third degree). The patient with a third degree was not treated for the LS during the first pregnancy, and there was some delayed healing. There were no issues with her second delivery when LS was quiescent. No patients developed LS in obstetric scars.

In a series of 40 patients studied for the effect of the oral contraceptive on LS, four became pregnant and reported complete remission of disease during pregnancy.[3] A study of 33 pregnancies in 29 patients with vulval LS reported 27 spontaneous vaginal births, two instrumental deliveries and 4 LSCS. Only one non-compliant patient required a section due to LS-related scarring.[4] One patient developed LS in a perineal scar. Two women with newly diagnosed LS and who delivered by normal spontaneous vaginal delivery without problems have been reported.[5]

The proportion of women with an intact perineum at delivery was 9.6% in nulliparae and 31.2% in multiparae.[6] In our population with LS, 10/21 nulliparae had tears and 9/21 had episiotomies; therefore, there was no increased incidence of perineal tears and episiotomies compared to the general population. No patients reported sexual dysfunction during follow-up of up to 4 years in many cases.

There is conflicting literature on the requirements for TCS during pregnancy and by extrapolation the severity of LS during pregnancy. There was no significant change in the TCS requirements in one study,[4] but in others the effect was variable.[7,8] In our cohort, 55% patients had the same TCS requirements during pregnancy but 45% decreased their use suggesting improvement in their LS.

In conclusion, LS does not worsen during pregnancy and can improve. When the disease is well controlled, there is no contraindication to vaginal delivery and women can be reassured about this.

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References

1 Goldstein AT, Marinoff SC, Christopher K *et al*. Prevalence of vulvar lichen sclerosus in a general gynecology practice. *J Reprod Med* 2005; **50**: 477–480.
2 Lee A, Bradford J, Fischer G. Long-term management of adult vulvar lichen sclerosus: a prospective cohort study of 507 women. *JAMA Dermatol* 2015; **151**: 1061–1067.
3 Gunthert AR, Faber M, Knappe G *et al*. Early onset vulvar lichen sclerosus and pre-menopausal women and oral contraceptives. *Eur J Obstet Gynecol Reprod Biol* 2008; **137**: 56–60.
4 Nguyen Y, Bradford J, Fischer G. Lichen sclerosus in pregnancy: a review of 33 cases. *Aust N Z J Obstet Gynaecol* 2018; **58**: 686–689.
5 Haefner HK, Pearlman MD, Barclay ML *et al*. Lichen sclerosus in pregnancy: presentation of two cases. *J Low Genit Tract Dis* 1999; **3**: 260–263.
6 Smith LA, Price N, Simonite V *et al*. Incidence of and risk factors for perineal trauma: a prospective observational study. *BMC Pregnancy Childbirth* 2013; **13**: 59.
7 Dalziel KL. Effect of lichen sclerosus on sexual function and parturition. *J Reprod Med* 1995; **40**: 351–354.
8 Helm KF, Gibson LE, Muller SA. Lichen sclerosus et atrophicus in children and young adults. *Pediatr Dermatol* 1991; **8**: 97–101.

DOI: 10.1111/jdv.15788

X-linked hypohidrotic ectodermal dysplasia: clinical and molecular genetic analysis of a large Russian family with a synonymous p.Ser267= (c.801A>G) splice site mutation

Editor

X-linked hypohidrotic ectodermal dysplasia (XLHED) is the most common form of ectodermal dysplasias.[1] Mutations in the *EDA* gene are the cause of XLHED.[2] This study describes a large Russian family with XLHED (Fig. 1).

The proband's examination revealed a large forehead with prominent supraorbital ridges and forehead bumps, wide cheekbones, small saddle-shaped nose with hypoplastic alae nasi, narrow and short maxillary regions, slightly deformed ears. The hair, eyebrows and eylashes were light-coloured, sparse and brittle. The skin was dry and pale. However, according to the mother, the proband had good heat tolerance. Examination revealed hypoplastic nipples, but nail structure was normal. The proband was susceptible to respiratory infections. Intraoral examination showed complete adontia. The mouth mucosa was pale, with noticeable hypersalivation. The maxilla and mandibula were hypoplastic with underdeveloped alveolar processes. X-ray orthopantomography revealed four rudimentary baby teeth and two rudimentary permanent central maxilla incisors.

The proband's *EDA* gene coding sequence and exon–intron junction analysis by Sanger sequencing revealed a synonymous p. Ser267= (c.801A>G) variant in exon 7. This variant was not present in the dbSNP or the Genome Aggregation Database but was described in HGMD (CS1614178) by Wohlfart *et al*.[3] The c.801A>G variant segregates with the disease: it was detected in all affected males in hemizygous state, in females – in heterozygous state and was not detected in a healthy blood relative (Fig. 1). The probability of the simultaneous inheritance of the XLHED and the revealed *EDA* gene variant in this family being a coincidence is about 0.01%.

