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Antenatal Biochemical Expression of Cystinuria and Relation to Fetal Hyperechogenic Colon

To the Editor:
Cystinuria is an inherited disorder characterized by impaired apical transport of cystine and dibasic amino acids (e.g., ornithine, lysine, and arginine) in the renal proximal tubule and the small intestine epithelia. The overall estimated prevalence is 1/7000 neonates (1) . Because of impaired renal cystine reabsorption, cystine precipitates and forms calculi that can produce urinary tract obstruction and may lead to renal insufficiency. Cystinuria is responsible for ~10% of all kidney stones observed in children; in ~50% of patients, stones form in the first decade of life. Diagnosis allows introduction of therapy to reduce stone formation and risk of renal impairment. Prenatal biochemical expression of the disease has not been described.

Fetal hyperechogenic small bowel (FHB), an infrequent ultrasound finding (0.1%–1.8% of pregnancies), is associated with severe fetal diseases such as cystic fibrosis and trisomy 21 (2). Even less common is fetal hyperechogenic colon (FHC) in which the hyperechogenicity is strictly limited to the colon. Recently, it has been suggested that FHC could be associated with cystinuria (3).

We found biochemical prenatal evidence of cystinuria and confirmed the association of cystinuria with FHC.

We retrospectively studied the clinical records of 782 pregnancies for which amniotic fluid (AF) was sent to our laboratory between January 2003 and May 2006 for biochemical investigation, because of digestive tract abnormalities (hyperechogenic bowel, dilated loops, and peritonitis) detected by routine ultrasound scan. Of them, 6 presented with FHC [see the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol53/issue1], detected in all cases at 3rd-trimester routine ultrasound examination, and normal AF digestive enzyme pattern. Thus, this study was restricted to the 3rd trimester of gestation and to AF with a normal digestive enzyme pattern (n = 197). We defined 3 groups: the 6 FHC cases (group 1), 12 FHB cases randomly selected from 175 (group 2), and a 3rd-trimester control group (group 3) consisting of 12 randomly selected AF samples from pregnancies followed for unrelated malformations detected at routine ultrasound scan. Informed consent was obtained for AF sampling in all cases. Samples were centrifuged (10 000g, 5 min at 4 °C) and divided into 2 aliquots, 1 of which was immediately stored at −40 °C, while the other was assayed for digestive enzymes. The frozen aliquot was analyzed by ion-exchange amino acid chromatography using an Aminotac analyzer (Jeol) after deproteinization by 10-fold dilution in 200 g/L sulfosalicylic acid.

Concentrations of half-cystine, lysine, ornithine, and arginine in AF (Fig. 1) were within the previously
described reference intervals (4) for gestational age for groups 2 and 3, except for a low concentration of arginine found in 1 FHB patient. In contrast, these amino acid concentrations were all above the reference values in group 1, except for 1 patient with normal lysine and arginine values.

Chromosomal aneuploidy and cystic fibrosis were systematically excluded in the FHC group. The 6 FHC pregnancies went to term, and the newborns had no apparent complications. For 4 of these cases (including the patient with normal AF lysine and arginine), urine samples were obtained at 1–3 years of age. Cystinuria was confirmed by urine amino acid chromatography (half-cystine, ornithine, lysine and arginine concentrations >10 times the upper limit of the reference interval), thus excluding transient hyperexcretion of cystine in the first months of life as an explanation (5).

Cystinuria is caused by defects in the amino acid transport system rBAT/b0,+AT of epithelial cells of the renal proximal tubule and small intestine (6). In the kidney, rBAT/b0,+AT is the main transport system for cystine reabsorption. During fetal life, tubular maturation begins after the 14th week. After 20 weeks, the kidneys provide >90% of the AF volume. Thus the AF amino acid chromatography in cystinuria-affected fetuses shows the same profile, as does postnatal urine.

The intestinal hyperexcretion in the 6 cystinuria-affected fetuses was not located in the small intestine, where the transporter is expressed, but downstream. During fetal life, the fetus continuously swallows AF. In the cystinuria-affected fetus, the renal reabsorption defect leads to an abnormally high concentration of cystine in AF. Because the rBAT/b0,+AT transport system is the only high-affinity system for cystine absorption in the small intestine, we propose that a progressive overload of the intestinal cystine transporter capacity occurs. Thus, unabsorbed cystine progressively concentrates and then precipitates in the intestinal tract. The occurrence of FHC after only 26 weeks can be explained by the progressive closure of the anal sphincter that commences after 22 weeks. Indeed, FHC resolved after evacuation of meconium in one of our cases.

These findings suggest that FHC should be studied as a potentially useful diagnostic indicator of cystinuria and could be regarded as a possibility to provide preventive medicine.

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References

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To the Editor:

We have discovered that the DNA sample used in our recent paper (1) as a control heterozygote at the sickle cell locus of β-globin (17A→T, also known as HBB c.20A→T by HUGO nomenclature) contained an additional variant. Subsequent sequencing revealed a double heterozygote, HBB c.9C→T; 20A→T). The HBB c. 9C→T is a silent variant for the 3rd amino acid, histidine. In view of this additional sequence variation, we reevaluated the heteroduplex scanning capabilities of the instruments, as reported in the original Fig. 3, to ascertain their ability to distinguish melting curves of heteroduplexes caused by single and double heterozygotes from melting curves of homoduplexes. The c. 9C→T is a common variant with an allele frequency of 38%, as determined in review of clinical samples submitted for β-globin sequencing (courtesy of Dr. Elaine Lyon, ARUP Laboratories).

The study was repeated as previously described (1), including both single and double heterozygotes. Eight instruments were evaluated for geno-