Evaluation of cytogenetic and DNA damage in mitochondrial disease patients: effects of coenzyme Q10 therapy

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Endogenous oxidative stress is believed to play a key role in the pathogenesis of mitochondrial diseases (MD). In this group of heterogeneous disorders the increased production of radical species caused by compromised mitochondrial respiratory function could affect both mitochondrial and nuclear DNA integrity. The aim of the present study was to assess the basal level of nuclear DNA (nDNA) damage in terms of chromosome and DNA alterations in leukocytes of 13 patients (age range 29–74 years) presenting several forms of MD. A further objective of this work was the evaluation of possible changes in nDNA in a subgroup of patients (10 individuals) before and after a 2 week therapy with ubidecarenone, a coenzyme Q10 analogue. The extent of cytogenetic damage, expressed as chromosome breakage and chromosome loss, was assessed employing the cytokinesis block micronucleus method in cultured peripheral blood lymphocytes, coupled with fluorescence in situ hybridization analysis using a digoxigenin-labelled pan-centromeric DNA probe. A modified version of the single cell gel electrophoresis assay was used to quantify primary and oxidative DNA damage in leukocytes. In MD patients an increased level of chromosome damage, expressed as frequency of micronucleated lymphocytes, was detected in comparison with healthy individuals of corresponding sex, age and lifestyle. The FISH analysis revealed a preferential occurrence of micronuclei arising from loss of whole chromosomes in patients, with no substantial difference in frequencies observed in matched controls. The Comet assay indicated a slightly higher level of primary DNA damage in patients compared with controls and also a difference in oxidative DNA damage, however, this was not statistically significant. Patients receiving ubidecarenone showed a statistically significant reduction in the frequency of micronucleated cells after therapy, while only a slight decrease was observed in the levels of both primary DNA damage and oxidized bases.

Introduction

Mitochondrial diseases (MD) are a wide group of disorders often characterized by impairment in energy production by means of the mitochondrial oxidative phosphorylation (OXPHOS) process. A common presenting feature of mitochondrial syndromes is the involvement of muscle and the central nervous system, tissues greatly relying on oxidative metabolism for their energy supply and so especially susceptible to its impairment (DiMauro and Moraes, 1993). These rare disorders are usually associated with a delayed age of onset, organ target selectivity and a progressive course. Over 100 point mutations and numerous rearrangements of mitochondrial DNA (mtDNA) have been associated with a wide spectrum of multi-systemic or tissue-specific disorders and mutations in nuclear genes responsible for alterations in individual respiratory chain complexes and for defects of intergenicomal signalling are now well known (Shoubridge, 2001). In some cases, as a consequence of the still incomplete knowledge of the underlying molecular defect, it is not possible to detect the exact pathogenetic mutation: in such instances the diagnosis is based on clinical, morphological and biochemical observations.

Primary or nucleus-driven mtDNA mutations are responsible for the observed decline in aerobic ATP production, but they also seem to lead to other important consequences. Indeed, several hypotheses have been proposed to explain the still unknown mechanisms underlying the pathophysiology of cell damage in mitochondrial disease. Among the factors invoked, increased endogenous oxidative stress seems to play a major role. Reactive oxygen species (ROS), such as the superoxide anion and hydrogen peroxide, are generated during the normal respiratory process in mitochondria through reactions with electrons that escape the electron transport chain. Enhanced production of ROS, elicited by OXPHOS defects and not followed by a comparable increase in antioxidant defence, seems to result in further persistent damage to cellular structures, including mtDNA itself and membrane lipids, thus generating a vicious cycle (Wei, 1998; Cassarino et al., 1999). Second, both nuclear DNA (nDNA) and mtDNA mutations, originating as a consequence of oxidative stress, may contribute to the initiation and progression of mitochondrial diseases and may also account for the great variation in clinical phenotypes. Enhanced production of radical species resulting from failure of the mitochondrial respiratory chain may also induce additional nDNA damage even in peripheral target tissues like blood cells. In fact, peripheral non-dividing leukocytes can be regarded as a valid indicator of the overall level of oxidative damage in the body.

This work aimed at assessing background levels of nDNA damage in peripheral leukocytes of 13 patients suffering from various forms of MD. Cytogenetic alterations, in terms of chromosome breakage and chromosome loss, were evaluated employing the cytokinesis block micronucleus method (CBMN assay) in cultured peripheral blood lymphocytes, followed by fluorescence in situ hybridization (FISH) using a digoxigenin-labelled alphoid DNA probe specific for the centromere of all human chromosomes. Micronuclei (MN) may originate fromacentric chromosome fragments or whole chromosomes lagging behind during cell division. The CBMN assay, especially when coupled to FISH analysis, is an effective tool for
assessing cytogenetic alterations (Norppa and Falk, 2003). This methodology was successfully applied to detect increased rates of chromosome damage in patients with degenerative diseases such as Alzheimer’s disease (Migliore et al., 1997; Trippi et al., 2001), Parkinson’s disease (Migliore et al., 2002) and cancer (Bolognesi et al., 2002).

