

## METABOLIC DISEASES

B. Parfait · P. de Lonlay · J. C. von Kleist-Retzow · V. Cormier-Daire ·  
D. Chrétien · A. Rötig · D. Rabier · J. M. Saudubray · P. Rustin · A. Munnich

## The neurogenic weakness, ataxia and retinitis pigmentosa (NARP) syndrome mtDNA mutation (T8993G) triggers muscle ATPase deficiency and hypocitrullinaemia

Received: 3 June 1998 / Accepted in revised form: 25 August 1998

**Abstract** Based on the study of three unrelated families, we report what we believe to be the first in vivo evidence of muscle ATPase deficiency in individuals carrying the neurogenic weakness, ataxia and retinitis pigmentosa (NARP) syndrome mtDNA mutation (T8993G). Since plasma citrulline was consistently low in 4/5 patients, we suggest that the NARP mutation caused complex V deficiency in the small intestine as well, thus reducing the availability of mitochondrial ATP required for citrulline synthesis.

**Conclusion** We suggest giving consideration to hypocitrullinaemia as a hallmark of the neurogenic weakness, ataxia and retinitis pigmentosa syndrome mutation and more generally of impaired oxidative phosphorylation in the small intestine in vivo.

**Keywords** T8993G mtDNA mutation · Hypocitrullinaemia · ATPase deficiency

**Abbreviations** *ATPase* ATP synthase · *CPS I* carbamoyl phosphate synthetase I · *NARP* neurogenic weakness, ataxia and retinitis pigmentosa · *OTC* ornithine transcarbamoylase

### Introduction

The T8993G mtDNA mutation in the ATP synthase subunit 6 (ATPase 6) gene was originally reported in the neurogenic weakness, ataxia and retinitis pigmentosa syndrome (NARP) [4]. The NARP mutation is also regarded as a cause of Leigh subacute necrotizing encephalopathy [12]. Yet, while a deficient ATPase activity has been reported in cultured cells of affected individuals [13, 14], no in vivo evidence of ATPase deficiency has been found in patients carrying the NARP mutation.

Here, we report on muscle ATPase deficiency in three unrelated families segregating the T8993G mtDNA mutation. Interestingly, hypocitrullinaemia was noted in 4/5 NARP patients. While its mechanism remains unclear, we suggest giving consideration to hypocitrullinaemia as a hallmark of the NARP mutation and more generally of impaired oxidative phosphorylation in the small intestine in vivo.

### Case reports

#### Pedigree 1

Patient 1 (III1) was born at term to unrelated healthy parents (birth weight 2.5 kg, length 47 cm). His mother's brother and a maternal uncle died in the neonatal period in unknown circumstances. He walked alone aged 2 years. At 2.5 years and 5 years of age respectively, he had recurrent attacks of hypotonia. At 4.5 years of age, he developed myoclonic seizures, cerebellar ataxia and pyramidal syndrome with CT scan evidence of vermis agenesis. The CSF lactate was normal. Heart ultrasound and electroretinogram were normal. He is now 11 years old and mildly mentally retarded.

His brother (III2) was born at term (birth weight 2.760 kg, length 47 cm) and developed severe hypotonia with major metabolic acidosis (blood pH 7.25, serum bicarbonate 15 mmol/l), hyperlactataemia (16 mmol/l, normal <2.2 mmol/l) and hyperammonaemia immediately after birth (270 µmol/l). He died at 3 days of age.

The third boy (III3) was born after a term pregnancy and normal delivery. Immediately after birth, he had hypotonia and tachypnoea with major metabolic acidosis (pH 7.15, serum bicar-

bonate 11 mmol/l), hyperlactataemia (7 mmol/l) and hyperammonaemia (110  $\mu$ mol/l). His neurological condition gradually worsened during the first 6 months of life and he developed retinitis pigmentosa with altered electroretinogram and increased CSF lactate (6 mmol/l, normal <1.7 mmol/l). Cerebral CT scan and MRI were normal but heart ultrasound showed a hypertrophic cardiomyopathy.

