

## An Adult with Type 2 Citrullinemia Presenting in Europe

**TO THE EDITOR:** Type 2 citrullinemia is an adult-onset, autosomal recessive disorder characterized by episodes of hyperammonemic encephalopathy. It is caused by mutations in the *SLC25A13* gene, which encodes the liver-specific isoform of the mitochondrial aspartate–glutamate carrier (AGC2).<sup>1,2</sup>

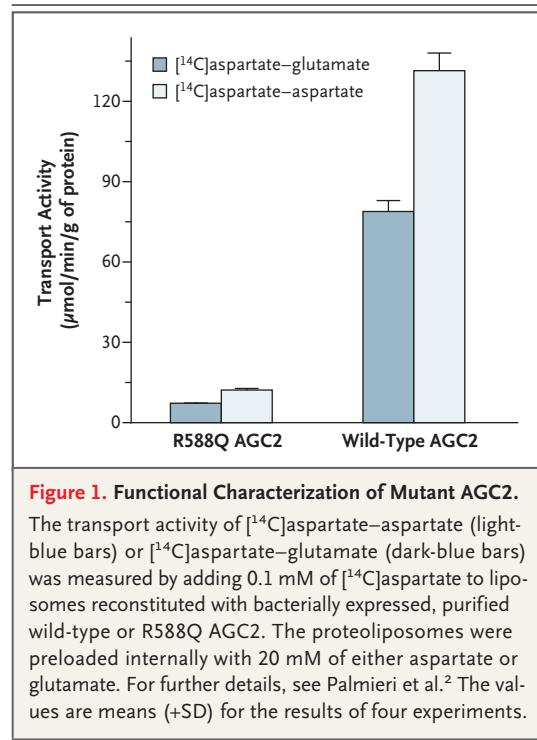
A 38-year-old Pakistani man with episodic confusion was found to have an elevated plasma ammonia level during an episode; citrullinemia and raised arginine, normal glutamine, and low serine levels were also noted, suggesting the diagnosis of type 2 citrullinemia. Unfortunately, despite aggressive treatment, the patient died from hyperammonemic encephalopathy.

Sequencing of the patient's *SLC25A13* gene revealed homozygosity for a novel point mutation, c.1763G→A (AF118838.1), which produced an Arg-to-Gln substitution at residue 588 of AGC2. Functional analysis of the mutant protein showed only about 10% of normal transport of aspartate and glutamate (Fig. 1).

The patient's parents, who were unrelated but came from the same village in Pakistan, were heterozygous for the mutation. Some members of the extended pedigree, which contained a number of first-cousin marriages, were screened; three members of the pedigree were heterozygous for the mutant allele, and two were homozygous. Both homozygotes were well and had no detectable biochemical abnormalities. The c.1763G→A mutation was not found in 104 unrelated control chromosomes.

An arginine residue is highly conserved in the equivalent position in all mitochondrial carrier proteins and is considered to be important for substrate binding.<sup>3</sup> The equivalent R275Q mutation in the human mitochondrial ornithine carrier 1 also abolishes transport activity and is pathogenic, causing the hyperornithinemia–hyperammonemia–homocitrullinuria syndrome.

These results directly demonstrate that a reduction in mitochondrial aspartate–glutamate transport in the liver can lead to type 2 citrullinemia. In exporting aspartate from the mitochondrion to the cytoplasm, AGC2 plays a crucial role in both urea synthesis and the transfer of



reducing equivalents from the cytosol to the mitochondria through the malate–aspartate NADH shuttle.<sup>4</sup>

Recent results in AGC2 knockout mice suggest that the cytosolic redox potential may be important in the generation of hyperammonemia seen in type 2 citrullinemia.<sup>5</sup> An increased cytosolic ratio of NADH to NAD<sup>+</sup>, perhaps triggered by dietary factors such as carbohydrate or alcohol intake, could lower cytosolic aspartate concentrations and precipitate hyperammonemia.

Although well recognized in East Asia, type 2 citrullinemia has rarely been reported elsewhere. It is important to consider this diagnosis in adults presenting with hyperammonemic encephalopathy, since the management of type 2 citrullinemia is very different from the management of classic urea-cycle defects. Patients with type 2 citrullinemia should not be treated with a low-protein diet or an emergency regimen that includes high amounts of carbohydrate. Given the dismal prognosis for this condition, patients

should be referred for consideration of liver transplantation as soon as possible after their first presentation with metabolic decompensation.

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1. Kobayashi K, Sinasac DS, Iijima M, et al. The gene mutated in adult-onset type II citrullinaemia encodes a putative mitochondrial carrier protein. *Nat Genet* 1999;22:159-63.
2. Palmieri L, Pardo B, Lasorsa FM, et al. Citrin and aralar1 are Ca<sup>2+</sup>-stimulated aspartate/glutamate transporters in mitochondria. *EMBO J* 2001;20:5060-9.
3. Robinson AJ, Kunji ER. Mitochondrial carriers in the cytoplasmic state have a common substrate binding site. *Proc Natl Acad Sci U S A* 2006;103:2617-22.
4. Palmieri F. The mitochondrial transporter family (SLC25): physiological and pathological implications. *Pflugers Arch* 2004;447:689-709.
5. Saheki T, Iijima M, Li MX, et al. Citrin/mitochondrial glycerol-3-phosphate dehydrogenase double-knockout mice recapitulate features of human citrin deficiency. *J Biol Chem* 2007;282:25041-52.

## HER2 Status and Benefit from Adjuvant Trastuzumab in Breast Cancer

**TO THE EDITOR:** Trastuzumab, an antibody against the protein product of the human epidermal growth factor receptor type 2 (*HER2*) gene, improves progression-free survival and overall survival when added to chemotherapy in patients with metastatic breast cancer.<sup>1</sup> Initial trials enrolled patients with tumors that had a staining intensity of 2+ or 3+ for *HER2* on immunohistochemical analysis, but in subsequent studies, the benefit was limited to tumors with *HER2* amplification as determined by fluorescence in situ hybridization (FISH). Trastuzumab also improves disease-free survival and overall survival in the adjuvant setting.<sup>2</sup>

On the basis of its mechanism of action, the *HER2* gene copy number was expected to predict benefit from adjuvant trastuzumab. National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-31, which compared standard chemotherapy of four cycles of doxorubicin and cyclophosphamide followed by four cycles of paclitaxel (ACT) with ACT plus trastuzumab (ACTH) in the adjuvant setting, provided an opportunity to test

this hypothesis.<sup>2</sup> Available tissue blocks were examined at a central site by means of a Food and Drug Administration–approved *HER2* FISH assay. We found no significant association between *HER2* copy number and benefit ( $P=0.60$ ). Even patients with normal gene copy numbers appeared to benefit (relative risk for disease-free survival, 0.40; 95% confidence interval [CI], 0.18 to 0.89;  $P=0.026$ ).

*HER2* status according to immunohistochemical analysis with the use of Herceptest (Dako) was also determined at a central site. Tumors that were negative on FISH and had an immunohistochemical staining intensity of less than 3+ were defined as “central *HER2*-negative,” as in our previous report.<sup>3</sup> Among the 1787 patients with follow-up data, 174 patients had breast cancers that were found to be central *HER2*-negative (9.7%), yet these patients also appeared to benefit from trastuzumab (relative risk for disease-free survival, 0.34; 95% CI, 0.14 to 0.80;  $P=0.014$ ) (Table 1).

To address the technical problems inherent in