J. Cassereau, MD
C. Casasnovas, MD, PhD
N. Gueguen, PhD
M.-C. Malinge, PharmD
V. Guillet, PhD
P. Reynier, MD, PhD
D. Bonneau, MD, PhD
P. Amati-Bonneau, MD
I. Banchs, BS
V. Volpini, MD, PhD
V. Procaccio, MD, PhD
A. Chevrollier, PhD

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 axonal neuropathy 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.

Supplemental data at www.neurology.org

Results. ***Mutation analysis.*** Patient II-8 and his asymptomatic daughter (III-15) were found to be heterozygous for the pathogenic p.R468H 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).

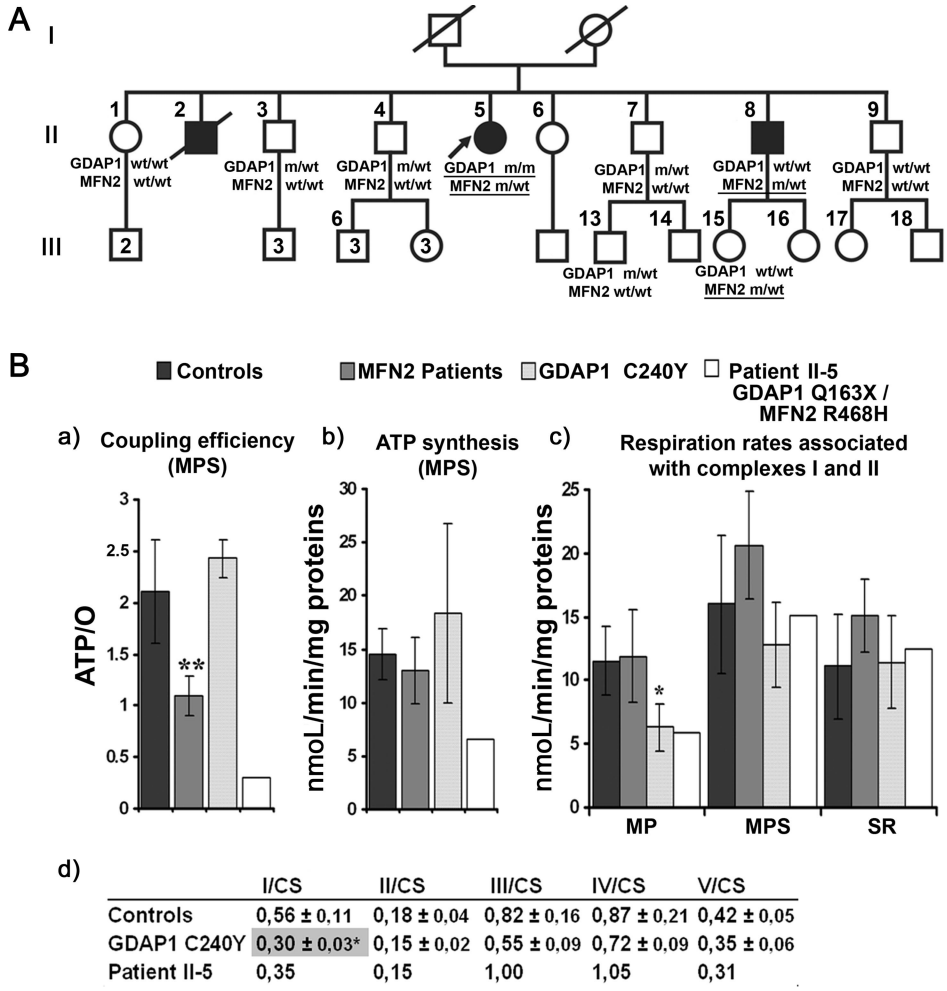
Mitochondrial imaging and biochemistry. No alteration of the mitochondrial network was revealed in MFN2: p.R468H fibroblasts and in MFN2:p.[R468H]+GDAP1:p.[Q163X]+[Q163X] fibroblasts (figure e-1).