In silico Human Splicing Finder and ESE finder analysis revealed that c.801A>G variant could break the site of an exonic splicing enhancer (ESE) and create a new exonic splicing silencer (ESS), resulting in pre-mRNA splicing alteration. However, three other splicing tools – BDGP, SplicePort and NetGene2 –

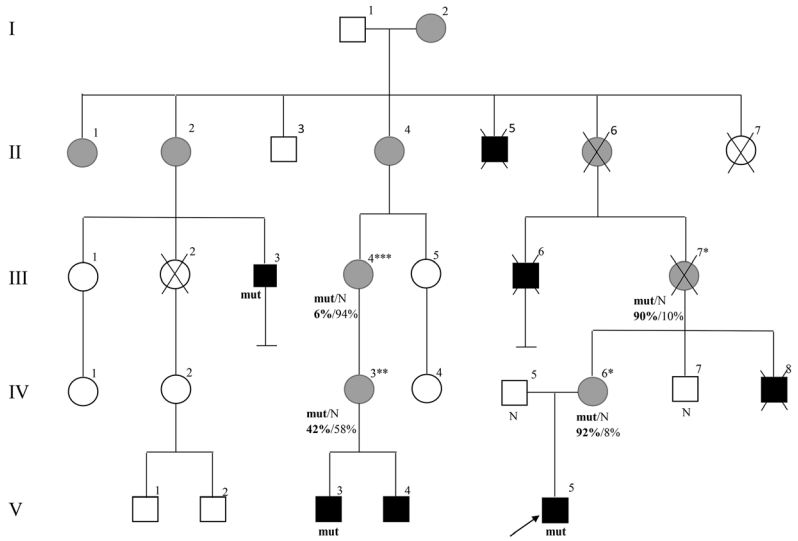

Figure 1 Pedigree of a Russian family with ectodermal hypohidrotic dysplasia. Squares and circles represent males and females, respectively. The black symbol indicates the affected member; the grey symbol, the female carriers; and the open symbols, the unaffected members. The patient above the arrow is the proband of this family. mut – p.Ser267= (c.801A>G) variant in the *EDA* gene. N – corresponds with *EDA* reference sequence. Numbers under the genotype of female carriers indicate the percentage of X chromosome inactivation: 92% – of the mutant X chromosome, 8% – of the intact X chromosome (e.g. proband's mother IV.6). The obtained data correlate with the severity of the clinical findings seen in female carriers. *mild clinical manifestations: slight dryness of the skin. **moderate clinical manifestations: micro and hypodontia, dry skin. ***severe clinical manifestations: hypotrichosis, hypodontia, hypohidrosis, dry skin with atopic eczema, poor mammary gland development, difficulty breastfeeding, increased susceptibility to infections.

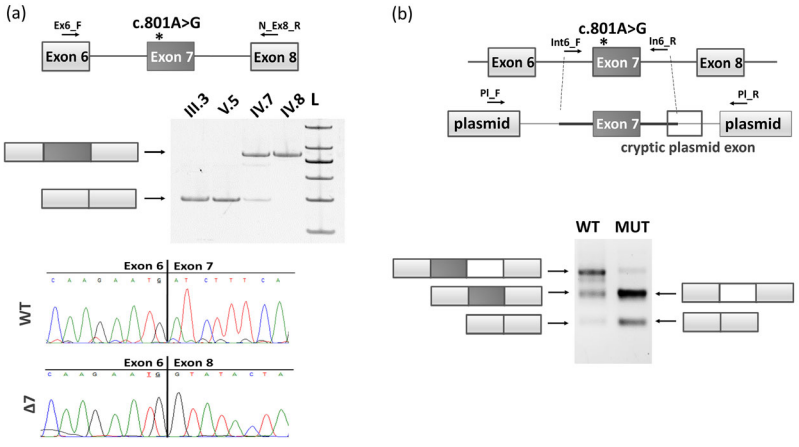

Figure 2 Functional pathogenicity evidence of the c.801A>G variant in the *EDA* gene. (a) RT-PCR analysis of *EDA* mRNA structure revealed that the c.801A>G variant leads to exon 7 skipping (Δ7). On the top, the scheme of analysed *EDA* locus is depicted. PAGE electrophoregram shows Δ7 *EDA* mRNA isoform in both patients' samples (III.3, V.5), wild-type (WT) and Δ7 isoforms in the mother's sample (IV.6) and WT isoform in the father's sample (IV.5). Sanger sequences of corresponding PCR products are shown below. (b) Results of minigene assay. The scheme of minigene cloning and analysis is depicted above. Agarose gel electrophoregram of RT-PCR fragments generated from minigene-spliced RNA of wild-type (WT) and mutant (MUT) constructions are depicted below. The empty rectangle represents the artificial exon created by a cryptic splice acceptor site of *EDA* intron 7 and cryptic splice donor site of the vector intron. Analysis revealed that mutant construction produces only transcripts with exon 7 sipping, while wild-type construction shows its inclusion.

