

Short Communication

Persistent hypocitrullinaemia as a marker for mtDNA NARP T 8993 G mutation?

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Severe hypocitrullinaemia (<10 µmol/L) is one of the main biochemical characteristics of two clinical situations: the disorders of mitochondrial urea-cycle enzymes (*N*-acetylglutamate synthase, carbamoylphosphate synthetase I (CPS-I) and ornithine carbamoyltransferase (OCT)) and the deficiency of pyrroline-5-carboxylate synthetase (Rabier and Kamoun 1995). Hypocitrullinaemia was also associated with Pearson syndrome (Ribes et al 1993). Here we report a new situation in which hypocitrullinaemia was observed in 5 patients with mt ATP6 NARP T 8993 G mutation. The NARP 8993 mutation impairs the function of the subunit *a* or ATPase 6 of the Fo segment of the complex V. This ATPase 6 polypeptide is involved in proton translocation through the inner mitochondrial membrane.

PATIENTS AND METHODS

Patients: The 5 patients described here are from three unrelated families. The first patient, a 4-year-old girl, was hospitalized at 1 year for psychomotor regression, axial hypotonia and myoclonic seizures. The second patient is a 2-year-old boy hospitalized at 10 months of age for psychomotor regression, hypotonia and abnormal CT scan. The three last patients are brothers born to unrelated parents. The first boy, 10 years old, was well up to 8 months of age and then showed psychomotor regression at 1 year and myoclonic seizures at 4 years. Now he has a pyramidal syndrome and a cerebellar syndrome with dysarthria. The second boy died at 3 days of life from a coma with ketoacidosis, hyperlactacidaemia and hyperammonaemia. The third boy, 10 months old, was hypotonic from birth and developed Leigh syndrome with retinitis pigmentosa. Hyperlactacidaemia was found in four patients and hyperammonaemia in two. Urinary organic acids were normal in four and showed

elevated lactate in one. CPS-I and OCT activities were in the normal range in two boys of the third family.

Methods: Plasma amino acids were measured by ion exchange chromatography on a Beckman 6300 amino acid analyser. Urinary organic acids were analysed by capillary gas chromatography after ethyl acetate extraction and silylation. Respiratory chain enzyme activities were determined spectrophotometrically on mitochondria isolated from skeletal muscle biopsies according to the method described by Rustin and colleagues (1994). NARP T 8993 G mutation was determined on total DNA extracted by standard techniques and amplified by PCR using 8581-8605 and 9226-9273 primers. The 715 bp PCR product was then digested by the restriction enzyme *Ava*I. Urea-cycle enzymes were determined as described elsewhere (Rabier et al 1989).

RESULTS AND DISCUSSION

Plasma amino acid analysis showed a mild intermittent increase of alanine and decrease of arginine. Plasma citrulline concentration was always low (0–13 $\mu\text{mol/L}$) (Table 1) with 20/22 values under 10 $\mu\text{mol/L}$. This hypocitrullinaemia seems to be a characteristic unique to NARP mutant patients. When we compared the percentages of plasma citrulline values under 10 $\mu\text{mol/L}$ we found 90% for NARP patients and between 0 and 20% for other respiratory chain deficiencies (Table 1). Among the patients with respiratory chain deficiencies hypocitrullinaemia was not consistent in an individual patient. Ribes and colleagues (1993) also described a permanent hypocitrullinaemia in a patient with Pearson syndrome, another mtDNA abnormality. However, in the four patients with Pearson syndrome we had the opportunity to investigate, only one had a severe and constant hypocitrullinaemia.

Because plasma citrulline concentration represents the balance between its synthesis by the gut mucosa and its utilization by the kidney, it can be assumed that severe hypocitrullinaemia results from a severe impairment of its synthesis at the intestinal level. CPS-I is one of the three mitochondrial urea-cycle enzymes involved in citrulline synthesis in the liver and in the intestinal mucosa. This enzyme is regulated both by the ATP/ADP ratio inside the mitochondrial matrix (Wanders et al, 1981) and by the *N*-acetylglutamate (NAG), its allosteric activator. Affinity of CPS-I for NAG is tightly related to ATP concentration (Alonso and Rubio 1983). For these reasons, CPS-I activity is directly bound to ATP-synthase activity.

