Letters to the Editor

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About the "Pathological" Role of the mtDNA T3308C Mutation…

To the Editor:

Numerous mtDNA mutations have been associated with the mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS) syndrome (MIM 540000). These include transitions at nucleotide positions (nt) 1642, 3243, 3252, 3256, 3271, 3291, 3308, and 9957, and a 4-bp deletion beginning at nt 14787. For some of these mutations (A3243G, C3256T, and T3271C), the causal relationship with the phenotype has been confirmed, whereas for others, the status is still provisional (MITOMAP). The T3308C mutation in the NADH dehydrogenase subunit 1 (ND1) is a member of the "provisional" group and was described in a Spanish subject affected by MELAS and bilateral striatal necrosis. This mutation changes the highly conserved methionine 1 to a threonine, was heteroplasmic in both the proband and her asymptomatic mother, and was absent in 130 normal and other-disease controls (Campos et al. 1997). More recently, a homoplasmic T3308C mutation has also been reported in a colorectal tumor, in which it was associated with two other somatic homoplasmic transitions, T710C and T1738C. It has been suggested that these mutations could have a functional effect in mitochondrial selection (Polyak et al. 1998). However, doubts about the pathological significance of the T3308C mutation have been raised by a study involving 37 Portuguese patients with a clinical phenotype of mitochondrial encephalomyopathies and 150 Portuguese control subjects. The T3308C mutation was observed in two patients and in four controls (Vilarinho et al. 1999). In all cases it was homoplasmic.

To better define the role of this putative pathological mutation, we did a detailed analysis of the mtDNA background on which the T3308C had been reported. By sequence analysis of several tRNA genes and their surrounding sequences, we determined that, in addition to the T3308C mutation, the mtDNA of both Portuguese patients harbored the combination of mutations T1738C, T5655C, G7521A, A10398C, and A14769G and a dinucleotide deletion at nt 514–515. We observed the same mutations in the two Spanish patients (in the meantime a second Spanish patient had been found) and in the four Portuguese controls who tested positive for the mutation. Thus, these results indicated that all these mtDNAs were members of the same mtDNA haplogroup and that most likely they shared the T3308C mutation by descent. Intriguingly, this haplogroup harbored the combination of mutations T3308C and T1738C, similar to the case reported by Polyak et al. (1998). The search in our samples for the third somatic mutation (T710C) found in the colorectal tumor was negative.

To identify the mtDNA haplogroup harboring the mutation T3308C, sequence analysis of the mtDNA control region between nt 16090 and 16375 was performed in the eight T3308C samples (table 1). This analysis revealed a consensus motif (16126–16187–16189–16223–16264–16270–16278–16293–16311) that is typical of the West African haplogroup L1b (Watson et al. 1997; Rando et al. 1998), thus allowing us to classify Portuguese and Spanish mtDNAs with the T3308C mutation within this haplogroup. It has been determined elsewhere, by high-resolution restriction analysis (Tor-

Table 1

mtDNA Control Region Variation in Iberian Patients and Controls

Sample ID	Origin	Control Region Variation[a]
1	Portuguese patient	126, 187, 189, 215T, 223, 264, 270, 278, 311
2	Portuguese patient	126, 187, 189, 223, 264, 270, 278, 293, 311
3	Spanish patient	126, 187, 189, 223, 264, 270, 278, 293, 311, 360
4	Spanish patient	126, 187, 189, 223, 264, 270, 278, 293, 311
5	Portuguese control	104, 187, 189, 223, 270, 278, 289, 293, 311
6	Portuguese control	126, 187, 189, 223, 264, 270, 278, 293, 311
7	Portuguese control	126, 187, 189, 223, 264, 270, 278, 293, 311
8	Portuguese control	126, 187, 189, 223, 264, 270, 278, 293, 311

[a] Nucleotide positions (−16000) between nt 16090 and 16375, different from the Cambridge Reference Sequence (Anderson et al. 1981). Mutations are transitions (T↔C, A↔G), unless the base change is specified explicitly.

roni et al. 1996, 1997), that haplogroup L1b is defined by the RFLP motif: +185 *Taq*I, +2349 *Mbo*I, −2758 *Rsa*I, +3592 *Hpa*I, −3693 *Mbo*I, −7055 *Alu*I, +10394 *Dde*I, +10806 *Hin*fI (Chen et al. 1995; Rando et al. 1998; A. Torroni, unpublished data). Therefore, we selected, among our African population samples, all those (a total of 48) who either by RFLP analysis or by control region sequencing had been classified as members of haplogroup L1b. Analysis of their status at nt 3308 revealed that all of them harbored the mutation. In contrast, control samples belonging to African haplogroups L1a, L1c, and L2 were found to lack the mutation. These results indicate that the T3308C mutation defines exclusively by descent haplogroup L1b mtDNAs, and it is very ancient since L1b probably originated in western Africa ~12,000–19,000 years ago (Watson et al. 1997; Rando et al. 1998). Thus, Spanish and Portuguese mtDNAs with the T3308C mutation are of African origin, and their presence probably reflects the arrival of North Africans during the Mesolithic Age (8000 B.C.) and/or during the Arabic rule that started at ~800 A.D. (Arnaiz-Villena et al. 1997). If we take into account that haplogroup L1b frequencies in populations of western Africa are in the range of 10%–20% (Watson et al. 1997; Rando et al. 1998), the observed frequency in the Portuguese population (~2%–3%) indicates a significant influence of North Africans in the Iberian gene pool.

