Noninvasive Prenatal Diagnosis of Hypohidrotic Ectodermal Dysplasia by Tooth Germ Sonography

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Noninvasive Prenatal Diagnosis of Hypohidrotic Ectodermal Dysplasia by Tooth Germ Sonography

Nicht-invasive vorgeburtliche Diagnose der hypohidrotischen ektodermalen Dysplasie durch sonografische Darstellung der Zahnanlagen

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Key words
- mouth
- ultrasound 2 D
- dysplasias
- fetus
- genetic defects

Abstract

Purpose: Hypohidrotic ectodermal dysplasia, a potentially life-threatening heritable disorder, may be recognized already in utero by characteristic features such as oligodontia and mandibular hypoplasia. As therapeutic options and prognosis depend on the time point of diagnosis, early recognition was attempted during routine prenatal ultrasound examinations.

Subjects and Methods: Fetuses of nine pregnant women (one triplet and eight singleton pregnancies) with family histories of hypohidrotic ectodermal dysplasia were investigated by sonography between the 20th and 24th week of gestation.

Results: In 4 male and 2 female fetuses reduced amounts of tooth germs were detected, whereas 5 fetal subjects showed the normal amount. Three-dimensional ultrasound evaluation revealed mandibular hypoplasia in 5 of the 6 fetuses with oligodontia. Molecular genetic analysis and/or clinical findings after birth confirmed the prenatal sonographic diagnosis in each subject.

Conclusion: In subjects with a family history of hypohidrotic ectodermal dysplasia, the diagnosis of this rare condition can be established noninvasively by sonography in the second trimester of pregnancy. Early recognition of the disorder may help to prevent dangerous hyperthermic episodes in infancy and may allow timely therapeutic interventions.

Zusammenfassung

Studienzweck: Die hypohidrotische ektodermale Dysplasie, eine potentiell lebensbedrohliche Erbkrankheit, führt bereits in utero zu charakteristischen Befunden wie Oligodontie und Unterkieferhypoplasie. Da sowohl die Behandlungsmöglichkeiten als auch die Prognose vom Zeitpunkt der Diagnosestellung abhängen, wurde eine Früherkennung im Rahmen pränatalsonografischer Routineuntersuchungen angestrebt.

Probanden und Methoden: Die Feten von 9 schwangeren Frauen (eine Drillings- und 8 Einlingsschwangerschaften) mit familiär bekannter hypohidrotischer ektodermaler Dysplasie wurden zwischen der 20. und 24. Schwangerschaftswoche sonografisch untersucht.

Ergebnisse: Bei 4 männlichen und 2 weiblichen Feten war eine deutlich verminderte Zahl an Zahnanlagen nachweisbar, während 5 Feten die normale Anzahl aufwiesen. 3D-Bildgebung offenbarte eine Unterkieferhypoplasie bei 5 der 6 Feten mit Oligodontie. Molekulargenetische und/oder klinische Befunde nach der Geburt bestätigten in allen Fällen die pränatalsonografische Diagnose.

Schlussfolgerung: Bei familiär bekannter hypohidrotischer ektodermaler Dysplasie ist durch Ultraschalluntersuchung im zweiten Schwangerschaftstrimenon eine nichtinvasive vorgeburtliche Diagnosestellung möglich. Die Früherkennung dieser Krankheit kann zur Vermeidung gefährlicher Hyperthermieepisoden im Säuglingsalter beitragen und rechtzeitige therapeutische Interventionen ermöglichen.

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Introduction

Hypohidrotic ectodermal dysplasia (HED), a rare heritable disorder, is characterized by a lack of sweat, sebaceous, submucous, meibomian and mammary glands, hypotrichosis, and oligodontia. Patients display typical facial features such as sparse eyelashes and eyebrows, prominent lips and hypoplasia of the mandible, and they suffer from disturbed thermoregulation [1, 2]. In early

childhood HED is a life-threatening condition [2, 3] mainly based on the risk for hyperpyrexia. Therapeutic options and prognosis clearly depend on the time point of diagnosis. There is a higher mortality in the first affected child within a sibship than in the later born affected subjects [2].

Characteristic features such as missing tooth germs and a hypoplastic lower jaw may allow prenatal sonographic recognition of HED, if the disease is known to the family or the doctor. Early awareness of the potential hazards related to this rare disorder is most helpful in preventing avoidable calamities, induced for example by placing the baby in an incubator after birth [4]. Detailed investigation of the sweating ability and molecular genetic analysis can be arranged later to make a definitive diagnosis [5].

