SIMULTANEOUS MFN2 AND GDAP1 MUTATIONS CAUSE MAJOR MITOCHONDRIAL DEFECTS IN A PATIENT WITH CMT

Mutations in the MFN2 gene are associated with Charcot-Marie-Tooth disease type 2A (CMT2A), a dominant axonal CMT, whereas mutations in GDAP1 are associated with recessive demyelinating CMT (CMT4A), recessive axonal CMT (AR-CMT2), and dominant axonal CMT (CMT2K). Both proteins are involved in energy metabolism and dynamics of the mitochondrial network.1-3 We have previously reported that, in fibroblasts from patients with CMT, MFN2 mutations resulted in a mitochondrial energy coupling defect,4,5 whereas dominant mutation in GDAP1 resulted in defective complex I activity.6

In this study, we investigated mitochondrial bioenergetics from a severely affected patient with CMT harboring combined mutations in both GDAP1 and MFN2 genes.

Methods. For details, see e-Methods on the Neurology® Web site at www.neurology.org.

Patients. Patient II-5 (figure 1A), a 71-year-old woman of Spanish origin, had severe distal muscle weakness from the age of 3, becoming wheelchair-bound during her third decade. Clinical examination showed severe weakness of limbs with proximal and distal amyotrophy, tactile and nociceptive hypoesthesia with a gloves-and-socks distribution, and abolition of the limb reflexes. She had pes cavus and moderate vocal cord paresis. Electrophysiologic studies (table e-1) indicated a severe sensory axonopathies characterized by a major reduction of motor action potential in the left median nerve (0.1 mV) with a slightly reduced motor conduction velocity (43 m/s).

Patient II-8, her 56-year-old brother, presented with a mild CMT2 clinical phenotype. Electrophysiologic examination showed a sensory axonal neuropathy (table e-1). His 2 daughters, aged 19 and 25 years, are currently asymptomatic. Patient II-2, who had a phenotype compatible with CMT, had died of respiratory failure.

Results. Mutation analysis. Patient II-8 and his asymptomatic daughter (III-15) were found to be heterozygous for the pathogenic p.Q163X mutation in MFN2, previously described.7 Individuals II-3, II-4, and II-7 were heterozygous for the p.Q163X mutation in GDAP1. Patient II-5 was found to be heterozygous for the p.R468H mutation in MFN2 and homozygous for the p.Q163X mutation in GDAP1 (figure 1A).

Mitochondrial imaging and biochemistry. No alteration of the mitochondrial network was revealed in MFN2: p.R468H fibroblasts and in MFN2:p.[R468H]+GDAP1:p.[Q163X]+[Q163X] fibroblasts (figure e-1).

In patient II-5 fibroblasts, a more severe energy coupling defect than in MFN2 patients was discovered with 85% reduction of the ATP/O ratio compared to controls (figure 1Ba). Mitochondrial uncoupling was associated with a 65% decrease of mitochondrial ATP production that was absent in fibroblasts with a single MFN2 mutation (figure 1Bb). Similarly to patients with dominant mutation in GDAP1, fibroblasts carrying mutations in both MFN2 and GDAP1 showed a 40% reduction of complex I activity compared with MFN2 patients and controls (see malate pyruvate complex I substrates value on figure 1Bc, and enzymatic complex measurements on figure 1Bd).

Discussion. We report a patient with CMT carrying simultaneous mutations in GDAP1 and MFN2. Initially, this patient was found to be heterozygous for the p.R468H mutation in MFN2. Individuals carrying this mutation usually have mild CMT phenotype or are asymptomatic.7 Hence the sole presence of this mutation could not be responsible for the severe clinical phenotype,7 suggesting the need for further genetic analysis, which revealed the existence of the p.Q163X homozygous mutation in GDAP1 as an explanation of the clinical severity.

We have previously reported that MFN2 mutations resulted in an energy coupling defect with normal mitochondrial ATP production,4,5 whereas dominant mutation in GDAP1 resulted in defective complex I activity with a decrease in ATP production when complex I substrates (malate and pyruvate) were used.6 A compensatory mechanism was postulated since the production of ATP was normal when succinate, the complex II substrate, was used (figure 1Bb). In a patient carrying mutations in GDAP1 and MFN2, we showed defective complex I activity as well as severe mitochondrial uncoupling. Interest-
ingly, the reduction of complex I activity was similar to that of fibroblasts with the dominant GDAP1 mutation. In addition, the ATP production with complex I and complex II substrates was significantly reduced compared to fibroblasts harboring either the MFN2 or GDAP1 mutations alone (figure 1Bb). These findings underscore the role of GDAP1 in the function of respiratory complex I and suggest that this combination of mutations has biochemical deleterious synergistic effects. In MFN2 patients, the mitochondrial uncoupling was associated with a higher respiratory rate involving complex II, serving as a compensatory mechanism (MPS value, figure 1Bc). However, in MFN2-GDAP1 double mutant fibroblasts, the complex I defect may have limited such compensation, leading to a decrease in ATP production.

