Hyaline Fibromatosis Syndrome: a case report

Thaís dos Santos Fontes Pereira , Jéssica Félix de Sales , Denise Vieira Travassos , Célia Regina Lanza , Wagner Henriques Castro , Carolina Cavaliéri Gomes , Felipe Paiva Fonseca , Tarcília Aparecida Silva , Ricardo Santiago Gomez

PII: S2212-4403(20)31081-6
DOI: https://doi.org/10.1016/j.oooo.2020.06.022
Reference: OOOO 4406

To appear in: *Oral Surg Oral Med Oral Pathol Oral Radiol*

Received date: 12 March 2020
Revised date: 11 May 2020
Accepted date: 26 June 2020

Please cite this article as: Thaís dos Santos Fontes Pereira , Jéssica Félix de Sales , Denise Vieira Travassos , Célia Regina Lanza , Wagner Henriques Castro , Carolina Cavaliéri Gomes , Felipe Paiva Fonseca , Tarcília Aparecida Silva , Ricardo Santiago Gomez , Hyaline Fibromatosis Syndrome: a case report, *Oral Surg Oral Med Oral Pathol Oral Radiol* (2020), doi: https://doi.org/10.1016/j.oooo.2020.06.022

Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology

Hyaline Fibromatosis Syndrome: a case report

--Manuscript Draft--

Manuscript Number:	TRIPLEO-D-20-00323R1
Article Type:	Case Report (online only)
Section/Category:	Oral and Maxillofacial Pathology
Keywords:	infantile systemic hyalinosis; juvenile hyaline fibromatosis; ANTXR2; CMG2; Oral lesions
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Abstract:	Hyaline Fibromatosis Syndrome (HFS) is a rare monogenic disease inherited in an autosomal recessive pattern and characterized by hyaline deposits on the skin, mucosa, and multiple organs, osteoporosis and joint contractures. This progressive condition is caused by mutations in the gene encoding the anthrax toxin receptor 2 protein (ANTXR2). HFS is a disabling disease, and patients progressively suffer from pain and disfiguring symptoms. Few case reports detailing the oral findings in patients with this condition have been published. The present case report describes a four-year-old female patient that shows a severe manifestation of HFS. The diagnosis was based on clinical and histopathological features and confirmed by molecular analysis. The present study emphasizes the oral manifestations, its histopathological aspects, the molecular pathogenesis, and the interdisciplinary management of patients affected by this condition.

5th May 2020

Faizan Alawi

Editor, Oral & Maxillofacial Pathology

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology

Dear Prof. Alawi,

We have received your decision letter, together with the issues raised by the reviewers. We are resubmitting a revised version of the manuscript " Hyaline Fibromatosis Syndrome: a case report". We have revised our manuscript according to the referees' suggestions.

We have answered each of the reviewers' queries, and all the changes in the revised version of the manuscript have been highlighted in red.

We would like to thank you and the referees for their time dedicated to the evaluation of the manuscript, which has contributed significantly to the final quality of the study. We hope our manuscript is now acceptable for publication in the Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology.

All co-authors are aware of and in agreement with the revisions.

On behalf of the co-authors,

Prof. Ricardo Santiago Gomez

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Comments from the Editors and Reviewers:

Reviewer #1

Hyaline fibromatosis syndrome (HFS) is a rare condition and its prevalence appears to be <1 / 1 000 000, approximately. Published data regarding what is known about oral findings of the disease it's even rarer. Therefore, this case report may represent a valuable contribution to the readership of the journal. Overall, the article is well structured, and the information is presented in a consistent manner. Below are a few pointers that are mainly related to English translations, construction of sentences and information related to overall prognosis and treatment.

Abstract

- Line 16, it is suggested that text (included in lines 16- 23) be condensed and re -written as it appears repetitive.

We re-wrote as suggested.

Introduction

- It is suggested that authors include information about the grading system such that it is based on severity of the disease and classified as mild, moderate, and severe.

We included the information as suggested.

- Line 50, substitute "in" for "with" feeding…

We changed to "with".

- Line 55, substitute disfiguring for disfigurement.

We substituted for "disfigurement".

Case report

- Line 18, it is suggested that a connector such as "notably" be used to introduce the sentence.

We added the connector "notably".

- Line 24, add "and" before restricted…

We added "and".

- Page 8, line 6 substitute bleeding for bled

We substituted for "bled".

- Page 8, line 11 substitute germs for tooth buds

We substituted for "a congenitally absent the absence of left lateral incisor…"as suggested by Reviewer #2.

- Page 8, line 18 substitute for: the patient underwent general anesthesia…

We substituted for "the patient underwent general anesthesia".

Discussion

- Page 13, line 13 please include how often the patient is seen for follow up appointments.

We included the information as follows: "The patient has been followed up every three months for over a year...".

- Page 13, line 28 please specify information regarding multi-professional follow up. Throughout the paper, it was not clearly evident what specialists were the members of the interprofessional team that cared for the patient.

We specified the information regarding the multi-professional team as follows: "In the present case, report, an interprofessional team involving dentists, nutritionists, pharmacists, dermatologists, palliative care and pediatricians, took care of the patient, demonstrating the importance of early multi-professional follow-up…".

