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*Clinical Report*

# A Familial Case of Achondrogenesis Type II Caused by a Dominant *COL2A1* Mutation and "Patchy" Expression in the Mosaic Father

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Achondrogenesis type II (ACG2) is the most severe disorder that can be produced by dominant mutations in *COL2A1*. We report on four pregnancies of an apparently healthy, nonconsanguineous young couple. The father had scoliosis as a child, and has slight body disproportion with short trunk. The first child was born at 32 weeks and died neonatally. In the second pregnancy, short limbs and fetal hygroma were noted on ultrasound at 17 weeks' gestation. Similar findings were observed in the third fetus. Clinical, radiological, and histological evaluation of the fetuses after termination of the pregnancies showed findings consistent with ACG2. Molecular analysis of genomic DNA extracted from amniotic cells of the second and third fetuses revealed heterozygosity for a 10370G > T missense mutation (G346V) in the *COL2A1* gene. This mutation was also found in the father, as a mosaic. The couple had a fourth pregnancy, and at 11 weeks fetal hydrops with a septated cystic hygroma were obvious. DNA from CVS demonstrated the same *COL2A1* mutation. © 2007 Wiley-Liss, Inc.

**Key words:** achondrogenesis type II; *COL2A1* mutation; lethal skeletal dysplasia; mosaicism; recurrence

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## INTRODUCTION

Achondrogenesis type II (ACG2) is a lethal skeletal dysplasia which is the most severe of the wide spectrum of disorders that can be produced by mutations in *COL2A1*, the gene coding for cartilage-specific collagen type 2. Mutations within the *COL2A1* gene also cause Hypochondrogenesis (OMIM 200610), Spondyloepiphyseal dysplasia (SED) congenita (OMIM 183900), SED Namaqualand type (OMIM 142670), mild SED with precocious osteoarthritis, spondyloepimetaphyseal dysplasia Strudwick type (OMIM 184250), Kniest dysplasia (OMIM 156550), multiple epiphyseal dysplasia with myopia and conductive deafness, spondyloperipheral dysplasia (OMIM 271700), and Stickler dysplasia type I (OMIM 108300). The mutations responsible for these phenotypes generally arise de novo—apart from Stickler syndrome type I, which is a milder condition and is frequently identified in family members as well. In rare cases, mutations that have resulted in Kniest dysplasia in children have been found in a mosaic parent who either had Stickler syndrome or mild spondyloepiphyseal dysplasia [Winterpacht et al., 1993; Spranger et al., 1994]. In another family, recurrence of ACG2 in two fetuses from the same clinically normal parents was interpreted as evidence for gonadal mosaicism [Faivre et al., 2004].

Here, we report on a family in which three fetuses were affected by ACG2, and the father, who carries the mutation in a mosaic state, shows an intermediate phenotype.

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## CLINICAL REPORT

The couple first came to our attention after the first child died soon after birth, and the second pregnancy was terminated. A *COL2A1*-related disease was suspected based on X-rays and postmortem examinations. Both parents were of normal height, but the father had scoliosis and showed slight body disproportion (see description below). Based on these findings, counseling included the potential for a recessive phenocopy. Somatic mosaicism was also considered, although deemed less likely. The couple had two additional pregnancies, which were terminated.

The father of this family is 36 years old, and in generally good health. He wore an orthopedic corset as a child for scoliosis, and a "genetic disorder of the spine" was suspected at that time, but no further examinations were performed.

He is now 165 cm tall and shows slight body disproportion with long limbs and short neck and trunk. X-rays (Fig. 1) show normal femoral heads, pelvis and lumbar vertebrae, and some thoracic vertebrae show mild flattening and anterior wedging.

