Archives of Oral Biology 150 (2023) 105689

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

A novel deletion of exon 4 in the *Ectodysplasin A* gene associated with X-linked hypohidrotic ectodermal dysplasia

Konstantinos Agiannitopoulos [a,*], Kevisa Potska [a], Anna Douka [b,c,d], Iphigenia Gintoni [b,c,d], Georgios N. Tsaousis [a], Eirini Papadopoulou [a], George Nasioulas [a], Christos Yapijakis [b,c,d,**]

[a] *Genekor Medical S.A, Athens, Greece*
[b] *Unit of Orofacial Genetics, University Research Institute for the Study of Genetic & Malignant Disorders in Childhood, School of Medicine, National Kapodistrian University of Athens, Athens, Greece*
[c] *Unit of Orofacial Genetics, 1st Department of Pediatrics, School of Medicine, National Kapodistrian University of Athens, "Aghia Sophia", Children's Hospital, Athens, Greece*
[d] *Cephalogenetics Center, Athens, Greece*

ARTICLE INFO

Keywords:
Ectodysplasin A gene
Ectodermal dysplasia
Whole exome Sequencing
Copy Number Variation
Deletion

ABSTRACT

Objective: Identify the disease-causing mutation in a patient with features of X-linked hypohidrotic ectodermal dysplasia, which is a genetic disorder characterized by hypodontia, hypohidrosis and hypotrichosis. It is caused by mutations in *Ectodysplasin A* gene, which encodes ectodysplasin A, a member of the tumor necrosis factor superfamily.

Design: Genetic analysis, was performed using chromosomal microarray analysis, whole exome sequencing and multiplex ligation-dependent probe amplification analysis in a 4-year-old boy with hypohidrotic ectodermal dysplasia features. Moreover, the boy's parents were tested for clinically significant findings identified in order to elucidate the pattern of inheritance of the finding detected in the proband.

Results: A novel deletion of entire exon 4 in *Ectodysplasin A* gene identified in the 4-year-old patient. This deletion was found in heterozygous state in the mother of the proband and was not detected in his father. RNA analysis revealed an in-frame deletion r.527_706del, p.(176_236del) in exon 4 of the *Ectodysplasin A* gene.

Conclusion: We identified a novel gross deletion in the *Ectodysplasin A* gene in a male patient with X-linked hypohidrotic ectodermal dysplasia. Clinical and molecular genetic analysis are crucial to set an accurate diagnosis in patients with hypohidrotic ectodermal dysplasia. These results highlight the importance of the collagen domain of Ectodysplasin A, encoded by exon 4, for its function in vivo.

1. Introduction

The ectoderm is one of the three layers formed during embryogenesis, and is important for the development of exocrine glands, nerves, hair, nails, epidermis of the skin and enamel (Ansari & Pillarisetty, 2022). Ectodermal dysplasias represent several types of congenital disorders due to abnormal development of certain ectoderm-derived structures (Sepulveda et al., 2003). The two most common forms of the disease are hypohidrotic and anhidrotic ectodermal dysplasia. Hypohidrotic ectodermal dysplasia (HED) is a genetic condition that occurs in about 1 of 10,000 newborns. It is characterized by hypodontia, characteristic facial features, and hypotrichosis, while the severity of the symptoms presented can be variable among patients (Keller et al., 2001).

Several genes have been found to be associated with Hypohidrotic ectodermal dysplasia - and disorder-causing mutations may display X-linked, autosomal dominant, and autosomal recessive inheritance patterns. Genes *EDA*, *EDAR*, *EDARADD* and *WNT10A* account for 90% of hypoidrotic/anhidrotic ectodermal dysplasia cases (Cluzeau et al., 2011). Accordingly, hypohidrotic ectodermal dysplasia -related genetic

Abbreviations: XHED, X-linked Hypohidrotic Ectodermal Dysplasia; WES, Whole Exome Sequencing; MLPA, Multiplex Ligation-dependent Probe Amplification; CNV, Copy Number Variation; NGS, Next Generation Sequencing.

* Corresponding author.
** Correspondence to: Unit of Orofacial Genetics, 1st Department of Pediatrics, National Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece.
E-mail addresses: kagiannitopoulos@GENEKOR.com (K. Agiannitopoulos), cyapi@med.uoa.gr (C. Yapijakis).

https://doi.org/10.1016/j.archoralbio.2023.105689
Received 9 January 2023; Received in revised form 23 March 2023; Accepted 23 March 2023
Available online 24 March 2023

testing, usually with whole exome sequencing, that represents a relatively economic and effective tool for the study of genetic diseases, seems to be necessary for accurate diagnosis in affected individuals as well as for a basis for prenatal diagnosis (Yapijakis et al., 2021).