Why do we observe such a discrepancy between citrulline levels in patients with deficient respiratory chain complexes and those with NARP mutation described here? In the first group of patients, mitochondrial ATP/ADP ratio is probably dependent on the residual activity of the different complexes, explaining the high variability of plasma citrulline observed for a same patient or between patients. In the NARP patients, ATP/ADP ratio could be very low owing to a preponderance of the mutant mtDNA in the intestinal mucosa (for example, 97% of mutant mtDNA was found in mucosa of one of our patients). In conclusion, persistent hypo-

Table 1 Absolute and relative concentrations of plasma citrulline in different respiratory chain disorders

Patients (numbers)		Concentration	Percentage	Number of determinations
		($\mu\text{mol/L}$; mean \pm SD) (range) [% values ≤ 10] ^a	total aminoacidaemia (mean \pm SD) (range) [% values ≤ 0.3] ^a	
NARP	(n = 5)	4 \pm 4 (0–13) [91]	0.14 \pm 0.11 (0–0.4) [86]	22
Complex I	(n = 55)	26 \pm 12 (0–63) [13]	1.0 \pm 0.6 (0–3.6) [7]	70
Complex II	(n = 6)	22 \pm 7 (11–30) [0]	0.9 \pm 0.3 (0.4–1.2) [0]	7
Complex III	(n = 15)	24 \pm 14 (4–60) [20]	0.9 \pm 0.5 (0.2–2.2) [15]	20
Complex IV	(n = 25)	22 \pm 13 (0–60) [14]	0.7 \pm 0.3 (0–1.6) [13]	56
Complex I + IV	(n = 14)	23 \pm 10 (2–46) [16]	0.8 \pm 0.4 (0.1–2.2) [13]	31
Generalized	(n = 7)	23 \pm 7 (8–39) [5]	0.9 \pm 0.3 (0.6–1.5) [0]	18
Pearson	(n = 4)	10 \pm 8 (1–20) [66]	0.5 \pm 0.3 (0.05–0.8) [33]	6
Controls	(n = 174)	26 \pm 7 (10–43)	1.0 \pm 0.3 (0.3–1.7)	174

^a Percentage of values below the lowest normal limit.

citrullinaemia should be considered as a biochemical marker of NARP mutation in patients with hypotonia, psychomotor regression, with or without retinitis pigmentosa and Leigh syndrome.

REFERENCES

- Alonso E, Rubio V (1983) Binding of *N*-acetylglutamate to rat liver carbamoylphosphate synthetase (ammonia). *Eur J Biochem* **135**: 331–337.
- Rabier D, Kamoun P (1995) Metabolism of citrulline in man. *Amino Acids* **9**: 299–316.
- Rabier D, Benoit A, Petit F, et al (1989) Ornithine carbamoyltransferase deficiency: a new variant with subnormal enzyme activity. *Clin Chim Acta* **186**: 25–30.
- Ribes A, Riudor E, Valcarel R, et al (1993) Pearson syndrome: altered tricarboxylic and urea cycle metabolites, adrenal insufficiency and corneal opacities. *J Inher Metab Dis* **16**: 537–540.

- Rustin P, Chretien D, Bourgeron T, et al (1994) Biochemical and molecular investigations in the respiratory chain deficiencies. *Clin Chim Acta* **228**: 35–51.
- Wanders RJA, Van Woerkom GM, Nootboom RF, Meijer AS, Tager JM (1981) Relationship between the rate of citrulline synthesis and bulk changes in the intramitochondrial ATP/ADP ratio in rat liver mitochondria. *Eur J Biochem* **113**: 295–302.