In conclusion, the T3308C mutation is an ancient marker of a common West African haplogroup, and all Iberian subjects with this mutation who were affected by mitochondrial encephalomyopathies harbored haplogroup L1b mtDNAs. This finding is difficult to reconcile with a role of this mutation in disease expression and further indicates that haplogroup classification of patients' mtDNAs, followed by a search for the putative disease mutation in phylogenetically closely related control mtDNAs, is a crucial step in the identification of mtDNA disease mutations. Furthermore, the observation that the elimination of the methionine codon AUA at position 1 of the ND1 subunit is common in some human populations suggests that the maintenance of that codon is not so critical in our species. Possibly this is because the third codon (AUG) of the human ND1 subunit also encodes for a methionine, and the ND1 subunit of L1b mtDNAs, although it might be shortened by two amino acids, apparently still retains its functionality. However, it is intriguing that the same combination, T3308C–T1738C, that characterizes haplogroup L1b has also occurred in a colorectal tumor as new somatic mutations. This is especially noteworthy when it is taken into account that T1738C occurs in the 16S rRNA, a gene involved in the translation process, and that the T3308C mutation might indeed affect the translation process of ND1 on non-L1b mtDNA backgrounds. This observation raises again the possibility of polygenic models in which certain mtDNA mutations can be functional and maintained in the population only if they occur in combination with other specific mtDNA mutations.

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Diaphragmatic Spinal Muscular Atrophy with Respiratory Distress Is Heterogeneous, and One Form Is Linked to Chromosome 11q13-q21

To the Editor:

Diaphragmatic spinal muscular atrophy (SMA) has been delineated as a variant of infantile SMA (SMA1 [MIM 253300]) (Mellins et al. 1974; Bertini et al. 1989). The most prominent symptoms are severe respiratory distress resulting from diaphragmatic paralysis with eventration shown on chest x-ray and predominant involvement of the upper limbs and distal muscles. In contrast to classic SMA1, in diaphragmatic SMA the upper spinal cord is more severely affected than the lower section. The *pmn* mouse presents with progressive motor neuronopathy and a disease that closely resembles diaphragmatic SMA (Schmalbruch et al. 1991). The *pmn* locus has been mapped to murine chromosome 13 (Brunialti et al. 1995).

Here we report on nine patients from three families with diaphragmatic SMA following autosomal recessive inheritance. The diagnosis of diaphragmatic SMA was made on the basis of clinical criteria (Rudnik-Schöneborn et al. 1996). Family 1 is of Lebanese origin; family 2, German origin; and family 3, Italian origin. We obtained DNA samples from these families after receiving informed consent, in accordance with the Declaration of Helsinki.

In family 1 (fig. 1*A*), the parents are first cousins. The first affected son died, at the age of 10 wk, of suspected sudden infant death syndrome (SIDS). One daughter presented, at the age of 6 wk, with feeding difficulties and progressive respiratory distress. Chest x-ray showed eventration of the diaphragm. Mechanical ventilation was initiated at the age of 8 wk. She developed progressive muscular atrophy with complete paralysis of the upper and lower limbs and mild contractures of the knee and ankle joints. Three other children, nonidentical twin daughters and the youngest daughter, died of respiratory failure—the twins at the age of 8 and 9 wk and the youngest daughter at the age of 8 wk. Autopsy specimens were taken from gastrocnemius muscle in both twins and from the upper spinal cord in one twin. Skeletal-muscle histology revealed neurogenic atrophy without signs of reinnervation. Ultrastructurally, the motor end plates lacked nerve terminals and showed postsynaptic degenerative changes characterized by deep invaginations. The diameter of anterior spinal roots was reduced in the upper spinal cord. The remaining motor neurons showed chromatolysis. These findings offer two different pathophysiological concepts: (1) degeneration of the anterior horn cells of the spinal cord with neurogenic muscular atrophy suggests dying-forward atrophy, and (2) presynaptic and postsynaptic signs of motor end-plate degeneration suggest dying-back atrophy. In family 2 (fig. 1*B*), the first child had severe muscular hypotonia and died, at the age of 9 wk, of cardiorespiratory failure. The third child has been mechanically ventilated since the age of 3 mo. In family 3 (fig. 1*C*), which has been reported in detail elsewhere (Novelli et al. 1995), the gene locus for SMA1, on chromosome 5q, has been excluded. Both affected sibs presented with respiratory insufficiency right after birth and with the typical signs of diaphragmatic SMA.

First, we confirmed that, in families 1 and 2, there is no linkage of the trait to markers of the SMA locus on 5q11.2-q13.3, as there is in family 3. Second, the orthologous regions corresponding to the murine *pmn* gene region on human chromosomes 1q and 7p were excluded as gene loci responsible for the disease (Grohmann et al. 1998).

To locate the gene locus for diaphragmatic SMA, a whole-genome scan was undertaken in family 1. Microsatellite analysis was performed, by standard semiau-