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Adult-onset liver disease and hepatocellular carcinoma in S-adenosylhomocysteine hydrolase deficiency

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ABSTRACT

Background: The etiology of liver disease remains elusive in some adults presenting with severe hepatic dysfunction.
Methods and results: Here we describe a woman of Pakistani descent who had elevated aminotransferases at age 23. She developed muscle weakness in her mid-20s, and was diagnosed with hepatocellular carcinoma at age 29. She died without a diagnosis at age 32 after having a liver transplant. Exome sequencing revealed that she was homozygous for a missense mutation (R49H) in *AHCY*, the gene encoding S-adenosylhomocysteine (SAH) hydrolase. SAH hydrolase catalyzes the final step in conversion of methionine to homocysteine and inactivating mutations in this enzyme cause a rare autosomal recessive disorder, SAH hydrolase deficiency, that typically presents in infancy. An asymptomatic 7-year old son of the proband is also homozygous for the *AHCY*-R49H mutation and has elevated serum aminotransferase levels, as well as markedly elevated serum levels of SAH, S-adenosylmethionine (SAM), and methionine, which are hallmarks of SAH hydrolase deficiency.
Conclusion: This report reveals several new aspects of SAH hydrolase deficiency. Affected women with SAH hydrolase deficiency can give birth to healthy children. SAH hydrolase deficiency can remain asymptomatic in childhood, and the disorder can be associated with early onset hepatocellular carcinoma. The measurement of serum amino acids should be considered in patients with liver disease or hepatocellular carcinoma of unknown etiology.

1. Introduction

The liver plays a key role in the metabolism of methionine, an essential amino acid in metazoa [1,2]. In mammals, the two pathways for methionine metabolism, the methionine cycle and the transsulfuration sequence, share the first three reactions in common. In both pathways, methionine is converted to S-adenosylmethionine, which functions as a methyl donor in diverse transmethylation reactions. These reactions yield methylated DNA, RNA and proteins plus *S*-adenosylhomocysteine, which is then cleaved to homocysteine and adenosine by S-adenosylhomocysteine (SAH) hydrolase. Approximately 50% of the methionine in humans is metabolized in the liver [1,2]. The medical importance of these pathways is revealed by the effects of genetic mutations that disrupt them in humans [3–6]. Such disruptive mutations lead to the accumulation of upstream metabolites, deficiencies in downstream metabolites, and a wide spectrum of clinical phenotypes, including neurological abnormalities, liver disease, and muscle weakness. A total of eight cases of SAH hydrolase deficiency has been described [6–12] and in all cases, the disease has manifested in infancy or early childhood.

Here we describe a family with a highly atypical presentation of SAH hydrolase deficiency. The proband is the offspring of a consanguineous mating who presented with liver disease of unknown etiology at age 23. She required liver transplantation at age 30 years and subsequent to her surgery, she developed severe muscle weakness and died at age 32 years. Exome sequencing performed post-mortem revealed homozygosity for a missense mutation in the gene encoding SAH hydrolase (*AHCY*). One of her two children, an asymptomatic 7-year old boy, is homozygous for the same mutation. Here, we describe the clinical course of the mother and the initial management of her affected son.

Abbreviations: AA, amino acid; CT, computed tomography; MAF, minor allele frequency; MRI, magnetic resonance imaging; ROH, runs of homozygosity; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

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2. Patients and methods

2.1. Human subjects

The study protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center. Venous blood was collected from the proband after obtaining written informed consent. Genomic DNA was extracted from the leukocytes using an Autopure LS DNA extractor (Qiagen, Germantown, MD). Plasma and serum were isolated, aliquoted and stored at −80 °C. The proband's medical history and family medical history were obtained from medical records and from interviews with relatives. Blood samples were also collected from her husband and two sons, who were referred to the pediatric metabolism clinic for clinical testing and treatment. No tissue samples were available from any of the other relatives of the proband.

2.2. Genotyping and DNA sequencing

The proband was genotyped using the Human-Omni5-4 BeadChip microarray (Illumina, San Diego, CA). Allele calls from the array data were generated using GenomeStudio. Of the 4,292,096 SNPs assayed, 4,283,655 (99.8%) were successfully called (GenCall Score > 0.15).

For exome sequencing, 3 μg of genomic DNA was sonicated using a Covaris S2 ultrasonicator (Covaris, Woburn, MA), purified, and assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). DNA was end-repaired, and 3′ ends were adenylated and barcoded with truncated adapters. PCR amplified libraries were purified with AmpureXP beads (New England Biolabs, Ipswich, MA) and assayed using an Agilent 2100 Bioanalyzer. A 750 ng aliquot of the fragment library was concentrated by vacuum to 3.5 μL and hybridized and captured with a SureSelect Human All Exon V4 kit (Agilent Technologies, Santa Clara, CA). Following hybridization the captured library was amplified and index tags were added to the adapters. DNA was again purified with AmpureXP beads, and fragment sizes were assayed using the Agilent 2100 Bioanalyzer. Paired-end sequencing (150 basepairs) was performed using an Illumina Hiseq 2000. Sequences were aligned to the human reference genome b37, and variants were called using the Genome Analysis Toolkit (GATK) HaplotypeCaller [13].

Homozygosity mapping was performed using PLINK. Runs of homozygosity (ROHs) were detected by a sliding window algorithm with a window size of 100 SNPs [14,15]. ROHs longer than 1 Mb were used to assist in filtering the variants identified by exome sequencing, since runs of this length are uncommon in the general population [16].