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-

Figure 1 Genetic and biochemical findings



d)

	I/CS	II/CS	III/CS	IV/CS	V/CS
Controls	0,56 ± 0,11	0,18 ± 0,04	0,82 ± 0,16	0,87 ± 0,21	0,42 ± 0,05
GDAP1 C240Y	0,30 ± 0,03*	0,15 ± 0,02	0,55 ± 0,09	0,72 ± 0,09	0,35 ± 0,06
Patient II-5	0,35	0,15	1,00	1,05	0,31

(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.

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).[5] 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.

From UMR INSERM (J.C., N.G., P.R., D.B., P.A.-B., V.P., A.C.), U771-CNRS6214, Angers; Departments of Neurology (J.C.) and Biochemistry and Genetics (N.G., M.-C.M., P.R., D.B., P.A.-R., V.P., A.C.), University Hospital of Angers, Angers; School of Medicine (J.C., V.G., P.R., D.B., V.P.), University of Angers, Angers, France; Neuromuscular Unit (C.C.), Neurology Department, Hospital Universitari de Bellvitge-IDIBELL, Barcelona; Molecular

Genetic Diagnosis Center of Inherited Disease-IDIBELL (C.C., I.B., V.V.), Barcelona; and Centro de Investigación Biomédica en Red Enfermedades Degenerativas (CIBERNED) (C.C.), IDIBELL, Barcelona, Spain.

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Address correspondence and reprint requests to Dr. Vincent Procaccio, Biochemistry and Genetics Laboratory, National Centre for Neurodegenerative and Mitochondrial diseases, CHU Angers, 4 rue Larrey, 49933 Angers, France; ViProcaccio@chu-angers.fr

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Zahra Pakzad
Tariq Aziz, BSc
Joel Oger, MD, FRCPC, FAAN

INCREASING INCIDENCE OF MYASTHENIA GRAVIS AMONG ELDERLY IN BRITISH COLUMBIA, CANADA

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 as a surrogate marker of MG.[4-6] The Neuro-Immunology Laboratory at the University of British Columbia is the sole laboratory in British Columbia (BC), Canada, offering anti-AChR antibody testing for clinical diagnosis.

The aims of this study were to evaluate the incidence and epidemiologic characteristics of anti-AChR antibody seropositivity in BC and to examine changes in incidence over time.

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 tests. A positive anti-AChR antibody test was necessary and sufficient for a patient to be included as a case. During these 25 years, BC's population increased from 2,947,181 to 4,381,603.

Data analysis. Incidence was defined as the annual number of first-time anti-AChR 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: ≤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.

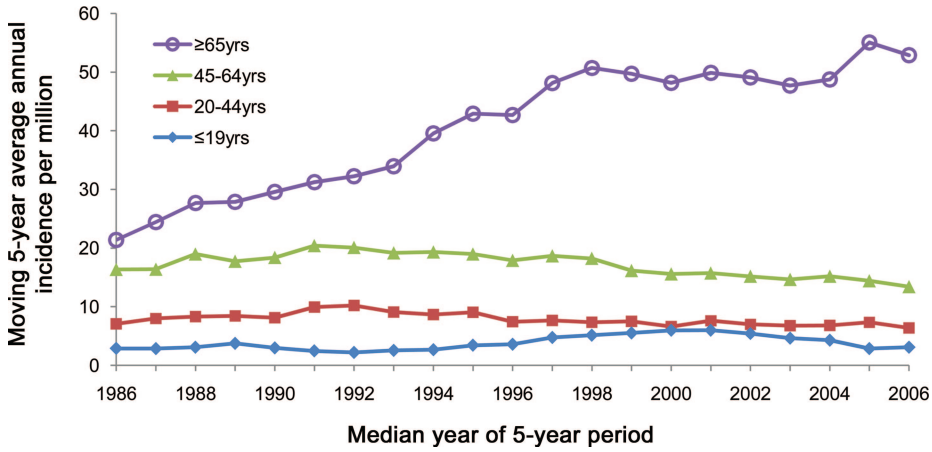
Results. Between January 1984 and December 2008, we identified 1,243 new anti-AChR seropositive individuals (648 women, 587 men, 8 unknown). The age at the first positive serum sample in women had a bimodal distribution with peaks at 45–55 and 70–85 years, whereas in men the distribution had a single 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.[3,5] 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)