The first pregnancy of the couple (maternal age 30 years) was evaluated at 28 weeks and 5 days for suspected abnormal development of fetal limbs and mild polyhydramnios. With transabdominal ultrasonography (ATL 3000, 5 MHz transducer), a viable fetus was recognized with circumferences of the head and abdomen appropriate for gestational age. All long bones had been measured in all extremities and showed that the limb shortening involved all segments: femur length 39 mm (<1st centile; range 49.8–60.1 mm); tibia 32 mm (<3rd centile; range 44.4–54.0 mm); fibula 31.7 mm (<3%; range 43.1–52.9 mm); humerus 36.8 mm (<3rd centile; range 45.5–55.0 mm); radius 31.9 mm (<3rd centile; range 37.8–47.3 mm); ulna 36.4 mm (<3rd centile; range 43.2–53.1 mm). The foot length was 48 mm (<3rd centile; range 51.4–64.3 mm) and the femur length/foot length ratio was 0.81 (<3rd centile).

Multiple anomalies included: micrognathia, fetal skin redundance in the neck and thorax region, and a "bell-shaped" thorax. The spine had an ovoid shape and flattening of vertebral bodies. This pregnancy ended with preterm labor at 32-week gestation and delivery of a 1,700 g male who died in the newborn period. There was a short, thick neck, flat face with a deep nasal bridge, micrognathia, and short limbs. Measurements were: total length 33 cm; vertex-sacrum 23 cm; upper limb: humerus 6 cm, forearm 6 cm, hand 4.5 cm; lower limb: femur 6.3 cm, lower leg 7 cm, foot 5 cm. Radiographs showed a bell-shaped thorax, platispondily, and wide femural and humeral metaphyses (Fig. 2). Autopsy identified a

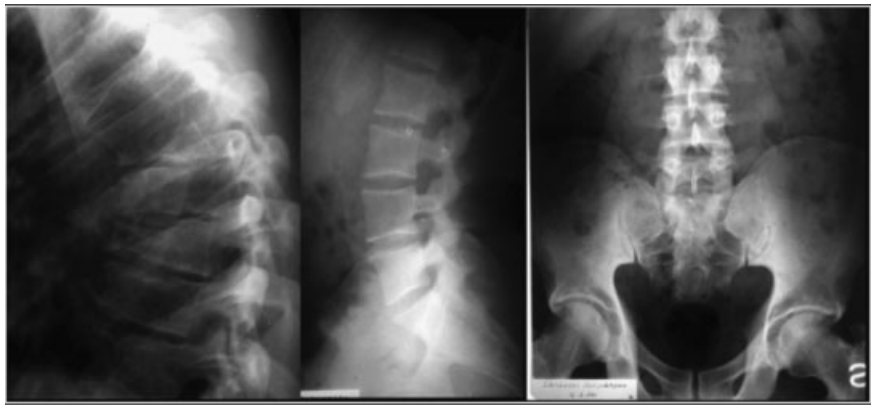

FIG. 1. Radiographs of father's spine. Note mild flattening and anterior wedging of some thoracic vertebrae and normal femoral heads, pelvis and lumbar vertebrae.

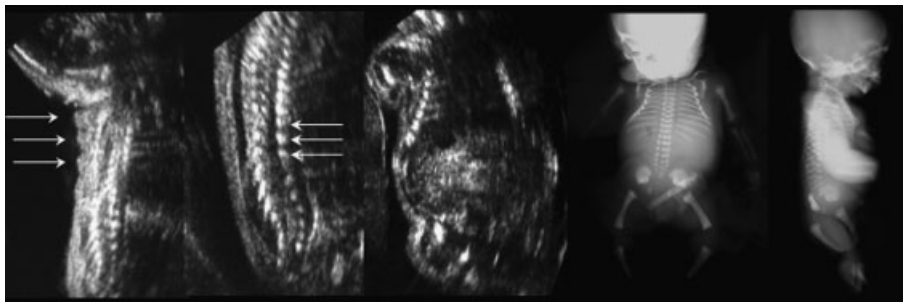

FIG. 2. Ultrasound scan and postnatal radiographs of fetus 1. Note redundant skin (arrows on the left), platispondily (arrows on the right), bell-shaped thorax, wide femural and homeral metaphises.