Finally, our study suggests that the clinical heterogeneity of CMT may be related to the simultaneous presence of mutant alleles in different CMT genes, emphasizing the need for extensive genetic investigation to provide accurate diagnosis.

Figure 1 Genetic and biochemical findings

(A) Pedigree of a family with Charcot-Marie-Tooth disease (CMT) showing the distribution of the nonsense p.Q163X mutation in GDAP1 and the missense p.R468H mutation in MFN2. Clear circles: unaffected females; clear squares: unaffected males; black circle: affected female; and black squares: affected males; m: mutant alleles; and wt: wild-type alleles. The arrow indicates the proband. (B) Biochemical findings. (a) Coupling efficiency (ATP/O), i.e., rate of ATP produced per nanomole of oxygen consumed with malate (M), pyruvate (P), and succinate (S) as substrates. (b) Mitochondrial ATP production with MPS. (c) Rate of oxygen consumption with malate pyruvate (MP) or MPS, or with S/Rotenone (R), a mitochondrial complex I inhibitor. (d) OxPhos enzymatic activities were normalized with the citrate synthase (CS) activity, a reference mitochondrial content. The results are expressed as mean values ± SD of 3 independent measurements. Statistical significance levels applied to the control, MFN2, and GDAP1 groups: *p < 0.05; **p < 0.01. Control group n = 7; MFN2 group n = 10 (7 different MFN2 mutations); GDAP1 group n = 3 (p.C240Y mutation). See e-Methods for more information.
Presence of anti-acetylcholine receptor (anti-AChR) antibodies is highly specific for myasthenia gravis (MG). These antibodies are detected in 85%–95% of sera from patients with generalized MG and 40%–70% of patients with ocular MG.1

An increasing incidence of MG, especially in the elderly population, has been described in Japan, the United Kingdom, and Denmark.2-4 Recent studies have also focused on the epidemiology of anti-AChR antibody seropositivity in the physiopathology of Charcot-Marie-Tooth disease type 2A. Exp Neurol 2009;218:268-273.

Methods. We performed a population-based study of the incidence of anti-AChR antibody-seropositivity in BC for the 25-year period of January 1, 1984, to December 31, 2008. Incident cases were ascertained by retrospectively identifying all first-time seropositive cases. Incidence rates (IRs) were calculated per 1 million inhabitants based on annual July population estimates (BCStats, www.bcstats.gov.bc.ca). Cases were stratified into 4 age groups based on age at the first positive test: 85-19, 20-44, 45-64, and ≥65 years. Age- and sex-stratified IRs were calculated based on population estimates of the corresponding age and sex group. Ninety-five percent confidence intervals (95% CI) were calculated using the Poisson distribution.

Results. Between January 1984 and December 2008, we identified 1,243 new anti-AChR seropositive individuals (648 women, 587 men, 8 unknown). The age at the first positive serum sample in women had a bimodal distribution with peaks at 45-55 and 70-85 years, whereas in men the distribution had a single peak at 70-80 years (figure e-1 on the Neurology® Web site at www.neurology.org). This age distribution resembles previous observations on the age at onset of MG.5 The average annual IR of first-time anti-AChR seropositive cases for the period of 1984-2008 was 13.2 per year per million (95% CI 12.5-14.0).

Mean annual IRs of the ≤19, 20-44, and 45-64 age groups did not change substantially over these 25 years: 3.6/year/million (95% CI 2.9-4.5), 7.7 (95% CI 6.8-8.7), and 16.8 (95% CI 15.1-18.7) (figure 1). In contrast, annual IRs of the ≥65 age group significantly increased from 21.4 during 1984-1988 (95% CI 15.2-29.3) to 52.9 during 2004-2008 (95% CI 45.1-61.9). Sex-adjusted IRs significantly increased for both men and women ≥65 from 1984-1988 to 2004-2008 as follows: from 26.4 (95% CI 16.3-40.4) to 63.5 (95% CI 50.8-78.7) for men and from 17.6 (95% CI 10.4-27.8) to 44.2 (95% CI 34.7-55.7)