X-linked hypohidrotic ectodermal dysplasia (XLHED, OMIM 305100) is the most common form of the disease and caused by mutations in ectodysplasin A (*EDA* or *EDA-1*) gene on chromosome Xq13.1. Several variants of the *EDA* gene have been reported and the most frequent types are missense and non-sense (Burger K et al., 2014).

In this study, we describe the clinical features and molecular characterization of a novel hemizygous deletion of exon 4 in the *Ectodysplasin A* gene in a Greek male with X-linked hypohidrotic ectodermal dysplasia and his family.

2. Methods

A case of a 4-year-old boy with hypohidrotic ectodermal dysplasia features and only four cone-shaped teeth (oligodontia) was referred for accurate clinical diagnosis and genetic counseling. The boy had average height and weight (55th percentile) for his age, thin blonde scalp hair and light blue eyes, oligodontia (Fig. 1), normal hand and foot nails. The ability of the patient to sweat was not quantified, but his mother reported that he was able to sweat and was tolerant to heat. His parents reported no family history of hypohidrotic ectodermal dysplasia, but his phenotypically normal mother lacked 6 teeth suggesting a possible X-linked mode of inheritance (Fig. 2). After extensive genetic counseling and signed informed consent, blood samples were obtained from the patient and his parents. This study has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). This study was ethically approved (number RPURI9002) by the respective Committee of the University Research Institute for the Study of Genetic & Malignant Disorders in Childhood at the School of Medicine of the National Kapodistrian University of Athens.

Genomic DNA was extracted from the patient followed by Whole Exome Sequencing and chromosomal microarray analysis and by Next Generation Sequencing technology. The presence of Copy Number Variations (CNVs) was investigated by use of the Multiplex Ligation-dependent Probe Amplification method (MLPA, P183-C1 EDA- EDAR-EDARAD), according to the manufacturer's instructions (MRC Holland, MLPA General Protocol Version-008). Electrophoresis was carried out on SeqStudio Genetic Analyzer (Thermo Fisher Scientific, Applied Biosystems) and analysis was performed using the Coffalyser.Net software by MRC-Holland (version of Coffalyser.Net™: v.220513.1739).

Total RNA was extracted from peripheral blood lymphocytes using Trizol (Invitrogen, Paisley, UK) following a standard protocol. cDNA was synthesized using the SuperScript™ VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific) as described by the manufacturer. The resulting cDNA was amplified by specific primers around the region of each variant and positioned at least two exons up- and down-stream of the target region (EDAX2F_RNA: 5′-TCTTCTTCCCTGATGAAAAGCC-3′ and EDAX6R_RNA: 5′-TTGAATTGCTGACCCTTGGC-3′). The PCR-products were purified using the NucleoFast® 96 PCR Cleanup kit (Macherey-Nagel GmbH and Co., Düren, Germany). The purified PCR product was used for each sequencing reaction, performed using the BigDye® Terminator v1.1Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing reaction products were purified prior to electrophoresis using the Montage™ SEQ96 Sequencing Reaction kit (EMD Millipore Corp., Billerica, MA, USA) and sequenced using SeqStudio Genetic Analyzer (Thermo Fisher Scientific, Applied Biosystems).

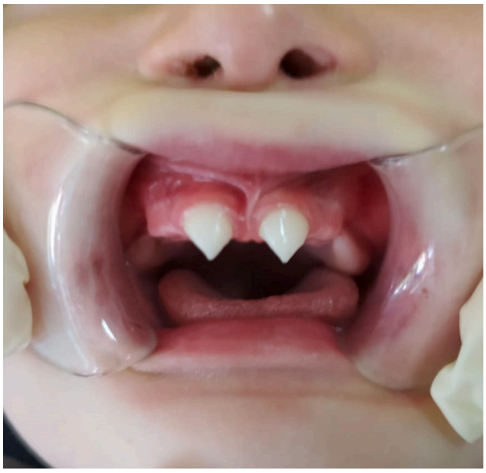

Fig. 1. Oral photo of the patient showing oligodontia.