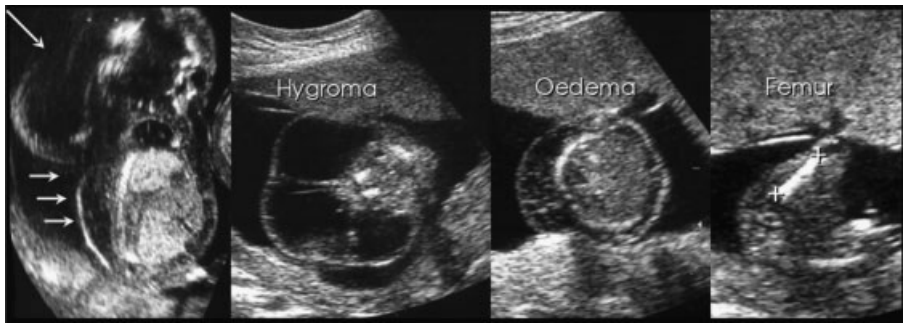


FIG. 3. Ultrasound scan of fetus 2. Note large and septated nuchal cystic hygroma (arrows; longitudinal and axial scans) and short femur.

patent ductus arteriosus, foramen ovale, and dilated left ureter.

The same 32-year-old mother was referred at 16 weeks of pregnancy for fetal karyotyping (amniocentesis), which was 46,XX. At ultrasonography, the fetus showed a large and septated nuchal cystic hygroma associated with hydrops and hyperechoic bowel (Fig. 3). Fetal biometry of the head was appropriate for gestational age. All long bones were below the 3rd centile (micromelia): femur length 13.5 mm (range 16.5–24.0 mm); tibia 10.3 mm (range 13.3–20.5 mm); fibula 10 mm (range 12.8–20.0 mm); humerus 13.5 mm (range 16.7–24.2 mm); radius 9.9 mm (range 13.0–20.3 mm); and ulna 10.4 mm (range 14.5–22.0 mm). Pregnancy was terminated at 17 weeks gestation.

The couple's third pregnancy (maternal age 33 years) underwent fetal karyotyping at 17 + 3/7 weeks. The circumferences of the head and abdomen were appropriate for gestational age. All long bones showed that only the proximal segments were shortened: femur length 20.4 mm (<3rd centile; range 19.4–27.2 mm) and humerus 18 mm (<3rd centile; range 19.6–27.2 mm). The measurements of the other long bones were between the 5th and the 10th centile. Micrognathia was also present.

The pregnancy was terminated at 18 weeks gestation. Postmortem examination showed a female fetus affected by a marked rhizomelic shortening of limbs. There was a prominent forehead, frontal bossing, and depressed nasal bridge. Measurements were: cranial circumference (CC) 17 cm; thorax circumference (TC) 14 cm; abdominal circumference (AC) 15 cm; upper limb: humerus 3 cm, forearm 3 cm, hand 2.4 cm; lower limb: femur 2.5 cm, foot 2.4 cm. Histologic examination of the knee growth plates showed reduced and disorganized columnar chondrocytes. Scans are shown in Figure 4.

The couple's fourth pregnancy was screened at 10.6 weeks for prenatal diagnosis by chorionic villus sampling. Transvaginal ultrasonography showed a septated nuchal cystic hygroma with enlarged jugular lymphatic sacs (see Fig. 5), and generalized fetal hydrops developed 1 week later. Amniotic fluid,