- It is recommended that the authors include information about the prognosis of the disease.

We included information about the prognosis, as suggested: "Little is known about the influence of clinical manifestations on the prognosis of HFS. Systemic involvement seems to be associated with a worse prognosis due to early complications, more commonly associated to infiltration of the intestines, leading to protein-losing enteropathy and diarrhea. Most patients have a fatal outcome before two years old in these cases.[4,45]"

- Although there is scarce information about treatment for the condition, it is suggested that the authors include information regarding physical therapy for joint contractures and splinting.

We included information about treatment for joint contractures, as suggested: "Joint contractures are a persistent and progressive stigma of the disease that are refractory to the treatments described so far. Some options, such as physiotherapy, capsulotomy, cortisone or

D-penicillamine, apparently benefited the patients, improving joint mobility and symptoms. [15,39,41–44] *However, there is no evidence that these treatments prevent joint deformities progression, and their effects appear to be only temporary..”*

Reviewer #2

In this case report, the authors describe the clinical and histopathological features of a 4-year-old girl with hyaline fibromatosis syndrome (HFS), confirmed via molecular analysis. The manuscript is very well-written and nicely documented with high-quality clinical, radiographic, and histological images. The discussion is thorough and informative. Although an extremely rare disorder, the authors' emphasis on maintenance of optimal oral hygiene and the unique challenges associated with managing HFS patients is appreciated. General and specific comments are presented below in abbreviated format.

General comments:

- Case report: Did the authors consider performing ancillary stains (e.g., congo red, fibrinogen) in this case? Was the cause of the patient's excessive bleeding during her biopsy explored/determined?

Periodic Acid-Schiff (PAS) positively stained the hyaline deposits and Congo Red was negative. We added this information in the paper. These stains associated with the clinical, radiographic characteristics and the patient's medical history would be sufficient for the diagnosis, therefore, no additional stains were performed.

As the patient's coagulogram values were within the normal range, excessive bleeding was attributed to the inflammatory gingival condition. We also included this information in the case report.

- Discussion: A summary of reported cases may be of interest to the readership.

Previous reported cases of HFS describe features involving multiple sites with different degrees of severity and extent. To summarize, the dermatological findings most commonly reported include: pearly papules on the skin,[10,15,17,19,28–35] *macular lesions over bony prominences,*[10,15,19,28,32–34,36] *perianal coalescent nodular lesions,*[10,15,17,19,28,29,32,33,35,36] *generalized skin thickening*[10,33,35] *and large subcutaneous nodules.*[15,28,35] *Skeletal findings result in significant orthopedic limitations and include: joint contractures, restricted movement of joints, osteoporosis/osteopenia, osteolysis and fractures.*[4,10,11,14,15,28] *Severe cases are associated with complications such as failure*

to thrive, recurrent infections and early death in infancy.4,35,36
Specific comments (most are minor suggestions; page 1 corresponds to page 1 of the submission pdf):
- Abstract, page 5, line 15: Consider revising to "…patient who shows severe manifestations of HFS."
We revised to"...patient who shows severe…"

- Introduction, page 6, lines 11-12: Consider revising to "…conditions represent differing severity on the spectrum…" if this does not alter the intent of the statement.
We revised to "...represent different severity degrees on the spectrum of diseases…" to not alter the intent of the statement.

- Introduction, page 6, line 18: Consider replacing "capillaries" with "capillary".
We replaced with "capillary".

- Introduction, page 6, lines 25-26: Consider replacing "similarly" with "similar".
We replaced with "similar".

- Introduction, page 6, lines 50-51: Consider revising to "…age, interfering with feeding…"
We revised to "...age, interfering with feeding..."

- Introduction, page 6, lines 54-57: Consider revising to "…pain and disfigurement. If treatment is ineffective, it is essential…"
We revised to "...pain and disfigurement. If treatment is ineffective, it is essential..."

- Case report, page 7, lines 23-24: Consider revising to "…flexural areas, and restricted facial…"
We revised to "...flexural areas and restricted facial..."
- Case report, page 7, lines 45-46: Consider replacing "compromising" with "compromised" if this does not alter the intent of the statement.
We replaced with "compromised".

- Case report, page 7 and 8, lines 60-61: Consider revising to "…surfaces scattered on the skin…elasticity, and limited mouth opening."

We revised to "...surfaces scattered on the skin…elasticity, and limited mouth opening."

- Case report, page 8, lines 10-13: Consider revising to "…revealed a congenitally absent left lateral incisor…"
We revised to "...revealed a congenitally absent left lateral incisor..."

- Case report, page 8, lines 18-19: Consider revising to "The patient received general anesthesia…"
We revised to "the patient underwent general anesthesia" as suggested by Reviewer #1.

- Case report, page 8, lines 20-22: Consider revising to "A fragment of the anterior mandibular gingiva, measuring…using an electric scalpel."
We revised to "A fragment of the anterior mandibular gingiva, measuring...using an electric scalpel."

- Case report, page 8, lines 23-24: Consider revising to "…procedure, the patient developed significant bleeding."
We revised to "...procedure, the patient developed significant bleeding."

- Case report, page 8, lines 47-48: Consider revising to "…overnight. The importance of oral hygiene procedures…"
We revised to "...overnight. The importance of oral hygiene procedures..."