X-linked hypohidrotic ectodermal dysplasia (XLHED [MIM 305 100]), the most common form of HED, is caused by the absence or dysfunction of the signaling molecule ectodysplasin A1 encoded by the gene *EDA* [6]. In mouse and dog models, prenatal and early postnatal ectodysplasin A1 replacement corrected the developmental abnormalities to a large extent [7 – 9]. Meanwhile the first human neonates with XLHED have been enrolled in a phase 2 trial of recombinant ectodysplasin A1 (www.clinicaltrials.gov NCT01 775 462). As dosing should be initiated in the first days of life, early perinatal diagnosis is a prerequisite for participating in this study. The trial is restricted to male subjects, because the clinical phenotype of XLHED is consistently severe in affected males and more variable in heterozygous females as the result of random X-chromosome inactivation.

In the human embryo, tooth development starts already 30 to 40 days after conception. Fetal teeth are normally detectable by sonography in the fourth month of pregnancy [10, 11]. Here we report for the first time experiences with the noninvasive prenatal diagnosis of XLHED by tooth germ sonography.

Subjects and methods

Fetuses of nine pregnant women with family histories of ectodermal dysplasia and known *EDA* mutations (one triplet and eight singleton pregnancies) were investigated during routine prenatal sonography between the 20th and 24th week of gestation. All women provided informed consent prior to sonography.

Postnatally the neonates were assessed for signs of XLHED. In case of any relevant symptoms, the children we referred to the German Competence Centre for Children with Ectodermal Dysplasias, where a blood sample was investigated by sequence analysis of the gene *EDA*.

Prenatal sonography was performed with high-end devices and a standard transducer (annular array probe, 5 MHz, with a sweep angle of 60°). Fetal tooth buds were observed in horizontal sections of the jaw and counted both in the maxilla and the mandible. The mean number of tooth germs detected by 2 D ultrasonography between the 20th and 24th week of gestation (six in the maxilla and six in the mandible) was considered normal. Apart from the number, sonomorphological aspects of the teeth were examined, particularly their calcification. The depiction of tooth buds was preceded by fetal biometry and detailed screening for malformations.

Postnatal sonography of the jaws was conducted with a high-end device (Acuson S2000, linear transducer – hockey stick 14L5SP, 14 MHz, Siemens, Erlangen).

Results

In all fetal subjects, tooth germs with mineralized parts of high echogenicity in the typical alveolar structures could be detected. Four male and two female fetuses showed a reduced number of tooth germs (**Table 1** and **Fig. 1**), whereas a normal amount was found in the remaining five subjects (three males, two fe-

Table 1 XLHED diagnosis by prenatal tooth germ sonography between 2010 and 2013.

patient	maternal EDA mutation	time point of prenatal sonography	sonographic diagnosis	postnatal diagnosis
M1	p.P17GfsX81	22nd week of gestation	male fetus, only two tooth germs detectable → XLHED	XLHED
M2	p.Y304C	22nd week of gestation	male fetus, normal amount of tooth germs	no XLHED
M3	p.P220_P225del	22nd week of gestation	male fetus, clearly reduced number of tooth germs → XLHED	XLHED
M4	Exon3del	21st week of gestation	male fetus, normal amount of tooth germs	no XLHED
M5	p.R155C	22nd week of gestation	male fetus, normal amount of tooth germs	no XLHED
M6	p. R156H	21st week of gestation	reduced number of tooth germs in one of the polyzygotic triplets (male fetus) → XLHED	XLHED
M7	Exon3dupl	24th week of gestation	male fetus, only two tooth germs detectable → XLHED	XLHED
F1	p. R156H	21st week of gestation	normal amount of tooth germs in one of the polyzygotic triplets (female fetus)	no XLHED
F1	p. R156H	21st week of gestation	reduced number of tooth germs in one of the polyzygotic triplets (female fetus) → XLHED	XLHED
F3	p.G85AfsX6	20th week of gestation	female fetus, normal amount of tooth germs	no XLHED
F4	p.P220_P225del	20th week of gestation	female fetus, reduced number of tooth germs → XLHED	XLHED

