missense	-	Rearranjo complexo e deleção de 487-kb no cromossomo 7p22.3	Nonsense, missense		-				Missense			-	Missense
c.1135G>A	-	46,XY.arr[hg19] 7p22.3	c.915C>A, c.803C>T		-				c.940C>T			-	c.1630C>T
6	-	-	4, 3		-				4			-	10
p.Gly379Arg	-	(36480-523731)x0	p.Tyr305X, p.Thr268Met		-				p.Pro314Ser			-	p.Arg544Trp
+	+	+	-	-	+	+	+	+	+	-	-	-	-
+	+	-	-	-	-	-	-	-	-	-	+	-	-
-	-	+	-	-	-	-	-	-	-	-	-	-	-
+	+	+	-	-	+	+	+	+	+	-	-	+	+
-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	-	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	-	+	+	+	+	+	-	+	+	-
+	+	+	+	+	+	+	-	+	+	+	-	-	-
-	+	-	-	-	-	-	-	-	-	+	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	-	+	-	-	-	-	-	-	+	-	-
-	+	+	-	-	+	+	-	-	+	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	+	-	-
-	+	+	+	+	-	-	-	-	-	-	-	+	-

-	-	+	+	+	-	-	-	-	-	-	+	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	+	-	-	-	-	-	-	-	-	-	-	-
-	+	+	+	-	-	-	-	-	-	-	-	+	-
+	+	+	+	+	+	+	+	-	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	+	-	+	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	-	-	-	-	+	+	+	+	-
-	+	+	+	-	+	+	-	-	+	-	+	+	-
-	+	+	+	+	+	+	+	-	-	+	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	+	-	-	-	+	+	-	-	+	-	-	+	+
-	-	-	+	+	-	-	-	-	-	+	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	-	-	-	-	-	-	-	-	-	-
-	-	-	+	+	-	-	-	-	-	-	-	-	-
-	-	+	-	-	-	-	-	-	-	-	+	-	-
+	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	-	+	+	-	-	-	-	-	-	+	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-	-	+	-

Simpson et al., 2009		**Chitayat et al., 2007**	**Simpson et al., 2007**				**Güneş *et al.*, 2005**	**Hülskamp et al., 2003**		
2		1	4				1	1		
Sim	Não	Não	Não	Sim	Não	Não	Sim	Sim		
1	1	1	1	1	1	1	1	3		
1	2	-	1	3	6	7	-	11	12	13
M	M	M	M	M	F	F	F	M	M	F
8	11	Pré-natal	Pós-natal				Pós-natal	Pós-natal	Pré-natal	Pré-natal
-	-	Pai português e mãe Judia Asquenaze	-	-	-	-	Turquia	Turquia		
-	-	Natimorto	2 horas	3 horas	> 1 semana	< 1 semana	2 horas	76 horas	Aborto (24 semanas de gestação)	5 dias
Não letal	Não letal	Letal	Letal	Letal	Letal	Letal	Letal	Letal		
-	-	Natimorto	NR	NR	NR	NR	-	Insuficiência respiratória	Aborto induzido	Insuficiência respiratória
HOM	HET COMP	-	-	HOM	HET COMP	HET COMP	-	HOM		
Substituição	Substituição	-	Rearranjo complexo	Substituição	Substituição; deleção	Inserção; deleção	-	Substituição		
Missense	Missense	-	Rearranjo do cromossomo 7 e microdeleção	Missense	Missense, Splice site	Splice site	-	Missense		
c.1309G>A	c.731T>A, c.796G>A	-	45,XYpsudic(7;7)	c.1093G>A	c.1094G>A, c.1322-2A>G*	c.914+5G>C*, c.1404-1G>A*	-	c.1121T>G[b]		
7	2, 3	-	-	6	6 8/7	4/4 9/8	-	6		
p.Asp437Asn	p.Ile244Asn, p.Gly266Arg	-	p22;p22	p.Gly365Arg	p.Gly365Glu	-	-	Leu374Arg		
-	+	-	-	-	-	-	+	-	+	-
+	+	+	-	-	-	-	-	+	+	+
+	+	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+
-	+	+	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+
-	-	+	-	-	-	-	-	-	+	+
-	-	-	+	-	-	-	+	+	-	-
+	+	+	-	-	-	-	+	-	+	+
-	-	+	-	-	-	-	-	-	+	-
+	+	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-