- Case report, page 8, lines 55-58: Can the authors kindly clarify the statement beginning "Despite this improvement…"? Do the authors intend to state that despite improvements in mouth opening, speech, and food intake, her oral health was still suboptimal due to functional limitations or that poor oral health is a functional limitation?
The improvements in mouth opening, speech and food intake were notable, but her oral health is still suboptimal due to the gingival hyperplasia and nodules that impose significant functional limitation. We re-wrote for better understanding: "Despite these notable is improvements, her oral health is still suboptimal due to the gingival hyperplasia and oral nodules that impose a significant functional limitation."

- Discussion, page 10, line 9: Consider replacing "is" with "are".

We replaced with "are".

- Discussion, page 10, lines 32-33: Consider revising to "…contractures and osteoporosis associated with osteolytic lesions…" if this does not alter the intent of the statement.
We revised to "...contractures and osteoporosis associated with osteolytic lesions..."

- Discussion, page 10, lines 40-41: Consider revising to "…progressed to pearly papules…"
We revised to "...progressed to pearly papules..."

- Discussion, page 10, lines 49-52: Consider revising to "lesions in areas…suggests that mechanical stress may be the trigger…"
We revised to "lesions in areas...suggests that mechanical stress may be the trigger..."

- Discussion, page 10, lines 55-59: The statement beginning "Hyaline deposits" is slightly confusing. Perhaps the microvascular alterations related to the ECM1 mutations can be discussed in a separate sentence?
We re-wrote the statement as suggested: "Hyaline deposits also result in papules in eyelids and skin lesions in frictional areas in a condition named Urbach–Wiethe disease (lipoid proteinosis). Microvascular alteration is caused by mutations in the extracellular matrix gene 1 (ECM1) in this condition.[20] The deposits corresponded to basal membrane-laminin (in skin laminin-10), type IV collagen, nidogen, and perlecan (IIF).[21]"

- Discussion, page 11, lines 5-6: Please change "Cong" to "Congo".
We changed to "Congo".

- Discussion, page 12, lines 51-53: Consider revising to "To date, there is no definitive treatment for this condition. The few possibilities include interventions…"
We revised to *"To date, there is no definitive treatment for this condition. The few possibilities include interventions..."*

Hyaline Fibromatosis Syndrome: a case report

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

This work was supported the National Council for Scientific and Technological Development (CNPq)/Brazil and Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 001).

WORD COUNT FOR THE ABSTRACT: 113

COMPLETE MANUSCRIPT WORD COUNT: 3674

NUMBER OF REFERENCES: 47

NUMBER OF FIGURES/TABLES: 4

ABSTRACT

Hyaline Fibromatosis Syndrome (HFS) is a rare monogenic disease inherited in an autosomal recessive pattern and characterized by hyaline deposits on the skin, mucosa, and multiple organs, osteoporosis and joint contractures. This progressive condition is caused by mutations in the gene encoding the anthrax toxin receptor 2 protein (ANTXR2). HFS is a disabling disease, and patients progressively suffer from pain and disfiguring symptoms. Few case reports detailing the oral findings in patients with this condition have been published. The present case report describes a four-year-old female patient who shows severe manifestations of HFS, emphasizing the oral manifestations, its histopathological aspects, the molecular pathogenesis, and the interdisciplinary management of patients affected by this condition.

Keywords: infantile systemic hyalinosis; juvenile hyaline fibromatosis; ANTXR2; CMG2; oral lesions

INTRODUCTION

Hyaline Fibromatosis Syndrome (HFS), OMIM # 228600, is a rare autosomal recessive condition that evolves with the accumulation of hyaline material on the skin and in various organs. More severe cases have been reported under the denomination "Infantile Systemic Hyalinosis" (ISH)[1], and the term "Juvenile Hyaline Fibromatosis" (JHF) has been reserved for milder cases with more prolonged survival.[2] Both conditions represent different severity degrees on the spectrum of diseases caused by mutations in Anthrax Toxin Receptor 2 (*ANTXR2*).[3] Therefore, Nofal et al. (2009) proposed the unifying term HFS and a grading system based on severity of the disease and classified as mild, moderate, and severe.[4]

The gene responsible for HFS, *ANTXR2*, was firstly associated with capillary formation, and it was initially named Capillary Morphogenesis Gene 2 (CMG2).[5] More recently, Scobie et al. (2003) identified a receptor for the anthrax toxin encoded by this gene, changing its official name.[6] In an animal model, ANTXR2 loss of function promotes collagen VI accumulation, similar to what happens in HFS.[7] To better characterize the mutational spectrum of HFS, genotype-phenotype studies have demonstrated an association of the phenotypic variability with different types of mutation. A mutational hotspot in *ANTXR2* exon 13 was identified, and frameshift mutations that lead to a premature stop codon and splicing mutations were typically associated with a more severe form of the disease.[3,8] Despite this association between mutation type and the degree of phenotypic severity, the molecular consequences of the mutations remain to be evaluated at the mRNA, protein, and functional levels.[9]

The abnormal accumulation of a hyaline substance in HFS individuals affects multiple organs, and prominent nodules are commonly present. Protein-losing enteropathy may result from the presence of nodules in the intestines and is characterized by diarrhea and weight loss.[10] The limitation of movement is frequently caused by joint stiffness and pain, evolving to deformities called contractures.[1] Gingival hypertrophy usually develops between 6 and 12 months of age, interfering with feeding and speaking.[11]

HFS patients progressively suffer from pain and disfigurement.[11] If treatment is ineffective, it is essential to make decisions about possible palliative interventions. The recognition and acknowledgment that a decision is required should be followed by the

integration of the available evidence with patients' values in the process of shared decision-making.[12]

The present case report describes the genetic and clinical features of HFS, emphasizing the oral manifestations, its histopathological aspects, and the interdisciplinary therapeutic approach.