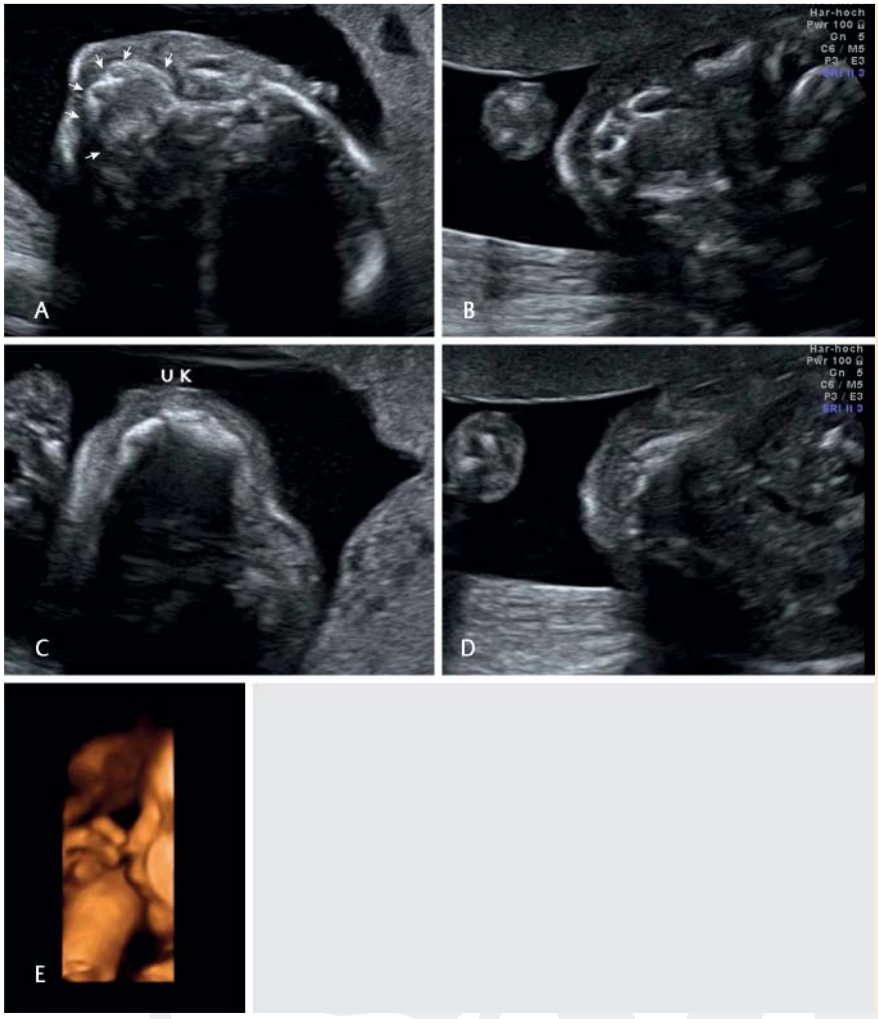

Fig. 1 Prenatal tooth germ sonography in the 22nd week of gestation. **A** upper jaw of fetus M1 with XLHED showing only two dental alveoli (round hypoechogenic areas with central white spots); **B** upper jaw of a healthy control fetus; **C** mandible of fetus M1 without any dental alveoli; **D** mandible of a healthy control fetus; **E** mandibular hypoplasia of fetus M1 in the three-dimensional image.

Abb. 1 Pränatalsonografie der Zahnanlagen in der 22. Schwangerschaftswoche. **A** Oberkiefer des Feten M1 mit XLHED, der nur zwei Zahnalveolen aufweist (runde hypoechogene Areale mit zentralen Aufhellungen). **B** Oberkiefer eines gesunden Kontrollfeten. **C** Unterkiefer des Feten M1 ohne Zahnanlagen. **D** Unterkiefer eines gesunden Kontrollfeten. **E** Mandibulahypoplasie des Feten M1 im dreidimensionalen Bild.

males). Three-dimensional ultrasound evaluation proved helpful for assessing the degree of mandibular hypoplasia which is commonly associated with oligodontia in the mandible. A hypoplastic lower jaw was evident in the four male fetuses and in one of the two female fetuses with oligodontia. The duration of the examination and accuracy of tooth germ counts depended mainly on the position of the fetal head.

The parents of five fetal subjects requested an amniocentesis and molecular genetic analysis of fetal cells to establish a definitive diagnosis. In one case of an apparently normal amount of tooth germs, fetal MRI was used to confirm the sonographic estimate (**Fig. 2**).