did not predict any splicing changes. To validate the influence of the synonymous variant c.801A>G on *EDA* pre-mRNA splicing, we performed reverse transcription with nested PCR analysis of total RNA obtained from patients' peripheral blood mononuclear cells (Fig. 2a). RT-PCR analysis revealed the shorter isoform of *EDA* mRNA with exon 7 skipping (Δ7) in the patients' samples, while the unaffected father had only wild-type (WT) isoform and the mother had both isoforms. To additionally prove the pathogenic role of the investigated variant, we performed a minigene splicing assay (Fig. 2b).[4]

The result confirmed that the *EDA* c.801A>G variant leads to exon 7 skipping. The absence of the exon 7 in the structure of mature *EDA* mRNA leads to a frame shift resulting in a truncated non-functional EDA protein p.(D265Gfs7*), lacking part of the conserved motifs in the TNF-related domain and the whole cysteine-rich C-terminal domain.[5] According to variant interpretation criteria,[6] the c.801A>G variant should be considered pathogenic: PVS1, PS3, PM1, PM2, PP3 and PP5. Thus, an independent comprehensive analysis of the synonymous p.Ser267= (c.801A>G) *EDA* gene variant's clinical significance in a large Russian family showed its critical splicing effect on ectodysplasin-A synthesis.

X-linked hypohidrotic ectodermal dysplasia shows incomplete penetrance in females. The probability of recognizing a female carrier via clinical examination is evaluated to be 60–70%.[5] The incomplete penetrance in female carriers can be caused by unequal X chromosome inactivation. The unequal X chromosome inactivation pattern analysis was performed for female carriers.[7] It is worth noting that two females in the studied family are confirmed as asymptomatic mutation carriers while other female relatives have variable HED signs (Fig. 1). The obtained data confirm the theoretical suggestions about XLHED pathogenic mechanisms in female carriers: there is an explicit correlation between the disease severity and the non-random X chromosome inactivation level in the studied family.

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The research was carried out within the state assignment of FASO Russia.

References

1 Dall'Oca S, Ceppi E, Pompa G, Polimeni A. X-linked hypohidrotic ectodermal dysplasia: a ten-year case report and clinical considerations. *Eur J Paediatr Dent* 2008; **4**(Suppl): 14–18.
2 Savasta S, Carlone G, Castagnoli R *et al.* X-linked hypohidrotic ectodermal dysplasia: new features and a novel EDA gene mutation. *Cytogenet Genome Res* 2017; **152**: 111–116.
3 Wohlfart S, Hammersen J, Schneider H. Mutational spectrum in 101 patients with hypohidrotic ectodermal dysplasia and breakpoint mapping in independent cases ofrare genomic rearrangements. *J Hum Genet* 2016; **61**: 891–897.
4 Filatova AY, Vasilyeva TA, Marakhonov AV, Voskresenskaya AA, Zinchenko RA, Skoblov MY. Functional reassessment of PAX6 single nucleotide variants by in vitro splicing assay. *Eur J Hum Genet* 2019; **27**: 488–493.
5 Vincent MC, Biancalana V, Ginisty D, Mandel JL, Calvas P. Mutational spectrum of the ED1 gene in X-linked hypohidrotic ectodermal dysplasia. *Eur J Hum Genet* 2001; **9**: 355–363.
6 Richards S, Aziz N, Bale S *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17**: 405–424.
7 Bolduc V, Chagnon P, Provost S *et al.* No evidence that skewing of X chromosome inactivation patterns is transmitted to offspring in humans. *J Clin Invest* 2008; **118**: 333–341.

DOI: 10.1111/jdv.15798

Incidence of cutaneous adverse events after exposure to tenofovir–emtricitabine in HIV-uninfected vs HIV-infected patients: pharmacovigilance within a large Midwestern U.S. patient population from the Research on Adverse Drug events And Reports program

Editor

Tenofovir disoproxil fumarate (TDF) combined with emtricitabine (FTC) for HIV pre-exposure prophylaxis (PrEP; FDA approval 07/2012) has been shown to dramatically decrease HIV acquisition rates in high-risk populations.[1–4] TDF-FTC is also used as part of multidrug combination antiretroviral (cART) regimens for treatment of HIV(+) patients (FDA approval 08/2004). When TDF-FTC is used as part of cART, cutaneous adverse events (cAEs), including serious cAEs, have been well-reported.[5,6] However, no cAEs were reported in premarketing trials for those exposed to PrEP.[1–3,7] The aim of this study is to determine the incidence of cAEs for TDF-FTC in HIV(−) persons compared to HIV(+) persons receiving TDF-FTC as a component of cART.

Using RADAR methodology,[8] retrospective data were extracted (2001—2017) from a medical record database for a large urban, Midwestern U.S. patient population of >6 million patients for persons prescribed TDF-FTC for HIV prevention (ICD9: V01, 79; ICD10: Z20.6), and compared to those with HIV/AIDS infection (ICD9: V08, 042; ICD10: Z21, B20-24) who were