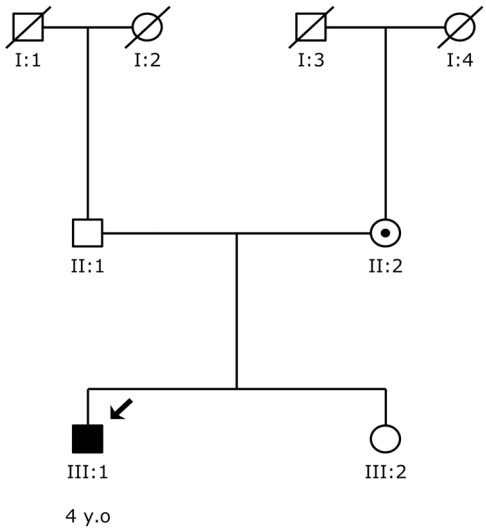

Fig. 2. Pedigree of the studied family with the patient (III-1) marked with an arrow.

3. Results

Initial attempts with Whole Exome Sequencing and chromosomal microarray analysis failed to detect clinically significant pathogenic variants. Consequently, the use of Multiplex Ligation-dependent Probe Amplification method analysis identified a hemizygous deletion of exon 4 in the *Ectodysplasin A* gene in our proband with HED (Fig. 3). The c.(526 +1_527–1)_(706 +1_707–1)del variant in *Ectodysplasin A* is a gross deletion of the genomic region encompassing exon 4 and was detected using the SALSA MLPA P183-C1 EDA- EDAR-EDARAD probe mix.

This variant is expected to lead to a deletion of 60 amino acid residues in the collagen-like domain of the EDA protein (Fig. 4). To our knowledge, this is the first time the aforementioned variant is described in a patient with X-Hypohidrotic ectodermal dysplasia. The identification of a hemizygous *Ectodysplasin A* pathogenic variant confirmed HED diagnosis.

The deletion was found in heterozygous state in the mother of the proband and was not detected in his father. RNA extracted from lymphocytes of the heterozygous mother followed by RNA analysis revealed an in-frame deletion r.527_706del, p.(176_236del) in exon 4 of the *Ectodysplasin A* gene (Fig. 5).

4. Discussion

X-Hypohidrotic ectodermal dysplasia is the most common type of HED and is caused by *Ectodysplasin A* gene mutations. EDA protein is a tumor necrosis factor (TNF) superfamily ligand consisting of a C-terminal tumor necrosis factor homology domain, a domain that is