-	-	+	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	+	-	-	-	-	-	-	+	+	+
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	+
+	-	-	-	-	-	-	-	-	-	-
+	+	-	-	-	-	-	-	+	-	-
+	-	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
+	+	+	-	-	+	+	+	+	+	+
-	-	+	-	-	-	-	+	+	+	+
+	-	+	-	-	-	-	+	-	+	-
-	-	-	-	-	-	-	-	-	-	-
+	-	+	-	-	-	-	-	-	+	+
+	+	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	+	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
+	+	-	-	-	-	-	+	-	-	-
-	-	-	-	-	-	-	-	-	-	+
+	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-

Al-Gazali et al., 2003	Mahafza et al., 2001	Acosta et al., 2000	Shalev et al., 1999	Al Mane et al., 1998	Rejjal et al., 1998	Al Mane et al., 1996	Kan and Kozlowski, 1992	Patel et al., 1992	Kingston et al., 1991	Raine et al., 1989
1	1	1	1	1	1	1	1	1	1	1
Não	Sim	Sim	Sim	Sim	Sim	Sim	Não	Sim	Sim	Não
1	1	1	1	1	1	1	1	1	1	1
-	-	-	-	-	-	-	-	-	-	-
M	M	M	F	F	M	M	F	M	M	F
Pós-natal	Pós-natal	Pós-natal	Pós-natal	Pré-natal	Pós-natal	Pós-natal	Pós-natal	Pós-natal	Pós-natal	Pós-natal
Egito	Jordania	Brasil	Palestina	Arábia Saudita	Arábia Saudita	Arábia Saudita	Caucasiano	Arábia Saudita	Caucasiano	Caucasiano
38 dias	8 dias	6 horas	27 dias	Natimorto	8 semanas	2 meses	45 min	-	4 horas	86 min
Letal	Letal	Letal	Letal	Letal	Letal	Letal	Letal	Letal	Letal	Letal
Insuficiência respiratória	Parada cardiopulmonar	NR	Insuficiência respiratória	Natimorto	Insuficiência respiratória	Insuficiência respiratória	Insuficiência respiratória	NR	Insuficiência respiratória	Insuficiência respiratória
HOM	-	-	-	-	-	-	-	-	HOM	-
Deleção	-	-	-	-	-	-	-	-	Missense	-
Splice site	-	-	-	-	-	-	-	-	Substituição	-
c.915-3C>G*[b]	-	-	-	-	-	-	-	-	c.1603C>T[b]	-
5/4	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	p.Arg535Trp	-
-	-	-	-	+	+	-	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	-	+	+	+
-	-	-	+	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	-	+	+
+	+	+	+	+	+	+	+	+	+	+
+	+	-	+	-	+	+	+	+	+	-
+	+	+	-	-	+	+	+	-	-	-
-	+	+	+	-	+	+	+	-	+	+
-	-	-	-	-	+	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
+	+	-	+	-	-	+	-	+	+	-
-	-	-	-	-	-	-	-	-	-	-
+	+	-	-	-	-	-	-	-	+	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	+	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	+	-	-	-

+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	+	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	+	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	+	+	+	+	+	-	-
+	+	+	+	+	+	+	+	-	+	+
+	+	+	+	+	-	+	+	-	+	+
-	-	-	-	-	-	-	-	-	-	-
-	+	-	+	-	-	-	+	-	+	+
-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-

Legenda: F – feminino; HET – heterozigose; HET COMP – heterozigose composta; HOM – homozigose; M – masculino; NR – não relatado; (+) – presente ou relatado; (-) – ausente ou não relatado. Este quadro foi realizado a partir dos relatos de casos incluídos na busca do Apêndice 1.

*Variante intrônica em *Splice site*.

[a] A criança relatada por Koob et al. (2011) foi pela primeira vez demonstrada e é irmã dos indivíduos relatados por Fradin et al. (2011).

[b] As caracterizações genéticas apresentadas nesta tabela nos relatos de Kingston et al. (1991), Al-Gazali et al. (2003) e Hülskamp et al. (2003) foram retiradas do artigo de Simpson et al. (2007)