#### Pedigree 2

A girl (III1) was born to healthy parents after a term pregnancy and normal delivery. At 4 months of age, she developed hypotonia and poor head control and died at 14 months of status epilepticus. Brain imaging showed abnormalities of basal ganglia typical of Leigh syndrome. Plasma and CSF lactate were elevated (3.8 and 5.4 mmol/l, respectively).

#### Pedigree 3

The proband (II3) was born after a normal pregnancy (birth weight 4.3 kg, length 53 cm). Two older sibs are healthy. He was hypotonic immediately after birth and developed severe hypotonia and swallowing difficulties during the 1st month of life. He was referred at 4 months of age for a generalized seizure and died aged 6 months. MRI revealed typical lesions of Leigh syndrome in both basal ganglia and brainstem. CSF lactate was elevated (4.4 mmol/l). Heart ultrasound was normal.

**Table 1** Plasma arginine, ornithine and citrulline concentrations and respiratory chain enzyme activities in skeletal muscle mitochondria of patients carrying the NARP mutation. Amino acid concentrations are given as extreme absolute values and as mean  $\pm$  2SD. For patient 1 (7 determinations) and patient 3 (12 determinations) the amino acid concentrations are given as extreme

## Methods

Urinary organic acids were analysed by gas chromatography-mass spectrometry [1] and plasma amino-acids were measured by ion exchange chromatography [10]. Protein loading tests (5 g/kg of protein, during meal) and ornithine loading tests were performed according to Rabier et al. [7].

NADH cytochrome c reductase, succinate cytochrome c reductase, decylubiquinol cytochrome c reductase, cytochrome oxidase and ATPase were measured spectrophotometrically in mitochondria isolated from skeletal muscle biopsies [9]. Carbamoyl phosphate synthetase I (CPS I) and ornithine transcarbamoylase (OTC) activities were assayed in jejunal and liver biopsies [6]. Screening for the T8993G mutation in the ATPase mtDNA gene was carried out using specific oligonucleotide primers (nt 8581-8604 and nt 9296-9273, amplification conditions: 30' at 94°C, 30' at 63°C, 30'' at 72°C for 30 cycles). Amplification products (715 bp) were then digested using Ava I restriction enzyme [4].