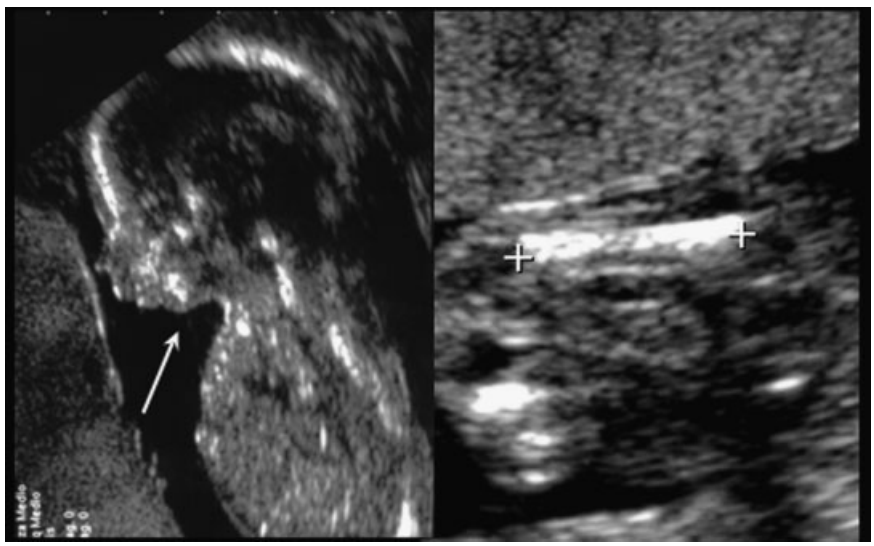

FIG. 4. Ultrasound scan of fetus 3. Note micrognathia (arrow) and short femur.

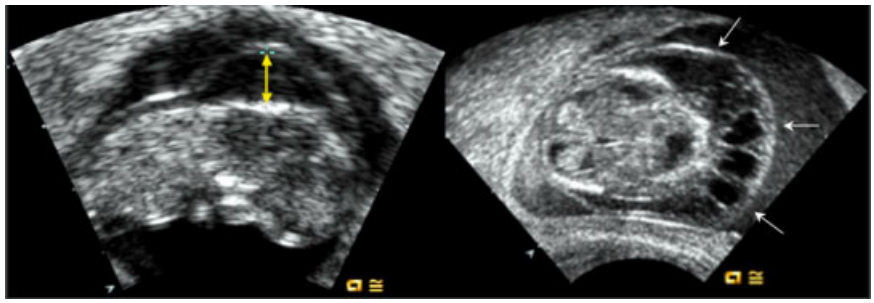

FIG. 5. Ultrasound transvaginal scan of fetus 4. Note increased nuchal translucency (left, longitudinal scan) and septated nuchal cystic hygroma (right, axial scan). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

fetal biometry (CRL 45 mm), and heart rate were normal. Pregnancy was terminated at 11 weeks gestation and analysis of CV DNA was performed for *COL2A1* mutations.

## MATERIALS AND METHODS

Ultrasonography has been performed with ATL 3000, 5-MHz transducer and with Acuson Sequoia—