In all 11 subjects the prenatal sonographic diagnoses proved to be correct as evidenced by pre- or postnatal molecular genetic analysis and/or clinical findings after birth. Postnatal sonography of the jaws was performed in two male neonates with reduced amounts of tooth germs and confirmed the prenatal diagnoses. The teeth of XLHED subjects were found to be pointed and much less mineralized than the teeth of the healthy control subjects (**Fig. 3**).

Discussion

During the last years, investigation of the fetal face has become more and more important for prenatal sonographers, as this may provide indications for fetal malformations and chromosomal abnormalities. To date, little attention has been paid to fetal tooth germs, although missing tooth germs may lead to an early recognition of heritable developmental disorders such as ectodermal dysplasia [10 – 12].

Our data clearly demonstrate that the diagnosis of XLHED can be established by noninvasive prenatal tooth germ sonography between the 20th and 24th week of gestation, at least in subjects with a family history of the disorder. This would eliminate the significant risks associated with invasive diagnostic procedures such as amniocentesis [13, 14] which may be requested by women known to carry a pathogenic *EDA* mutation. Prenatal diagnosis of this rare condition is reasonable to take appropriate postnatal measures that prevent potentially life-threatening hyperthermic episodes soon after birth. However, as XLHED patients require dental prostheses and later tooth implants, are predisposed to atopy presenting with eczema and asthma, chronic sinusitis, and dry eye complications [15], suffer from self-esteem issues and the impact of their susceptibility to hyperthermia on outdoor activities [16] and occupational choices, early therapeutic interventions which may provide a sustained health benefit are most desirable.

The role of ectodysplasin A1 and its receptor during odontogenesis has not been clarified entirely. Tooth morphogenesis is regulated by complex epithelial-mesenchymal signaling cascades involving a number of molecules of the fibroblast growth factor

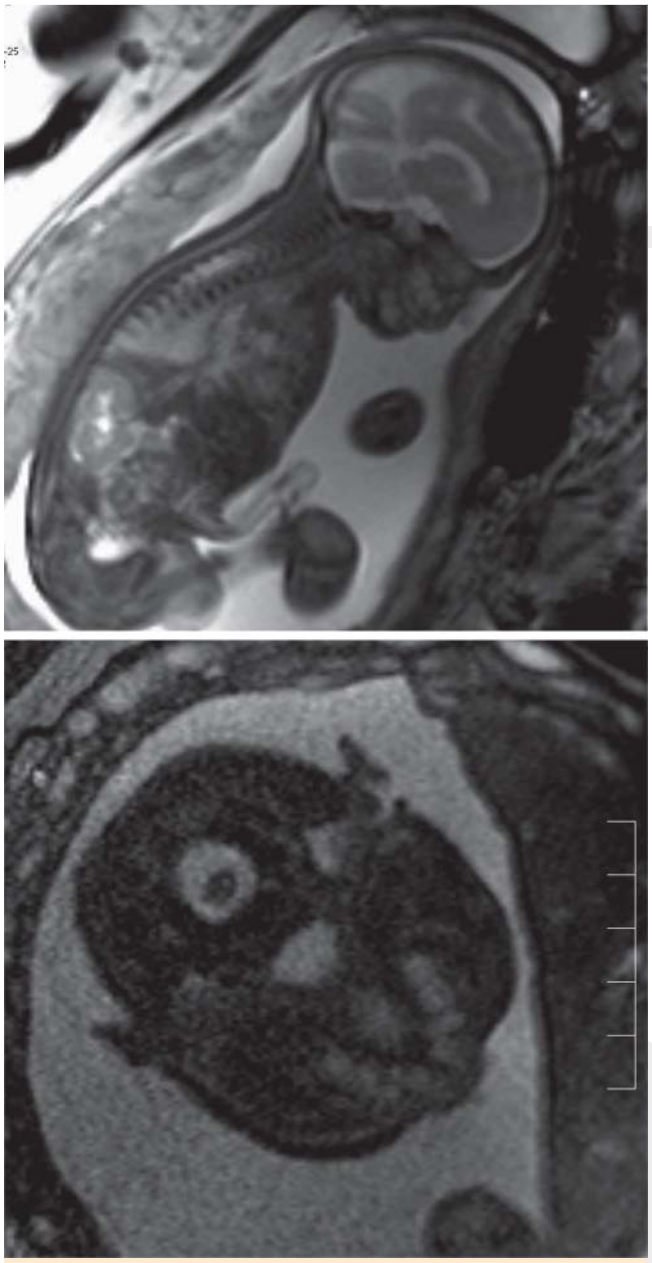

Fig. 2 Tooth germs of subject M2 visualized by fetal MRI.