## Results

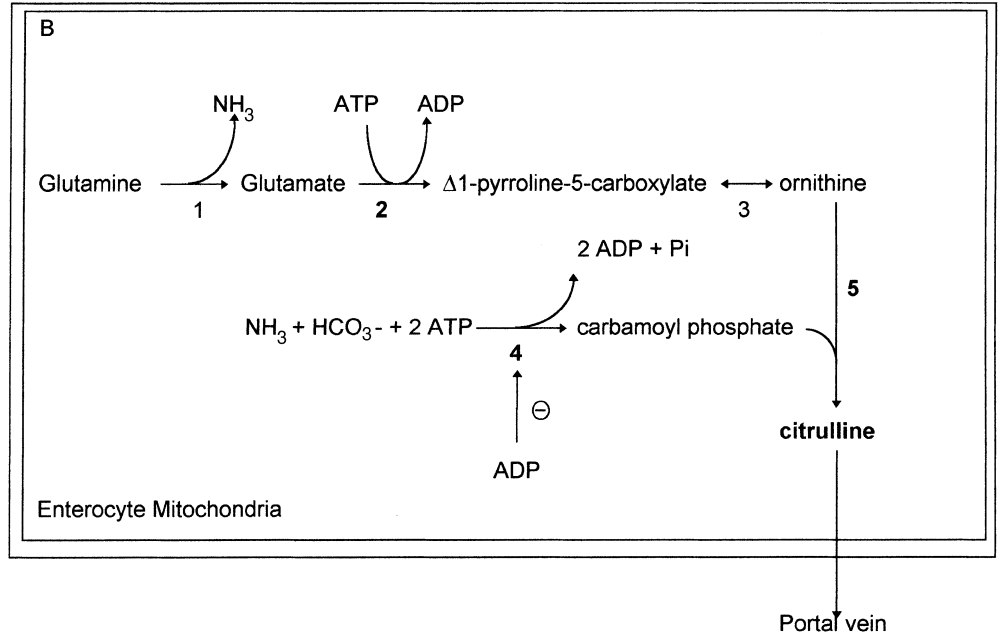
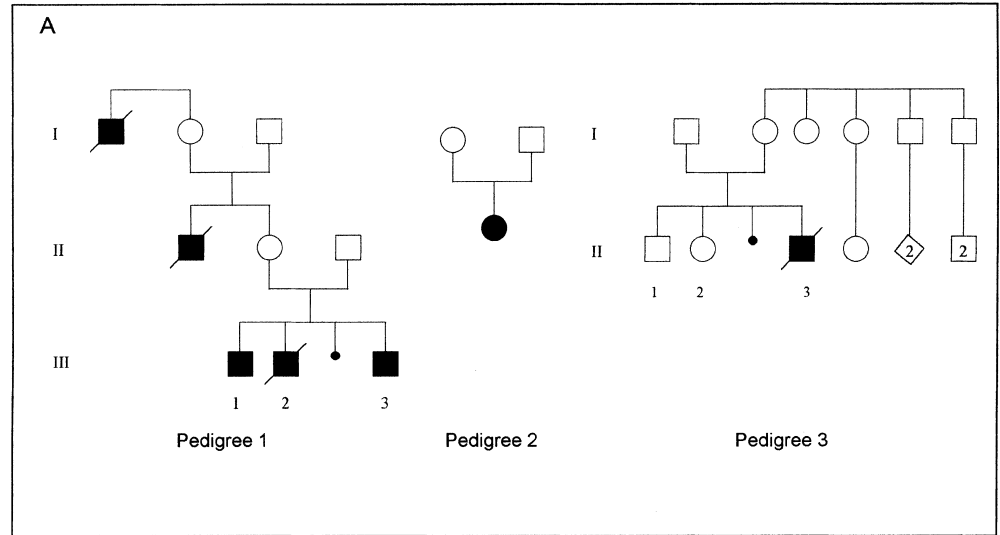
The oligomycin-sensitive ATPase activity was markedly reduced in muscle mitochondria of patients 3–5 (Table 1) while other respiratory chain enzyme activities were normal. No muscle biopsy was available for patients 1, 2 and their mother. Interestingly, plasma citrulline was consistently low in patients 1–4 while

absolute values and as mean  $\pm$  2SD. Control respiratory chain enzyme activities are given as extreme absolute values. *n* number of controls. (ATPase ATP synthase, COX cytochrome c oxidase, NCCR NADH cytochrome c reductase, QCCR decylubiquinol cytochrome c reductase, SCCR succinate cytochrome c reductase)

	Mother III	Pedigree 1			Pedigree 2	Pedigree 3	Control values
		Patient 1 III1	Patient 2 III2	Patient 3 III3	Patient 4 II1	Patient 5 II3	
Plasma concentration, $\mu$ mol/l							
Arginine	63	24–47 36 $\pm$ 7	99*	18–70 29 $\pm$ 14	110–156	30	26–155 <i>n</i> = 174 77 $\pm$ 20
Ornithine	74	23–54 39 $\pm$ 11	191*	20–73 44 $\pm$ 14	144–53	36	31–179 <i>n</i> = 174 72 $\pm$ 25
Citrulline	9	3–5 3.8 $\pm$ 0.7	13*	0–13 3.2 $\pm$ 4	4–3	19	19–33 <i>n</i> = 174 26 $\pm$ 7
Number of plasma concentration determinations	1	7	1	12	2	1	
Respiratory chain enzyme activities muscle mitochondria, nmol/min/mg protein							
NCCR				423	160	196	138–549 <i>n</i> = 225
SCCR				286	188	161	171–679 <i>n</i> = 305
QCCR				1051	356	671	421–1654 <i>n</i> = 210
COX				1051	564	643	607–2419 <i>n</i> = 285
ATPase				117	170	172	220–862 <i>n</i> = 159