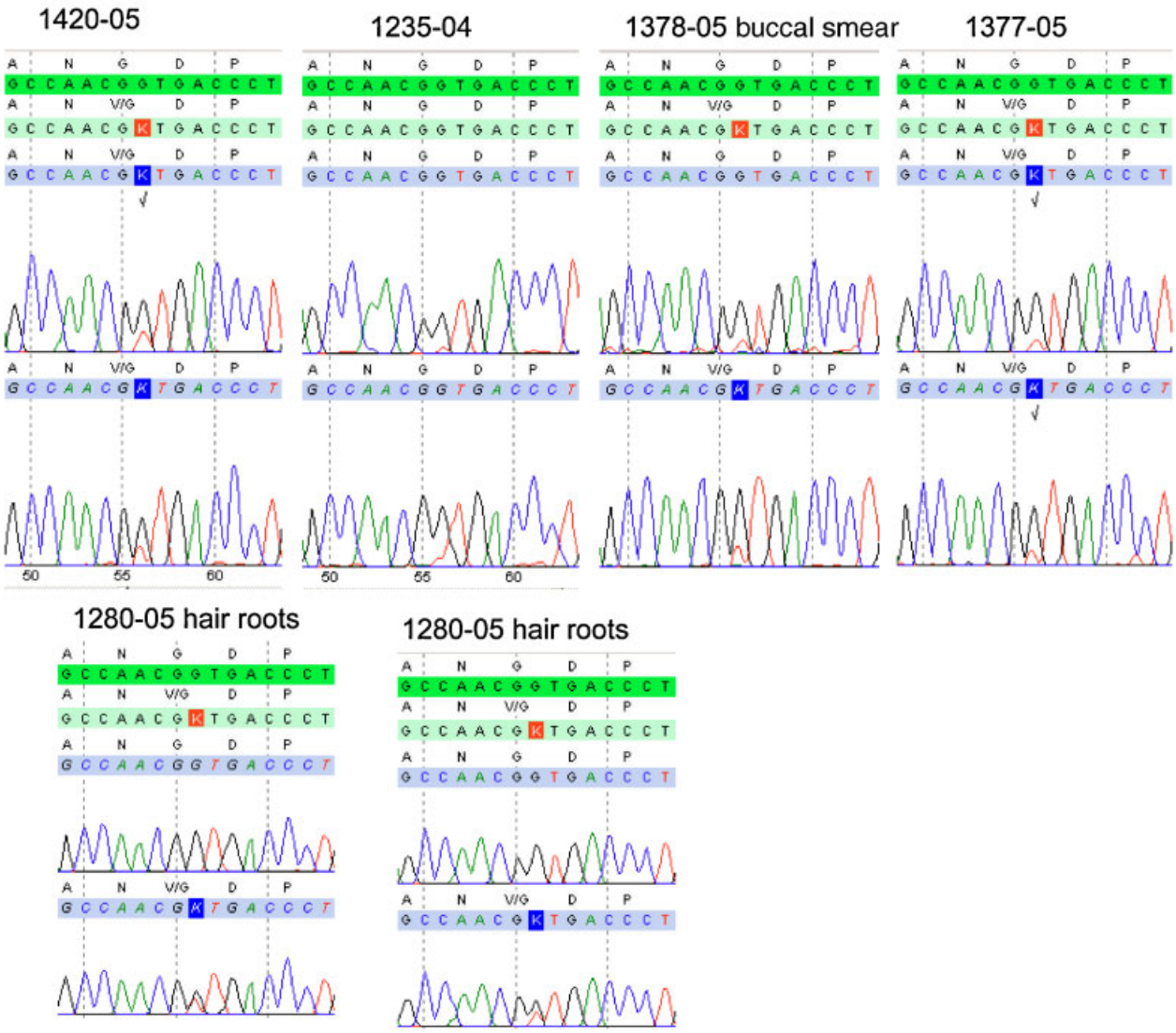


FIG. 6. DNA sequence analysis of exon 23 of the *COL2A1* gene in different tissues. The same GGT → GTT mutation is shown in all the tissues examined. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Siemens (Mountain View, CA), 6–10 MHz in all pregnancies.

As an index case, the DNA of fetus 2 was screened for mutations in the *COL2A1* gene. Exons 21–49 were amplified by polymerase chain reaction (PCR) and sequenced. Further analysis of other family members was performed by the amplification of exon 23 by PCR and direct sequencing. Exons 21–24 were amplified using the forward primer 5′-CTG AAA CAG TTG CCA AGG CTA C-3′ and the reverse primer 5′-TCA CAG TAC TTC AGG CCT CCC-3′. Sequence analyses of exon 23 were carried out with the forward primer 5′-CAC AGC TGC TCC CCA GAA ATT G-3′ and the reverse primer 5′-ATC CTG GCA GTG CAG GGC TG-3′. Fibroblasts were obtained by skin biopsy and cultured with a Hamm's f10, bovine fetal serum 25%, glycerol medium.

Hair roots from the father were taken from different parts of his body, in particular: head (front and occiput), thorax, right shoulder, upper limb (forearm, wrist, and hand right and left), lower limb (knee, leg right and left), and pubis. We also extracted DNA from buccal smears and urine samples. Sperm collection was refused by the patient.

## RESULTS

We first screened exons 21–49 of *COL2A1* in fetus 2. Molecular analysis revealed a heterozygous *COL2A1* point mutation in exon 23 (G1037T). The mutation alters codon 346 (GGT → GTT) and results in the aminoacid substitution of glycine to valine (G346V).

No alterations were found in the mother, while the father's blood DNA harbored a low mutation signal consistent with mosaicism. In order to better define the degree of mosaicism, we performed DNA analyses on fibroblasts, hair roots, buccal smear, and urine. Comparison of the G/T peaks at the mutation site revealed weak mosaicism in all tissue samples (Fig. 6 and Table I). In hair root samples 2, 9, 12, and 13, the ratio of G/T was approximately 50%, whereas hair root samples 1, 5, 6, and 14 only harbored the wild type allele.