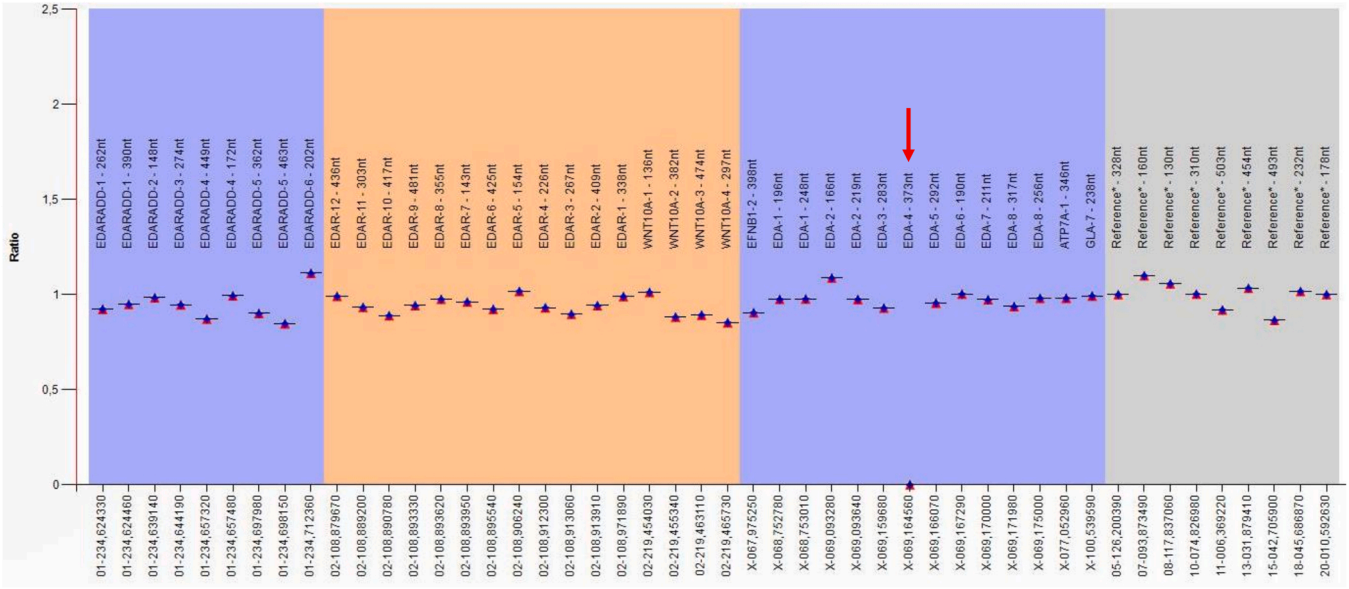

Fig. 3. Representative plots of the Multiplex Ligation-dependent Probe Amplification (MLPA) analysis by Coffalyser.Net showing the probe ratios with 95% confidence intervals as error bars for all exons of the *EDA- EDAR-EDARAD* genes. Calculated ratios are reported on the Y-axis and probes on the X-axis. Red dots highlight the hemizygous deletion of exon 4 in the *Ectodysplasin A* gene. The ratio of each individual reference probe in the normal reference sample is between 0.80 and 1.20.

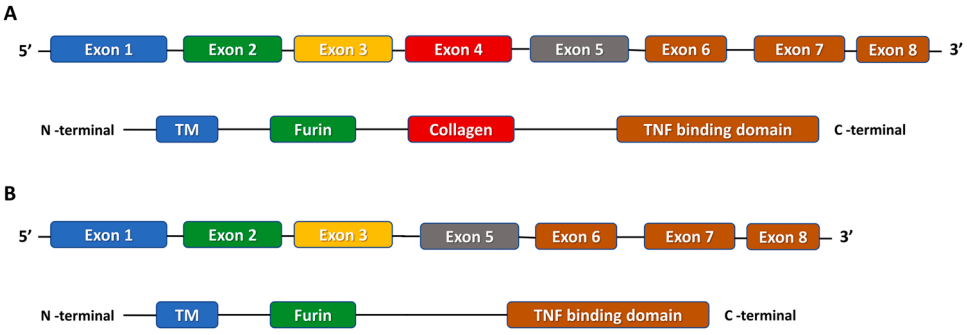

Fig. 4. Diagram of the *Ectodysplasin A* gene and of EDA1 protein. A. Wildtype B. Mutant TM: transmembrane domain; Furin: furin cleavage site; Collagen: collagen-like domain; TNF: tumor necrosis factor homology domain.

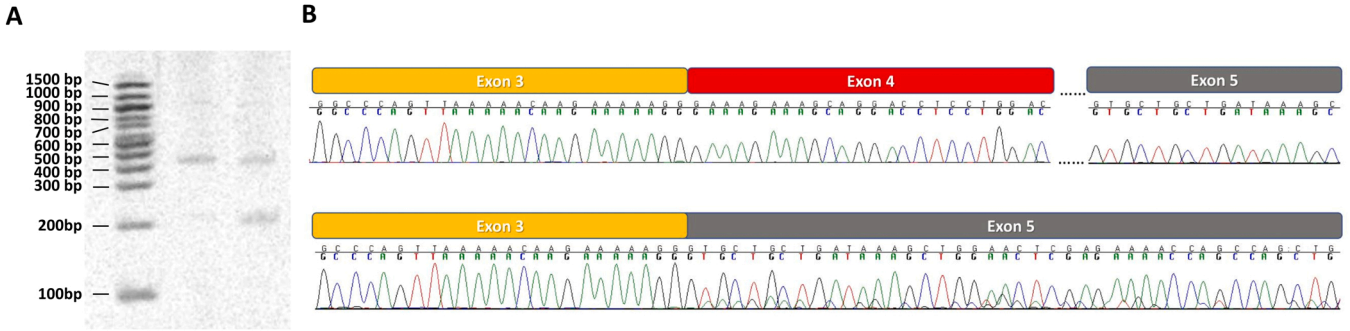

Fig. 5. A. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis. RNA was isolated from peripheral blood lymphocytes of heterozygous mother of patient and an unrelated healthy individual, converted to cDNA and amplified by PCR in the region of exon 4. PCR products were size fractionated by gel electrophoresis and submitted to Sanger sequencing. Lane 1, DNA length standard; Lane 2, control cDNA; Lane 3, mother of patient cDNA. B. Sequence analysis of RT-PCR revealed the deletion of exon 4 in *Ectodysplasin A* gene, resulting in an in-frame deletion (the bottom chromatograms) compared to the analysis of a wild type sample (top chromatogram).