CASE REPORT

A four-year-old female patient was the only child of healthy consanguineous, first cousins, parents. The mother reported that she had a full-term pregnancy, and all exams in the prenatal period showed no alterations. Fifty hours after delivery, the mother and child were discharged with no complications. Notably, the newborn usually cried when handled, starting to concern the parents.

At the age of six months, the mother started to notice erythematous areas in the skin around the nose and eyes, hyperpigmentation at flexural areas and restricted facial and body movements. The patient was referred for specialized medical attention. Clinically, small pearly papules appeared in pressure areas, dorsum, neck, and face. Nodules with pink-reddish discoloration were present in the perianal region. Short stature, limited joint movement, and pain were important complaints at the time. Diffuse osteopenia and articular contractures of elbows, knees, and hands were radiographically observed. Cognitive development was not compromised. The patient was diagnosed with HFS at the age of one year and five months based on clinical and radiographic findings. The patient evolved with significantly joint contractures, making locomotion impossible and fixing the limbs in a "frog-leg position". Larger subcutaneous nodules grew bilaterally on the scalp. The mentioned physical features are shown in Figure 1.

The patient's dental history included acute pain and abscess, extensive gingival hyperplasia, covering the surfaces of deciduous teeth, and compromised nutrition and oral hygiene. The oral findings were associated with moderate fever, and she was treated with antibiotics and anti-inflammatory drugs. The patient´s parents also reported that she had frequent diarrhea episodes and weight loss. The patient was then referred to the dental service of the Clinics Hospital of the *Universidade Federal de Minas Gerais* when she was three years old. The main complaint in the first appointment was the difficulty of oral feeding, due to the presence of extensive gingival hyperplasia and poor condition of her teeth. The physical exam revealed small papules with smooth, pink surfaces scattered on the skin around the lips and eyelids, prominent lips with reduced elasticity and limited

mouth opening. Submucosal nodules were diffusely distributed on the lips and buccal mucosa bilaterally. On intraoral examination, extensive and massive gingival hyperplasia was observed covering the dental surfaces. The gingival mucosa was erythematous and bled at the slightest manipulation. The oral hygiene was poor, and bacterial biofilm was observed in all teeth. The upper incisors were decayed and showed pulp exposure. Her oral condition also negatively influenced speech development. Panoramic radiography revealed a congenitally absent left lateral incisor and multiple teeth cavities (Figure 2).

Considering the damage caused by gingival hyperplasia on the patient's oral and systemic health, a surgical approach was proposed, involving gingivectomy and excision of intraoral nodules. The patient underwent general anesthesia and nasotracheal intubation. A fragment of the anterior mandibular gingiva, measuring 1.5 cm in the largest diameter, was removed using an electric scalpel. However, during the procedure, the patient developed significant bleeding. Local hemostatic measures were insufficient to control bleeding, and the patient lost 120 ml of blood, evolving with hypotension. As the patient's coagulogram values were within the normal range, excessive bleeding was attributed to the inflammatory gingival condition. The surgical procedure was interrupted, and the excised tissues were submitted to histopathological examination.

Microscopically, the fragment of gingival tissue was covered by stratified parakeratinized squamous epithelium. The connective tissue was highly collagenized, showing accumulation of extracellular matrix and a sparse population of stromal cells. The connective tissue cells showed intracellular vacuoles. Chronic inflammatory infiltrate was focally observed (Figure 3). Periodic Acid-Schiff (PAS) positively stained the hyaline deposits and Congo Red was negative.

After the surgical intervention, outpatient dental care was instituted for oral health promotion and to remedy the inflammatory gingival condition. The parents were instructed to use a cotton swab soaked in 0.12% chlorhexidine solution to clean the gingival grooves and the dental surfaces three times a day and 0.2% chlorhexidine gel overnight. The importance of oral hygiene procedures was emphasized in comprehensive care, including nutritional guidance.

During a year of follow up, the patient progressively improved her mouth opening, speech, and oral food intake capacity. However, the gingival inflammatory condition remained, although to a lesser extent. Despite these notable improvements, her oral health is still suboptimal due to the gingival hyperplasia and oral nodules that impose a

significant functional limitation. We also observed the recurrence of gingival hyperplasia in the anterior region of the mandible nine months after the biopsy (Figure 2).