## DISCUSSION

ACG2 is a lethal skeletal dysplasia characterized by severe micromelia, relatively large head, small thorax, and protuberant abdomen. Radiologically, incomplete ossification of vertebral bodies and skull can be observed in association with shortening of the tubular bones and hypoplastic pelvis. Almost all screened cases have de novo mutations in the *COL2A1* gene. Faivre et al. [2004] first reported a recurrence of the disease within a family, but no mutation was found in the parents. This suggested gonadal mosaicism in one of the parents.

We describe the first case of proven somatic mosaicism for a *COL2A1* mutation that led to a lethal skeletal dysplasia in four offspring who inherited the mutation.

Germline or somatic mosaicism is not per se an exceptional finding in autosomal dominant skeletal dysplasias, since it has been previously documented for *FGFR3* [achondroplasia, Fryns et al., 1983], *COMP*

TABLE I. DNA Sequence Analyses of Blood, Fibroblast, Buccal Smear and Hair Root Samples. Mosaicism is Present in All Cell Lines, Although at Different Levels

| | Nucleotide in position c.1037 exon 23, gene *COL2A1* | RPA (result file peak area) | |
|---|---|---|---|
| | | T | G |
| 1235-04 blood | G/T | 0.076 | 0.581 |
| 1235-04 blood ek 1 | G/T | 0.182 | 0.924 |
| 1235-04 blood ek 2 | G/T | 0.171 | 0.930 |
| 1235-04 blood ek 3 | G/T | 0.152 | 0.858 |
| 1235-04 blood ek 4 | G/T | 0.152 | 0.932 |
| 1420-05 fibroblasts | G/T | 0.267 | 0.744 |
| 1378-05 buccle smear | G/T | 0.174 | 0.735 |
| 1377-05 urine | G/T | 0.164 | 0.834 |
| 1280-05 hair roots (1) | G/T | 0.247 | 0.712 |
| 1280-05 hair roots (2) | G/T | 0.295 | 0.668 |
| 1280-05 hair roots (3) | G | 0.001 | 0.997 |
| 1280-05 hair roots (4) | G | 0.062 | 0.993 |
| 1280-05 hair roots (5) | G/T | 0.229 | 0.793 |
| 1280-05 hair roots (6) | G/T | 0.164 | 0.862 |
| 1280-05 hair roots (7) | G | 0.020 | 0.898 |
| 1280-05 hair roots (8) | G | 0.010 | 1.000 |
| 1280-05 hair roots (9) | G/T | 0.356 | 0.649 |
| 1280-05 hair roots (10) | G | 0.023 | 0.928 |
| 1280-05 hair roots (11) | G | 0.000 | 1.034 |
| 1280-05 hair roots (12) | G/T | 0.370 | 0.583 |
| 1280-05 hair roots (13) | G/T | 0.415 | 0.533 |
| 1280-05 hair roots (14) | G/T | 0.248 | 0.674 |

[pseudoachondroplasia, Ferguson et al., 1997], and *COL1A1* [osteogenesis imperfecta, Cohn et al., 1990] mutations. When somatic mosaicism was identified in a parent, the manifestations, if any, ranged from minimal to severe phenotypes. Different phenotypes also include mild Stickler syndrome and SED in parents with mosaic *COL2A1* mutations who conceived children affected by a severe Kniest dysplasia [Spranger et al., 1994].

The father of the family in this report has a regional, "patchy" expression of *COL2A1*-related abnormalities, with some bones affected while others are entirely unaffected.

This suggests that somatic mosaicism can lead to a milder but generalized clinical phenotype, which might be determined by the time of onset and the body region or segment in which the somatic mutation arises.

Finally, this case of *COL2A1* mosaicism reinforces the need to consider mosaicism when counseling families with newly recognized *COL2A1*-related disorders. We recommend diagnostic prenatal ultrasound screening as follow-up for subsequent pregnancies, especially when a gene mutation is not identified or when the parents refuse invasive examinations, Recurrence of the disease can be identified by ultrasound at the earliest around the 12th week of gestation [Soothill et al., 1993], and include polyhydramnios, nuchal edema, and shortening of limbs.

## ACKNOWLEDGMENTS

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