Abb. 2 Darstellung der Zahnanlagen des Feten M2 mittels Magnetresonanztomografie.

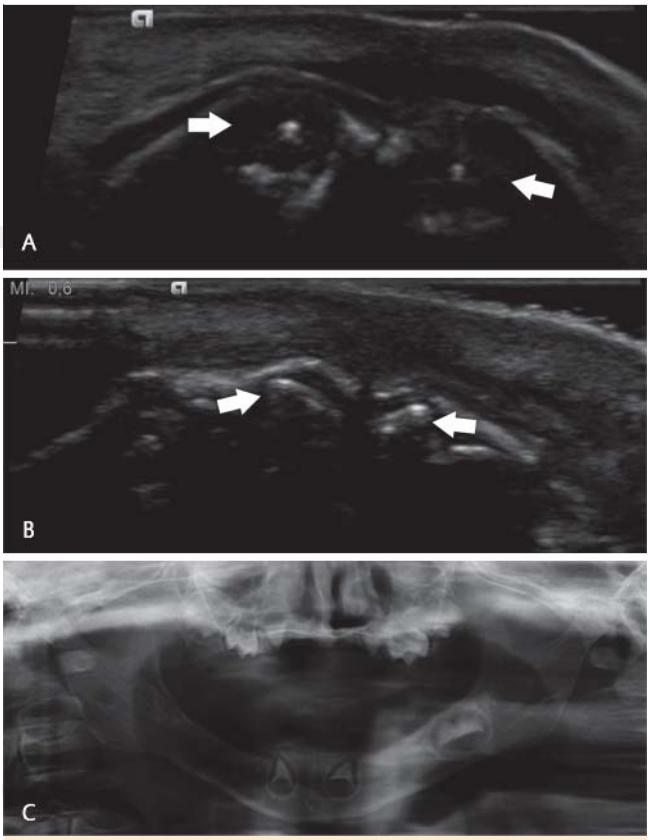

Fig. 3 Postnatal confirmation of tooth germ estimates by neonatal sonography or later X-ray examination. **A** neonatal sonography of the upper jaw of subject M7 with XLHED showing only two round hypoechogenic tooth germs (arrowed) with nearly unmineralized pointed teeth; **B** neonatal sonography of the upper jaw of a healthy control subject with normal tooth germs characterized by hyperechogenic, normally shaped teeth (arrowed) and small hypoechogenic margins; **C** dental panoramic radiograph of subject M6 with XLHED at an age of two years allowing reliable tooth quantification and demonstrating oligodontia.

Abb. 3 Postnatale Bestätigung der vorgeburtlich bestimmten Zahl der Zahnanlagen durch Sonografie am Neugeborenen oder spätere Röntgenuntersuchung. **A** Oberkiefersonografie des neugeborenen Probanden M7 mit XLHED, bei der nur zwei runde, hypoechogene Zahnanlagen darstellbar sind (Pfeile), die nahezu unmineralisierte, spitze Zähne enthalten. **B** Oberkiefersonografie eines gesunden Neugeborenen mit normalen Zahnanlagen, charakterisiert durch hyperechogene, normal geformte Zähne (markiert) und schmale hypoechogene Randbereiche. **C** Dentale Panoramaschichtaufnahme des Probanden M6 mit XLHED im Alter von zwei Jahren zur zuverlässigen Zahnzählung bei Oligodontie.

(FGF), Wnt, hedgehog und TGF-β families [17, 18]. Ectodysplasin A1, which generally promotes placodal cell fate during early development of ectodermal organs [19], has major downstream effectors such as FGF20 which affect ectodysplasin-regulated characteristics of tooth morphogenesis, including the number, size and shape of teeth [20]. In murine and canine XLHED models, an ectodysplasin A1 replacement protein administered *in utero* or in the early newborn period has been shown to correct many of the phenotypic characteristics of ectodysplasin A1 deficiency, producing normal teeth and a sustained health improvement of the treated animals [7 – 9]. Data of the current phase 2 trial in male human neonates with XLHED will become available in the near future. If treatment with recombinant ectodysplasin A1 proves to be safe, it could be given already in pregnancy. Thus, prenatal diagnosis of this condition may soon become even more important and should be based on prenatal sonography.

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