To better elucidate the syndrome's etiopathogenesis and to provide genetic counseling, direct sequencing of the exon 13 of *ANTXR2*, a hotspot region recognized in HFS, was performed. Briefly, genomic DNA (gDNA) was isolated from the formalin-fixed paraffin-embedded (FFPE) hyperplastic gingival tissue using the Deparaffinization Solution (Qiagen, Germany) and the DNA FFPE Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. Buccal swabs were collected from the patient and both parents after the signature of free and informed consent. gDNA was isolated from the swabs using DNeasy Blood & Tissue Kit (Qiagen, Germany). Spectrophotometer (Nano- DropTM 2000 instrument; Thermo Fisher Scientific, USA) was used for assessing DNA quantity and purity.

The exon 13 of *ANTXR2* was amplified by standard PCR with primers designed using Primer- BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast)[13]: forward primer (5'-TACACATCCTTACTATCTCCTCTCT-3') and reverse primer (5'-TTTGGGCATGGTATCTGCATT-3'). ExoSAP- IT™ PCR Product Cleanup Reagent (Life Technologies, CA, USA) was used for purification of PCR products. DNA sequencing reactions were carried out using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and then sequenced on an ABI 3730 DNA Analyzer (Applied Biosystems, USA). The sequences were visualized in the software SnapGene viewer, and the chromatograms were manually inspected, comparing to the reference sequence.

The patient's DNA sequencing revealed a homozygous frameshift mutation (c.1074del; p.Ala359Hisfs*50) characterized by the deletion of T (thymine), which causes the substitution of alanine by a histidine and frameshift. The father and the mother carried the same heterozygous deletion. A homozygous single nucleotide polymorphism (SNP rs12647691) was also detected in the DNA sequence of the proband and the mother, while in the father, this SNP was heterozygous. Additionally, the mother had the heterozygous synonymous SNP rs72653288. The molecular features detected in the patient and the parents are further characterized in Figure 4.

DISCUSSION

HFS was classically reported in the literature under two different terminologies, ISH and JHF. ISH was described as a more severe condition compared to JHF, associated with early-onset and lower survival.[14] However, both conditions share important similarities, and overlapping features occur in some borderline cases.[15,16] Although the clinical features of the present case are consistent with ISH, we have adopted the unifying term, HFS, following the concept of clinical overlap previously highlighted by Nofal et al. (2009).[4]

In the present case, the patient showed the initial signs of the condition in the first six months of age, revealing an early onset. The parents reported that the child cried continuously, which was later associated with pain and restricted joint movements. Decreased limbs movement and crying when manipulated were the earliest findings reported by Felix et al. (2004). Due to the restriction of movement, imaging exams were conducted and demonstrated diffuse osteoporosis.[14] Diffuse osteopenia and articular contractures were radiographically observed in our patient at the initial diagnostic tests, and its progression caused severe physical disability. Osteolytic lesions, mainly in long bones, associated with cortical erosions were also reported in a subset of JHF cases, but skeletal involvement is often milder compared to reports of ISH.[16,17] Articular contractures and osteoporosis associated with osteolytic lesions are also components of the Winchester syndrome, caused by mutations in membrane-bound matrix metalloprotease 14 gene (*MMP14*).[18]

Skin lesions in our patient were initially noticed as erythematous rash and progressed to pearly papules and nodules in various parts of the body. The cutaneous involvement is an important stigma of this condition and frequently reported as papuloerythematous rash, polypoid, and nodular pearly lesions.[14,19] In the present case, these lesions were located on the perianal and nuchal skin, surrounding the nose and eyes, on external ears and other areas subjected to pressure. The high frequency of lesion in areas associated with repeated movement or pressure suggests that mechanical stress may be the trigger for deposition of perivascular hyaline matrix originating the cutaneous lesions.[11,15]

Hyaline deposits also result in papules in eyelids and skin lesions in frictional areas in a condition named Urbach–Wiethe disease (lipoid proteinosis). Microvascular alteration is caused by mutations in the extracellular matrix gene 1 (*ECM1*) in this condition.[20] The deposits corresponded to basal membrane-laminin (in skin laminin-10),

type IV collagen, nidogen, and perlecan (IIF).[21] Hyaline is a widely used term and includes different biochemical compositions. In amyloidosis, deposits of amorphous, hyaline, acellular material may occur locally or involve multiple organs, showing apple-green polarization on Congo red stain.[22] Hyaline depositions are also observed in biopsy specimens of erythropoietic protoporphyria, a condition associated to painful photosensitivity. [23] Different diseases, such as ligneous periodontitis,[24] juvenile colloid milium,[25] lichen *sclerosus*[26], and localized scleroderma,[27] also exhibit hyaline material, and clinicopathological correlation is essential for the differential diagnosis.