\* Post mortem measurement

**Fig. 1 A** Pedigree of family 1, 2 and 3. **B** Enterocyte mitochondrial citrulline synthesis: 1: glutaminase, 2:  $\Delta^1$ -pyrroline-5-carboxylate synthetase, 3: ornithine  $\delta$ -aminotransferase, 4: CPS I, 5: OTC



plasma arginine and ornithine were within normal ranges (Table 1). No increased ammoniaemia was noted following an oral protein load in patient 1 (not shown), but an ornithine loading test failed to increase the level of plasma citrulline in patient 1 (plasma citrulline =  $3 \mu\text{mol/l}$ , control =  $19\text{--}33 \mu\text{mol/l}$ ). OTC and CPS I activities in jejunum and/or liver were normal in patients 1 and 2 (not shown).

The PCR amplification and Ava I digestion of the mitochondrial ATPase 6 gene detected a heteroplasmic T8993G mtDNA mutation in the muscle of patients 3–5. Further investigations detected high levels of mutated mtDNA (90–99%) in a number of tissues including leucocytes (patients 1, 3 and 5), cultured skin fibroblasts (patient 2) and gut (patient 1, not shown). The asymp-

tomatic mother of patients 1–3 also carried a heteroplasmic T8993G mtDNA mutation in her circulating leucocytes (40% of mutated molecules).

## Discussion

Here, we report on hypocitrullinaemia in 4/5 patients with ATPase deficiency ascribed to the NARP (T8993G) mtDNA mutation. This mutation changes a leucine into an arginine in the ATPase 6 gene, thus altering the membrane-spanning domain of the protein with a 50% decrease in the enzyme activity [11]. While the NARP mutation has been previously shown to trigger ATPase deficiency in lymphoblastoid cell lines [13] and cultured

skin fibroblasts [14], this is, to our knowledge, the first report of an ATPase deficiency in muscle mitochondria of NARP patients.

The mechanism of hypocitrullinaemia in patients carrying the NARP mutation and its possible relationship with respiratory enzyme deficiency are unclear. Citrulline is a non protein amino-acid formed from glutamine in liver and gut mitochondria (see for review: [5]). In the liver, citrulline is involved in ammonia elimination and urea synthesis and is not exported. By contrast, citrulline produced by enterocytes can be exported and circulating citrulline is uptaken by kidney to synthesize arginine.

In the intestine, citrulline is synthesized by three mitochondrial matrix enzymes:  $\Delta 1$ -pyrroline-5-carboxylate synthetase, OTC and CPS I (Fig. 1). CPS I requires two ATP molecules for activity and is regulated by the concentration of ATP [2] while ADP acts as an inhibitor [3].  $\Delta 1$ -pyrroline-5-carboxylate synthetase is also dependant on ATP for activity.