responsible for binding with the receptor EDAR, activating the NF-κB pathway, and regulating the development of ectodermal organs. EDA is expressed by keratinocytes, hair follicles and sweat gland. EDA contains a transmembrane domain, putative furine cleavage site, collagen-like domain and tumor necrosis factor homology domain (Fig. 4) (Kowalczyk-Quintas & Schneider, 2014). Several variants in *EDA* gene have been reported in these regions, possibly affecting the secretion of EDA, the formation of the trimer EDA, or the receptor binding activity. The

alterations include missense, small deletion, insertion, non-sense, and splice-site variant as well as complete gene deletion (Liu et al., 2019). More than 60% of point mutations are identified in exons 7, 8, and 9, which encode for the tumor necrosis factor homology domain (Mues et al., 2010).

In our patient with X- Hypohidrotic ectodermal dysplasia features, an in-frame deletion of exon 4 occurs in the collagen-like domain of the EDA protein. The collagen-like domain plays an important role in the specific oligomerization of the tumor necrosis factor domain of EDA, which is crucial for protein function. The in-frame deletion of exon 4 deletes 60 amino acids comprising the entire collagen domain of EDA, but does not affect its furin processing site and its receptor-binding domain. Without the multimerization potential of the collagen domain, activation of EDAR by this mutant is probably reduced but not abolished. This would be in line with the mother's observation that the patient could sweat sufficiently to avoid heat intolerance, and with the finding that patients with hypomorphic mutations of EDA can sweat to a certain extent, while others with null mutations cannot sweat at all (Burger et al., 2014). Smaller In-frame deletions in the collagen-like domain in exon 4 of *Ectodysplasin A* gene have been identified in patients with X-Hypohidrotic ectodermal dysplasia (Martínez-Romero et al., 2019). Other gross deletions in the *Ectodysplasin A* gene have also been described in the literature (Sun et al., 2021).

X- Hypohidrotic ectodermal dysplasia is a serious disease that influences the physical and mental health of patients. The recommended cures for Hypohidrotic ectodermal dysplasia patients are prosthetic dental treatment, psychological treatment, and speech therapy to improve life quality (Bildik et al., 2012). The mother of the patient who has mild symptoms (hypodontia) was heterozygous for the exon 4 deletion in the *Ectodysplasin A* gene. That phenotype is within the phenotypic range of female carriers which varies from an absence of clinical symptoms to classic X- Hypohidrotic ectodermal dysplasia abnormalities of ectodermal structures, including teeth, nails, hair, and sweat glands (Savasta et al., 2019). This knowledge is important to be discussed during genetic counseling for future pregnancies and prenatal diagnosis in this and other X-Hypohidrotic ectodermal dysplasia families.

In conclusion, we identified a novel gross deletion in the *Ectodysplasin A* gene in a male patient with X-Hypohidrotic ectodermal dysplasia. The early recognition of the Hypohidrotic ectodermal dysplasia phenotype and molecular genetic diagnosis are very important to provide accurate genetic counseling in similar cases. Moreover, genetic testing in patients with Hypohidrotic ectodermal dysplasia is essential to include a full assessment of all genes by next generation sequencing technology and copy number variation analysis.

CRediT authorship contribution statement

Konstantinos Agiannitopoulos: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Kevisa Potska**: Investigation, Data curation, Methodology, Writing – review & editing. **Anna Douka**: Investigation, Writing – review & editing. **Iphigenia Gintoni**: Investigation, Writing – review & editing. **Georgios N. Tsaousis:** Investigation, Writing – review & editing. **Eirini Papadopoulou:** Conceptualization, Investigation, Writing – review & editing. **George Nasioulas**: Conceptualization, Writing – review & editing, Supervision. **Christos Yapijakis**: Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

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

Data availability

The data used to support the finding of this study are available from the corresponding authors upon request.

Acknowledgements

We thank all who participated in this study for their contribution.

References

Ansari, A., & Pillarisetty, L. S. (2022). Embryology, Ectoderm. StatPearls [Internet]. *Treasure Island (FL)*. StatPearls Publishing.