Previous reported cases of HFS describe features involving multiple sites with different degrees of severity and extent. To summarize, the dermatological findings most commonly reported include: pearly papules on the skin,[10,15,17,19,28–35] macular lesions over bony prominences,[10,15,19,28,32–34,36] perianal coalescent nodular lesions,[10,15,17,19,28,29,32,33,35,36] generalized skin thickening[10,33,35] and large subcutaneous nodules.[15,28,35] Skeletal findings result in significant orthopedic limitations and include: joint contractures, restricted movement of joints, osteoporosis/osteopenia, osteolysis and fractures.[4,10,11,14,15,28] Severe cases are associated with complications such as failure to thrive, recurrent infections and early death in infancy.[4,35,36]

A genome-wide linkage search in four affected individuals suggested that the gene for JHF was on chromosome 4q21.[37] From the locus identification, the disease-causing mutations in *ANTXR2* was later characterized in both conditions, JHF and ISH, defining them as part of the same spectrum of diseases.[35] *ANTXR2* encodes a single-pass type I transmembrane protein that include an extracellular von Willebrand factor A (vWA) domain with a metal ion-dependent adhesion site motif.[38] The investigation of differential gene expression in human capillary morphogenesis revealed a potential role of *ATXR2* in basement membrane matrix synthesis. *ANTR2* is upregulated during endothelial cells morphogenesis.[5] The encoded protein is primarily targeted to the endoplasmic reticulum and shows strong binding to collagen type IV and laminin.[5]

HFS is inherited in an autosomal recessive pattern, so this condition is frequently reported among consanguineous families, as in the present case.[10,39,40] The geographical prevalence of HFS is suggested to be influenced by the degree of consanguineous marriages.[40,41] Cases of non-consanguineous parents were also reported.[14,29] The parents in the present case were heterozygous for a pathogenic *ANTXR2* mutation (c.1074delT), and the proband was found to be homozygous for the same genetic mutation. Knowledge

about the genetic bases of this condition may contribute to the genetic counseling of these families.

HFS is a monogenic disease caused by different types of mutations spread throughout the *ANTXR2* gene. Molecular analysis showed that point mutations in gene sequences encoding the ectodomain and transmembrane region resulted in the retention of the protein in the endoplasmic reticulum due to a folding and assembly defect.[42] Our patient was homozygous for a single nucleotide deletion in exon 13, a frameshift mutation that affects the cytosolic tail of the protein and leads to a premature stop. The analysis of the molecular effect of frameshift mutations in exon 13 revealed that these genotypes resulted in decreased abundance of ANTXR2 at mRNA and protein levels, suggesting that the mRNAs may be recognized by the nonsense-mediated mRNA decay pathway and degraded.[43] Functional studies indicated that the mutation c.1074delT also leads to defects at a protein-folding level.[9]

HFS is caused by homozygous or compound heterozygous mutation in the gene *ANTXR2* (608041) on chromosome 4q21. The variety of possible disease-causing mutations in HFS could explain the different levels of phenotypic severity. Thus, in a study that evaluated the genotype-phenotype correlation, missense and in-frame mutations in the cytoplasmic domain were associated with milder cases. While in cases described as the infantile form of the condition, patients showed an insertion/deletion mutation that altered the open reading frame. Severe cases also showed missense and truncating mutations affecting the extracellular vWA domain.[3] However, the multiplicity of possible allelic combinations makes this correlation increasingly complex. The mutation found in our patient has been previously described in a homozygous state as well as a compound heterozygote.[29,44] Interestingly, a significant variation in severity can be seen in individuals harboring this mutation. Homozygosis has been reported in severe cases, including two affected individuals of a Kuwaiti family and one of an Egyptian family. [3,44] On the other hand, an eight-year-old Moroccan patient also showed the homozygous state, but the manifestation of the disease was moderate.[41] Affected individuals showing compound heterozygosis ranged from moderate to lethal grades. This range of clinical severity may be, at least in part, a consequence of the different mutations in the second allele.[29,34,44]

The nature and location of the *ANTXR2* mutation influences not only the phenotype but also the molecular treatment possibilities. Mutations that map the extracellular domain were previously demonstrated to affect folding, leading to

degradation.[42] Nevertheless, the functional protein could be partially rescued by the treatment with proteasome inhibitors.[43] Further studies, analyzing the specific c.1074delT mutation, demonstrated that it affects both the mRNA and protein folding levels, leaving gene replacement as the only future alternative of molecular treatment.[9]

To date, there is no definitive treatment for this condition. The few possibilities include interventions to minimize symptoms and prevent complications. Surgical excision of skin and oral lesions was considered in some cases to prevent ulceration, pain, and infection.[15,16,45] However, the risks of such treatment choice must be carefully weighed. In the present case, the patient had severe complications during the excision of the oral lesions, and the procedure had to be aborted. Excessive bleeding during surgical removal of gingival hyperplasia was also reported by Stucki *et al.* (2001).[11] Local recurrence is often reported, showing that this approach may represent a short-term benefit in some cases.[29,30] In the present case, recurrence in the biopsied region was observed after nine months. Therefore, some authors suggest that surgical interventions should be reserved only for ulcerated, infected lesions or those that cause significant functional impairment to the patient.[29,31] In line with this recommendation, a less invasive approach has been instituted in the present case after the unsuccessful surgical procedure. The patient has been followed up every three months for over a year and has no acute foci of infection. The guided oral hygiene program was well accepted by the parents and tolerated by the patient.

Joint contractures are a persistent and progressive stigma of the disease, and they seem to be refractory to the treatments described so far. Some options, such as physiotherapy, capsulotomy, cortisone or D-penicillamine, apparently benefited the patients, improving joint mobility and the symptoms. [15,31,33,45–47] However, there is no evidence that these treatments prevent joint deformities progression, and their effects appear to be only temporary.