Variant alleles with a minor allele frequency (MAF) of less than 1% in the 1000 Genomes Project (http://www.1000genomes.org) and the Exome Aggregation Consortium (ExAC) [17] databases that were homozygous in the proband were collated and filtered for potentially pathogenic variants (missense, nonsense, splice-site, or frameshift) located within ROH intervals >1 Mb. Selected mutations were confirmed by Sanger DNA sequencing PCR-amplified fragments containing the nucleotide substitution.

The oligonucleotides used to confirm the mutation identified in *AHCY* were the following: 5′-TGCGGTGACAGAGTGCTAAG-3′ and 5′-ACCGAGTGAGAGGGAGGAAC-3′.

2.3. Clinical chemistry

Serum amino acids, homocysteine, and aminotransferases were measured using standard biochemical methods at Children's Medical Center, University of Texas Southwestern Medical Center. SAH and S-adenosylmethionine (SAM) levels were measured by tandem mass spectrometry (Shimadzu Nexera LC System interfaced with a 5500QTRAP® Sciex) at the Institute for Metabolic Disease at Baylor University Medical Center, Dallas, TX as previously described [18].

3. Results

3.1. Hepatocellular carcinoma of unknown etiology

The proband (III.2, Fig. 1) was a 32 year old woman of Pakistani descent, whose parents were first-cousins. She was the product of a term pregnancy and developed normally. She had a self-limited episode of jaundice at age 7 that was attributed to hepatitis A, though no clinical testing was performed at the time. She was not a good student, starting in elementary school. She never underwent psychometric or intelligence testing. At age 23 she married and migrated to the United States where she worked in the home. Soon thereafter she developed pneumonia and was found to have elevated aminotransferase (AST 56 U/L [ref. range 10–40 U/L], ALT 107 U/L [ref. range 7–56 U/L]), and serum creatine phosphokinase levels (615 to 1256 U/L [ref. range: <145 U/L]) with a normal level of bilirubin (0.7 mg/dL [ref. range 0.2–1.5 mg/dL]) and reduced plasma albumin level (2.1 g/dL [ref. range 3.5–5.5 g/dL]). Her coagulation studies were abnormal. Her PT was 37 s (ref. range 9.5–13.5 s) with an INR of 3.7 (ref. range 0.8–1.3). Her PTT was 25 s (ref. range 25–35 s). She tested negative for hepatitis B surface Ag and core IgM, hepatitis C Ab, and hepatitis A IgM. She tested positive for hepatitis A IgG, thus indicating a prior exposure to this virus. Computed tomography (CT) and ultrasonography of her liver revealed no abnormalities. Six months later, her INR was 1.7, PT was 19 s, PTT was 25 s, and GGT was 13 U/L (ref. range <30 U/L).

At age 24, when she was 20 weeks pregnant, she presented with thrombocytopenia accompanied by a hypochromic, microcytic anemia. Her reticulocyte count was normal but her serum aminotransferase levels were elevated (AST 56 U/L, ALT 99 U/L). Two months later, her AST was 159 U/L, ALT was 98 U/L and her plasma albumin was 3.0 g/dL (ref. range in the third trimester 2.3–4.2 g/dL), which fell further to 2.5 g/dL during the pregnancy. Abdominal ultrasound showed a normal liver with moderate splenomegaly. After delivery, her aminotransferase levels returned to baseline, but her plasma albumin level remained slightly decreased (3.4 g/dL). Therefore, she underwent a liver biopsy, which revealed normal histology without any evidence of fibrosis or inflammation. She gave birth to a second son at age 26. After the birth, she noted increasing muscle weakness and fatigue, though she did not have difficulty walking or taking care of her children.

At age 29, she presented with elevated levels of aminotransferases (AST 63 U/L, ALT 105 U/L), an increased INR (3.4) and reduced plasma levels of albumin (1.9 g/dL). She had a markedly elevated level of serum alpha-fetoprotein (1426 ng/mL [ref. range <50 ng/mL]). Her rheumatoid factor was 36 U/mL (ref. range <15 U/mL), and her plasma levels of copper, ceruloplasmin and α1-antitrypsin were normal. She did not have any detectable antibodies to mitochondria, Jo-1, centromeres, dsDNA, SCL-70, SSA, SSB, RNP, Smith antigen and smooth muscle antigen. Ultrasonography, CT scan, and magnetic resonance imaging (MRI) of her abdomen showed several focal hepatic lesions. Biopsies of one of the lesions revealed a well-differentiated hepatocellular carcinoma. Histology was negative for PAS and iron staining. She underwent liver transplantation at age 30.

Over the ensuing 14 months, she developed muscle weakness that was initially attributed to her immunosuppressive therapy, which included glucocorticoids. She could walk only short distances. Cessation of glucocorticoid treatment failed to improve her muscle strength. Her creatine kinase level was 3069 U/L 6 months after liver transplantation, and two weeks later the level remained elevated at 639 U/L. Electromyography and nerve-conduction studies showed a myopathic pattern in the proximal musculature with no irritative features, and a mild sensorimotor neuropathy with primarily axonal features. MRI of the legs showed muscular atrophy, but no inflammation. A muscle biopsy from the soleus was performed and showed myofiber type 2 atrophy and a possible very low-grade neuropathic process. There was no myocyte necrosis, myocyte regeneration, inflammatory infiltrates, or evidence of vasculitis. No ragged red fibers were seen. Electron