Response to Shen and Zou

Since publication of our previous paper on aminoacyl-tRNA synthetase (ARS) deficiencies,[1] the number of recognized patients suffering from these diseases has steadily increased, putatively through progressively genetic diagnostic testing, aided by recognition of the clinical phenotype. To further improve disease recognition, counseling, prognostic prediction, and potential treatment development and evaluation, further understanding of the disease mechanism, genotypes, and clinical phenotypes remains crucial.

Shen et al. provide interesting additional clinical information on QARS deficiency,[2] one of these ARS deficiencies. They show that serum protein levels were consistently decreased in their three patients with QARS deficiency, while albumin was only slightly and inconsistently decreased, which they attribute to the relatively low glutamine content of albumin (3.3%). Hypoalbuminemia is common to multiple ARS deficiencies,[1] including IARS, LARS, MARS, and possibly KARS. However, the abundance of the corresponding amino acids varies (isoleucine: 1.5%, leucine: 10.5%, methionine: 1.1% methionine, and lysine: 9.9%). Therefore, a more general mechanism seems more plausible.

Of the QARS deficient patients we previously described,[1,3] the first[1] displayed normal serum albumin concentrations at ages 1.5 years (40 g/l, normal values: 35–50 g/l), and 3 years (43 g/l), but decreased concentrations at age 5.5 years (28 g/l). The second patient[3] also displayed mostly normal concentrations except at age 12 years (31 g/l, normal values: 34–42 g/l). Total plasma protein concentration was only measured once in both patients and was normal (63 g/l, normal 60–80 g/l) at age 1.5 years in the first patient[1] and decreased (54 g/l, normal 60–80 g/l) at age 13 years in the second.[3] In this second patient, serum prealbumin concentrations were normal, and marginally reduced once (185 g/l, normal 186–335 g/l) at age 9 years. When looking at our other ARS deficient patients,[1] IARS deficient patient 1 also had low plasma protein levels (33 and 34 g/l at age 1 month and 2.5 months, respectively), but IARS deficient patient 2 had normal levels (80 g/l) at age 4 years and so did KARS deficient patient 4 (64 g/l) at age 1 year. Plasma protein concentration was not determined in our LARS deficient patients. Collectively, these data suggest that both plasma albumin and protein concentrations may be decreased in ARS deficiencies, putatively through a common mechanism shared among the ARS deficiencies involving either insufficient protein synthesis, mistranslation, and/or increased peptide/protein degradation. Affected proteins may depend on the specific amino acid corresponding to the ARS deficiency.

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Since QARS deficiency is a predominantly neurological disease, Shen et al. sought to extend the hypothesis of decreased protein synthesis to the central nervous system.[2] However, they did not find decreased protein levels in cerebrospinal fluid (CSF). Similarly, our QARS deficient patients[1,3] did not have decreased CSF protein levels (0.36 g/l, normal range 0–0.40, and 0.36 g/l, normal values 0.10–0.35 g/l, respectively), nor did our KARS deficient patient (0.31 g/l) and our IARS deficient patient (0.75 g/l; but this sample was contaminated with >80,000 erythrocytes and >400 leukocytes). Shen et al. then propose an interesting hypothesis of excitotoxicity induced by increased glutamate/glutamine ratios due to QARS deficiency.[2] Indeed, severe neurological symptoms have been associated with increased glutamate/glutamine ratios in several inborn errors of metabolism.[4] However, CSF amino acid analysis in our reported QARS patients[1,3] revealed normal glutamate (3 μmol/l, normal range 0–7.8 μmol/l and <8.5 μmol/l, normal range: <8.5 μmol/l, respectively) and nearly normal glutamine concentrations (488 μmol/l, normal range 363–785 μmol/l and 688 μmol/l, normal range 260–684 μmol/l, respectively). The first patient[1] also had slightly decreased taurine concentrations (2 μmol/l, normal range 4–13 μmol/l) and the second[3] marginally decreased serine concentrations (24.4 μmol/l, normal 25–56 μmol/l). Although the proposed disease mechanism would be QARS specific, the underlying mechanistic hypothesis would suggest alterations in the corresponding amino acids in CSF of other ARS deficiencies. However, CSF amino acids were also normal in our KARS deficient patient.

As a global central nervous system fluid, CSF may not reflect local concentrations of amino acids in the brain. We performed brain magnetic resonance (MR) spectroscopy in the white matter of our QARS patient,[1] which revealed low N-acetyl aspartic acid peaks compatible with white matter disease, and prominent glutamate/glutamine peaks. Unfortunately, these 3-Tesla MRI scans do not allow distinction between glutamate and glutamine. Further studies with more advanced spectroscopy in different brain areas may provide further insight in this hypothesis. Similarly, as also mentioned by Shen et al.,[2] the response to treatments affecting the glutamate/glutamine balance (like glutamine supplementation) and/or excitotoxicity (like memantine, which antagonizes the interaction between glutamate and the NMDA receptor and pyridoxine, which is required for the conversion of glutamate to GABA) may provide further support for this hypothesis and guide therapeutic strategies. Nevertheless, since neurological symptoms are shared among many cytosolic, combined cytosolic and mitochondrial, and mitochondrial ARS deficiencies, a more common translational deficiency or mitochondrial dysfunction may also contribute to the neurological phenotype.

When interpreting genetic variations, their potential pathogenicity, associated clinical phenotype. and response to

specific treatments, their precise genetic location is crucial. This is the reason we dedicated Fig. 5 of our previous article[1] to the genetic locations of all reported *ARS* variants. We have updated this figure (Supplemental Fig. 1) with novel pathogenic variants,[5–10] and hope this genetic information, the additional clinical features, and deduced mechanistic hypotheses will aid other researchers and clinicians to improve understanding of ARS deficiencies and care for this expanding group of patients.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-01014-8) contains supplementary material, which is available to authorized users.

DISCLOSURE

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