Little is known about the influence of clinical manifestations on the prognosis of HFS. Systemic involvement seems to be associated with a worse prognosis due to early complications, more commonly associated with infiltration of the intestines, leading to protein-losing enteropathy and diarrhea. Most patients have a fatal outcome before two years old in these cases.[4,36]

HSF is a disabling condition that does not yet have viable treatment alternatives. The growing knowledge of the genetic bases of this disease has enabled the identification of potential therapies that depend on further studies for the development of individualized

treatment protocols. The molecular investigation is helpful to confirm the diagnosis and family genetic counseling. In the present case, an interprofessional team involving dentists, nutritionists, pharmacists, dermatologists, palliative care and pediatricians, took care of the patient, demonstrating the importance of early multi-professional follow-up to increase the survival and quality of life of individuals affected by the disease.

Figure Legends

Figure 1: Physical appearance of the four year-old HFS patient. The limbs were fixed in a "frog-leg position" (a). Multiple small pearly papules on the skin near the nose and eyelids (b). Subcutaneous large nodules grew on the scalp bilaterally (c). Pearly papules were observed in flexural areas, as the nuchal skin (d). Nodules were present on the external ears (e).

Figure 2: Clinical and radiographic images of the oral condition of HFS patient. Massive gingival hyperplasia affected the maxilla and mandible (a), bilaterally (b and c). Panoramic radiography shows the deciduous dentition affected by multiple cavities (d).

Figure 3: Histopathological features of the biopsy of the gingival lesions. Gingival specimen was covered by stratified parakeratinized squamous epithelium (a: hematoxylin–eosin, original magnification: 20×). The connective tissue was highly collagenized with hypocellular areas showing eosinophilic and amorphous material (c and d: hematoxylin–eosin, original magnification: 50× and 200×). Clear spaces give the spindle cells a vacuolar aspect (d: hematoxylin–eosin, original magnification: 400×).

Figure 4: Molecular study of HFS patient and parents. *ANTXR2* is located on chromosome 4q21. Exon 13 is a hotspot of mutations implicated in HFS pathogenesis and was sequenced in the present case. The chromatogram of the patient's DNA sequencing exhibits the frameshift mutation c.1074del in homozygosis state (indicated by a red arrow), in addition to a SNP (rs12647691). The sequencing chromatogram of the father shows the same frameshift mutation c.1074del in heterozygosis state, as well as the SNP (rs12647691). The mother is also heterozygous for the frameshift mutation c.1074del and shows the the SNPs rs12647691 and rs72653288. The SNPs are indicated by gray arrows.

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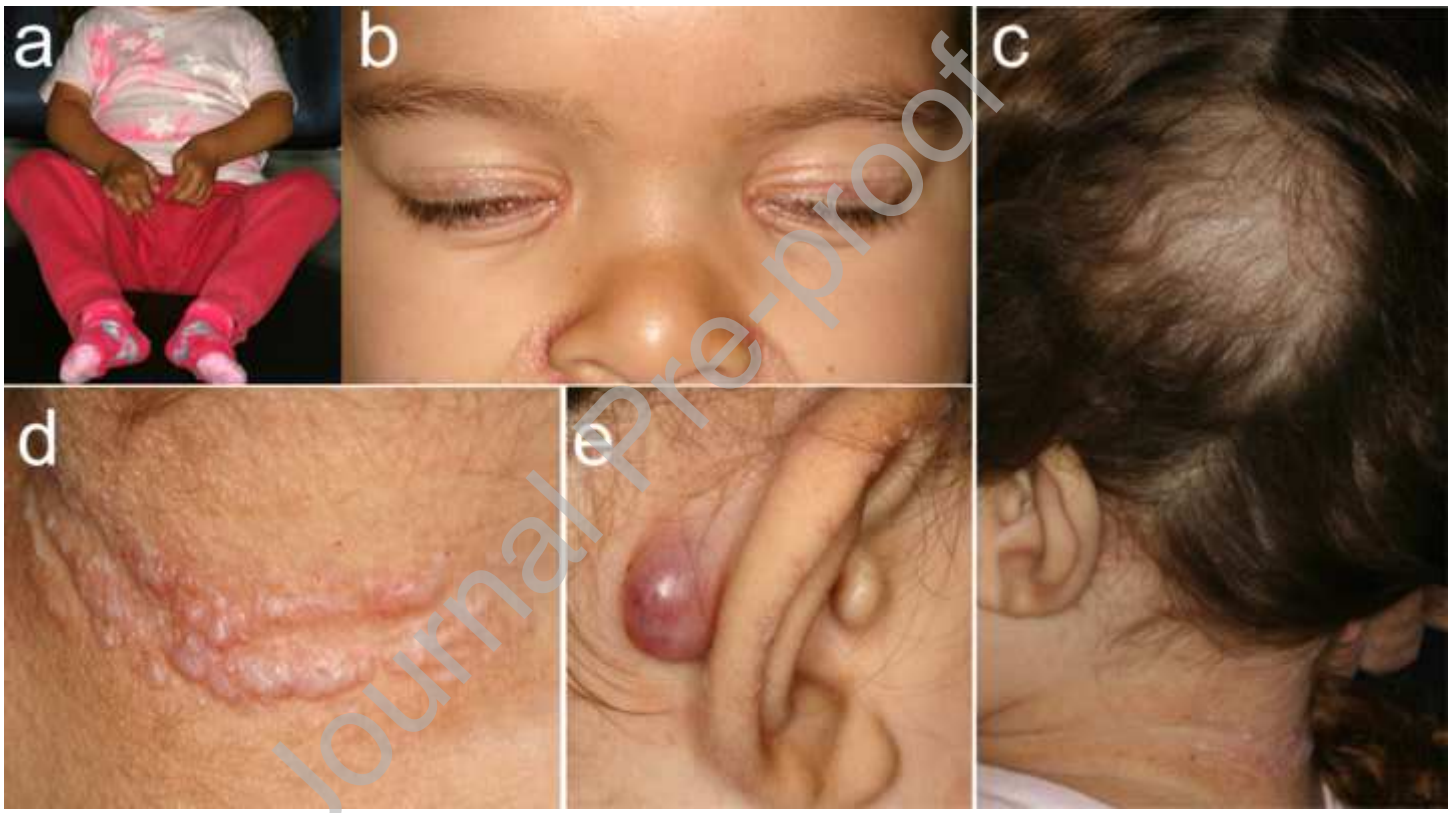
gure 1