Hypocitrullinaemia could result therefore from either a deficient enzyme activity or intestinal atrophy. Normal intestinal histology in patient 1 ruled out intestinal atrophy. Normal plasma ornithine and OTC activity suggested that neither  $\Delta 1$ -pyrroline-5-carboxylate synthetase nor OTC were involved in hypocitrullinaemia. However, the abnormal response to an oral ornithine load suggested that hypocitrullinaemia could result from an impaired formation of citrulline from carbamoyl phosphate. Because the *in vitro* CPS I activity was normal and the ATP concentration is controlled by the respiratory chain, we suggest that the NARP mutation caused complex V deficiency in the small intestine thus reducing the availability of mitochondrial ATP required for CPS I activity. Along these lines, it is worth remembering that hypocitrullinaemia and hypo-ornithinaemia have been already reported in Pearson syndrome [8], a feature ascribed to the secondary deficiency of ATP-dependent enzymes involved in biogenesis of citrulline. It is possible therefore that hypocitrullinaemia could be a non-specific hallmark of respiratory enzyme deficiency in the small intestine. Since hypocitrullinaemia was not observed in one of our five patients, we suggest that the level of heteroplasmy in enterocytes had a threshold effect on citrulline synthesis in our patients. We suggest giving consideration to hypocitrullinaemia as a possible clue to a NARP mutation and more generally, of respiratory enzyme deficiency with intestinal involvement.

## References

1. Chalmers RA, Lawson AM (1982) In: Chalmers and Lawson (ed) *Organic acids in man*, Chapman and Hall, London, 46–55
2. Elliot KRF, Tipton KF (1974) Kinetic studies of bovine liver carbamoylphosphate synthase. *Biochem J* 141:807–816
3. Elliot KRF, Tipton KF (1974) Product inhibition studies on bovine liver carbamoylphosphate synthetase. *Biochem J* 141:817–824
4. Holt IJ, Harding AE, Pety RKH, Morgan-Hughes JA (1990) A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 46:428–433
5. Rabier D, Kamoun P (1995) Metabolism of citrulline in man. *Amino Acids* 9:299–316
6. Rabier D, Benoit A, Petit F, Chekoury A, Bonnefont JP, Saudubray JM, Kamoun P (1989) Ornithine carbamoyl transferase deficiency : a new variant with subnormal enzyme activity. *Clin Chim Acta* 186:25–30
7. Rabier D, Nuttin C, Poggi F, Padovani JP, Abdo K, Bardet J, Parvy P, Kamoun P (1992) Familial joint hyperlaxicity, skin hyperelasticity, cataract and mental retardation with hyperammonemia and low citrulline, ornithine and proline. A new disorder of collagen metabolism. Communication 30th SSIEM symposium
8. Ribes A, Riudor E, Valcarel R, Salva A, Castello F, Murillo S, Dominguez C, Rötig A, Jakobs C (1993) Pearson syndrome: altered tricarboxylic acid and urea-cycle metabolites, adrenal insufficiency and corneal opacities. *J Inherit Metab Dis* 16:537–540
9. Rustin P, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, Munnich A (1994) Biochemical and molecular investigations in the respiratory chain deficiencies. *Clin Chim Acta* 228:35–51
10. Slocum RH, Cummings JG (1991) Amino acids analysis of physiological samples. In: Hommes FA (ed) *Techniques in diagnostic human biochemical genetics*. Wiley-Liss, New York, 87–126
11. Tatuch Y, Robinson BH (1993) The mitochondrial DNA mutation at 8993 associated with NARP slows the rate of ATP synthesis in isolated lymphoblasts mitochondria. *Biochem Biophys Res Commun* 92:124–128
12. Tatuch Y, Christodoulou J, Feigenbaum A, Clarke JTR, Wherret J, Smith C, Rudd N, Petrova-Benedict R, Robinson BH (1992) Heteroplasmic mtDNA mutation T8993G can cause Leigh disease when the percentage of abnormal mtDNA is high. *Am J Hum Genet* 50:852–858
13. Tatuch Y, Pagon RA, Vlcek B, Roberts R, Korson M, Robinson B (1994) The 8993 mtDNA mutation: heteroplasmy and clinical presentation in three families. *Eur J Hum Genet* 2:35–43
14. Vazquez-Memije ME, Shanske S, Santorelli FM, Kranz-Eble P, Davidson E, DeVivo DC, DiMauro S (1996) Comparative biochemical studies in fibroblasts from patients with different forms of Leigh syndrome. *J Inherit Metab Dis* 19 (1):43–50