Supplemental data at www.neurology.org

Figure 1 Age-specific incidence rates of anti-acetylcholine receptor antibody seropositivity in British Columbia from 1984 to 2008



The rates are age group adjusted and calculated per million population as 5-year simple moving averages.

for women (figure e-2). Moreover, our results also indicate that the incidence of anti-AChR seropositivity in the ≥65 age group has increased at a significantly greater rate than the rate of increase in the proportion of people ≥65 in the BC population (figure e-2).

Discussion. The presence of anti-AChR antibodies is considered a good surrogate marker for the diagnosis of MG. The overall average anti-AChR seropositivity IR of 13.2 per million in BC is among the highest reported, with only a higher IR of 18 per million in the United Kingdom.[3]

Our data indicate that the probability of becoming anti-AChR seropositive significantly increases with age (figure 1). Previous studies have also indicated an increasing incidence of elderly onset MG.[2-4,7] From 1984 to 2008, the incidence of anti-AChR seropositivity in BC remained relatively constant in younger age groups, but more than doubled in the ≥65 population for both men and women (figures 1 and e-2). This trend cannot simply be attributed to increasing longevity and an aging population, since our results show that the incidence of anti-AChR seropositivity in the ≥65 age group has increased at a significantly greater rate than the rate of increase in the proportion of people ≥65 in BC (figure e-2). One can argue that our observations are due to greater awareness of MG or increased anti-AChR antibody testing. However, the IRs of the 3 younger age groups have remained stable. Other contributing factors could be improved diagnosis of elderly patients or environmental and lifestyle changes.

Prominent features of our study include a centralized source of data, a large sample size which renders IRs more reliable, and data spanning 25 years. This study confirms the trend of increasing incidence of elderly onset anti-AChR seropositive MG observed in previous studies and its results are important for future health care planning.

From the Neuro-Immunology Laboratory, Department of Medicine, Division of Neurology, University of British Columbia, Vancouver, BC, Canada.

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Address correspondence and reprint requests to Dr. Joel Oger, UBC Hospital, Room S-159, 2211 Wesbrook Mall, Vancouver, BC, Canada V6T 2B5; oger@interchange.ubc.ca

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Classification scheme requirements for therapeutic questions

Class I. A randomized, controlled clinical trial of the intervention of interest with masked or objective outcome assessment, in a representative population. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class II. A randomized, controlled clinical trial of the intervention of interest in a representative population with masked or objective outcome assessment that lacks one criterion a-e in Class I or a prospective matched cohort study with masked or objective outcome assessment in a representative population that meets b-e in Class I. Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences.

Class III. All other controlled trials (including well-defined natural history controls or patients serving as their own controls) in a representative population, where outcome is independently assessed, or independently derived by objective outcome measurements.

Class IV. Studies not meeting Class I, II, or III criteria including consensus or expert opinion.

AAN classification of recommendations

A = Established as effective, ineffective, or harmful (or established as useful/predictive or not useful/predictive) for the given condition in the specified population. (Level A rating requires at least two consistent Class I studies.)

B = Probably effective, ineffective, or harmful (or probably useful/predictive or not useful/predictive) for the given condition in the specified population. (Level B rating requires at least one Class I study or two consistent Class II studies.)

C = Possibly effective, ineffective, or harmful (or possibly useful/predictive or not useful/predictive) for the given condition in the specified population. (Level C rating requires at least one Class II study or two consistent Class III studies.)

U = Data inadequate or conflicting; given current knowledge, treatment (test, predictor) is unproven.