gure 2

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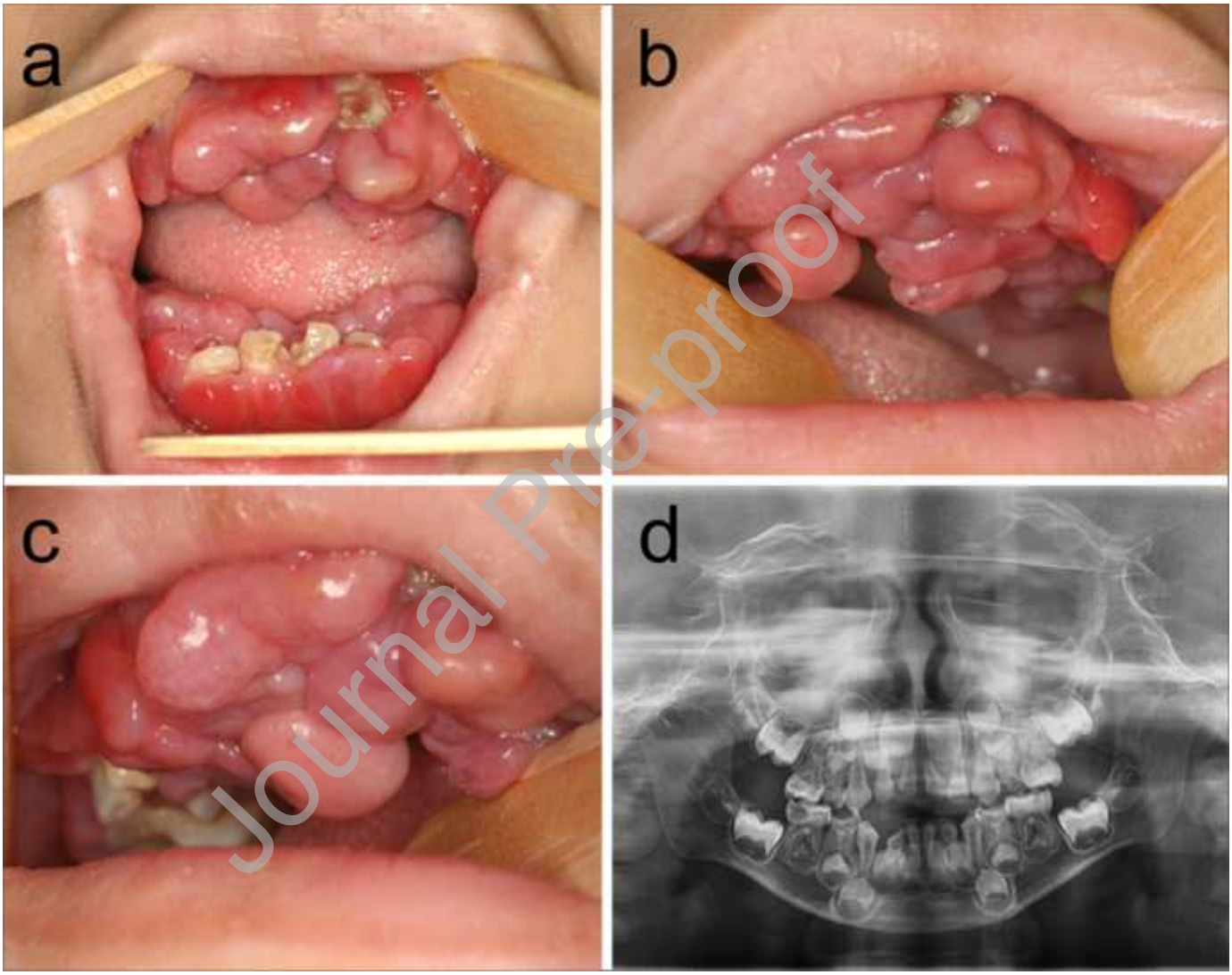

gure 3

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