Bildik, T., Ozbaran, B., Kose, S., Koturoglu, G., Gokce, B., Gunaydin, A., & Altintas, I. (2012). Hypohidrotic ectodermal dysplasia: A multidisciplinary approach. *The International Journal of Psychiatry in Medicine, 44*, 225–240. https://doi.org/10.2190/PM.44.3.d

Burger, K., Schneider, A. T., Wohlfart, S., Kiesewetter, F., Huttner, K., Johnson, R., & Schneider, H. (2014). Genotype-phenotype correlation in boys with X-linked hypohidrotic ectodermal dysplasia. *The American Journal of Medical Genetics - Part A, 164A*, 2424–2432. https://doi.org/10.1002/ajmg.a.36541

Cluzeau, C., Hadj-Rabia, S., Jambou, M., Mansour, S., Guigue, P., Masmoudi, S., Bal, E., Chassaing, N., Vincent, M. C., Viot, G., Clauss, F., Manière, M. C., Toupenay, S., Le Merrer, M., Lyonnet, S., Cormier-Daire, V., Amiel, J., Faivre, L., de Prost, Y., Munnich, A., Bonnefont, J. P., Bodemer, C., & Smahi, A. (2011). *Human Mutation, 32*, 70–72. https://doi.org/10.1002/humu.21384

Keller, M. D., Petersen, M., Ong, P., Church, J., Risma, K., Burham, J., Jain, A., Stiehm, E. R., Hanson, E. P., Uzel, G., Deardorff, M. A., & Orange, J. S. (2001). Hypohidrotic ectodermal dysplasia and immunodeficiency with coincident NEMO and EDA mutations. *Frontiers in Immunology, 2*, 61. https://doi.org/10.3389/fimmu.2011.00061

Kowalczyk-Quintas, C., & Schneider, P. (2014). Ectodysplasin A (EDA) - EDA receptor signalling and its pharmacological modulation. *Cytokine Growth Factor Reviews, 25*, 195–203. https://doi.org/10.1016/j.cytogfr.2014.01.004

Liu, G., Wang, X., Qin, M., Sun, L., & Zhu, J. (2019). A novel missense mutation p.S305R of EDA gene causes XLHED in a Chinese family. *Archives of Oral Biology, 107*, Article 104507. https://doi.org/10.1016/j.archoralbio.2019.104507

Martínez-Romero, M. C., Ballesta-Martínez, M. J., López-González, V., Sánchez-Soler, M. J., Serrano-Antón, A. T., Barreda-Sánchez, M., Rodriguez-Peña, L., Martínez-Menchon, M. T., Frías-Iniesta, J., Sánchez-Pedreño, P., Carbonell-Meseguer, P., Glover-López, G., & Guillén-Navarro, E. (2019). EDA, EDAR, EDARADD and WNT10A allelic variants in patients with ectodermal derivative impairment in the Spanish population. *Orphanet Journal of Rare Diseases, 14*, 281. https://doi.org/10.1186/s13023-019-1251-x

Mues, G., Tardivel, A., Willen, L., Kapadia, H., Seaman, R., Frazier-Bowers, S., Schneider, P., & D'Souza, R. N. (2010). Functional analysis of Ectodysplasin-A mutations causing selective tooth agenesis. *The European Journal of Human Genetics, 18*, 19–25. https://doi.org/10.1038/ejhg.2009.127

Savasta, S., Carlone, G., Castagnoli, R., Chiappe, F., Bassanese, F., Piras, R., Salpietro, V., Brazzelli, V., Verrotti, A., & Marseglia, G. L. (2019). X-LInked hypohidrotic ectodermal dysplasia: New features and A Novel EDA gene mutation. *Cytogenetic and Genome Research, 152*, 111–116. https://doi.org/10.1159/000478922

Sepulveda, W., Sandoval, R., Carstens, E., Gutierrez, J., & Vasquez, P. (2003). Hypohidrotic ectodermal dysplasia: Prenatal diagnosis by three-dimensional ultrasonography. *Journal of Ultrasound in Medicine, 22*, 731–735. https://doi.org/10.7863/jum.2003.22.7.731

Sun, J., Chen, L., Han, S., He, Y., Dumitru, A. G., Zhou, W., Cai, J., & Qi, M. (2021). Characterization of a novel gross deletion and insertion mutation in EDA gene causing hypohidrotic ectodermal dysplasia. *The International Journal of Dermatology, 48*, e29–e30. https://doi.org/10.1111/1346-8138.15653

Yapijakis, C., Gintoni, I., & Chrousos, G. (2021). Prenatal genetic testing for X-linked hypohidrotic ectodermal dysplasia. *Advances in Experimental Medicine and Biology, 1339*, 337–340. https://doi.org/10.1007/978-3-030-78787-5_41