In recent years, single cell gel electrophoresis (SCGE), also known as the Comet assay, a rapid, simple and sensitive device, was introduced to quantify genomic damage at the individual cell level and specifically for detecting DNA strand breaks (Singh et al., 1998; Trippi et al., 1994; Moraes et al., 1995). As far as clinical characteristics were concerned, eight subjects were affected by chronic progressive external ophthalmoplegia (CPEO), in one of them associated with ataxia, one by a complex picture of encephalomyopathy, one by myoneurogastrointestinal encephalopathy (MNGIE) syndrome and one by myoclonic epilepsy. From a molecular point of view, all patients but one (patient code MD1, mtDNA mutation unknown) carried a single or multiple large scale mtDNA deletion(s) (Table I). Patients did not follow any pharmacological therapy at the beginning of the study. In addition, 12 healthy individuals, matched for gender, age and smoking habit with MD patients, were selected as control subjects.

Heparinized peripheral blood samples were collected by venipuncture from individuals included in the study. All subjects were interviewed to fill in a detailed questionnaire on their personal data, occupational and medical history and lifestyle (smoking habit, diet, etc.); they had no previous history of exposure to toxic substances or recent X-ray examination. Informed consent for participation in the study was obtained from each subject and the study was approved by our local Ethical Committee.

A subset of 10 patients (six males and four females) gave consent to receive a 2 week therapy with CoQ10 (100 mg/day). Two blood samples were obtained from these individuals: the first, prior to the treatment and the second at least 1 month after the first sampling, i.e. 2 weeks after the end of the therapy.

### Table I. Study population details

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<th>Code</th>
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### Materials and methods

#### Subjects

The study was performed with 13 patients affected by several forms of MD diagnosed at the Department of Neuroscience, University of Pisa. The diagnosis of mitochondrial disease was established according to accepted clinical, morphological, biochemical and molecular genetic criteria (Schon et al., 1994; Moraes et al., 1995). As far as clinical characteristics were concerned, eight subjects were affected by chronic progressive external ophthalmoplegia (CPEO), in one of them associated with ataxia, one by a complex picture of encephalomyopathy, one by myoneurogastrointestinal encephalopathy (MNGIE) syndrome and one by myoclonic epilepsy. From a molecular point of view, all patients but one (patient code MD1, mtDNA mutation unknown) carried a single or multiple large scale mtDNA deletion(s) (Table I). Patients did not follow any pharmacological therapy at the beginning of the study. In addition, 12 healthy individuals, matched for gender, age and smoking habit with MD patients, were selected as control subjects.

The CBMN assay was performed according to the procedure described by Migliore et al. (1999). Briefly two paired, independent lymphocyte cultures were set up in RPMI 1640 (Gibco BRL, Milan, Italy) supplemented with 20%...
Effects of coenzyme Q10 therapy

foetal bovine serum (Gibco BRL), 1.5% phytohaemagglutinin (Gibco BRL) and 1% penicillin/streptomycin (Gibco BRL) for each whole blood sample collected. Cytochalasin B (Sigma, St Louis, MO) at a final concentration of 6 
ng/ml was added to each tube 4 h after the cultures were established, to block the cytokinesis of dividing cells. Binucleated lymphocytes were harvested 72 h after culture setting. Cells were treated with a hypotonic solution (0.075 M

KCl) to lyse erythrocytes, prefixed in 3:5 methanol:acetic acid, washed once with methanol and twice with 7:1 methanol:acetic acid fixative and dropped onto cold glass slides. The slides were stained in 4% Giemsa solution or hybridized for the FISH analysis.

Two thousand binucleated cells per experimental point were examined, following the scoring criteria adopted by the HUman MicroNucleus Project (Bonassi et al., 2001). We evaluated the binucleated micronucleated cell frequency (BN MN %) as number of binucleated lymphocytes containing one or more MN per 1000 binucleated cells. The ratio of per cent binucleated cells to total cells scored (BN %) was used as an index of cell proliferation in culture.

FISH analysis

A digoxigenin-labelled α-satellite DNA probe specific for the centromeres of all human chromosomes (Appligene Oncor, France) was used for FISH analysis. Pre-warmed slides were denatured in 70% formamide, 2× SSC (saline sodium citrate buffer), pH 7.0, at 70°C for 2 min and then dehydrated through an ethanolic series. The centromeric probe was denatured at 70°C for 5 min, placed on the slides and incubated overnight at 37°C in a moist box. Post-hybridization washes were performed, first in 2× SSC, pH 7.0, for 5 min and then in 4× SSC, 0.05% Tween 20 (SSCW) (Sigma-Aldrich, Italy) for 5 min. Slides were then incubated with three antibodies: mouse anti-digoxigenin (Boehringer Mannheim, Italy), TRITC-conjugated rabbit anti-mouse and TRITC-conjugated goat anti-rabbit (Sigma-Aldrich, Italy). Antibodies were diluted in immunological buffer (4× SSC with 5% non-fat dry milk) and alternatively incubated at 37°C for 1 h and 30 min step was performed in enzyme buffer (PBS, pH 7.0, for 3 min), washed, placed on the slides and incubated overnight at 37°C. The slides were placed in this tank side by side with the agarose end facing the anode and with a horizontal gel electrophoresis tank was filled with freshly made 4

°C alkaline buffer for a duration of at least 1 h at 4°C. A horizontal gel electrophoresis tank was filled with freshly made 4°C alkaline buffer (300 mM NaOH, 1 mM Na3EDTA, pH > 13). The slides were placed in this tank side by side with the agarose end facing the anode and with the electrophoresis buffer at a level of ~0.25 cm above the slide surface. The slides were left in this high pH solution for 20 min to allow DNA unwinding and expression of alcali-labile sites. The DNA fragments were then separated by electrophoresis for 20 min at 25 V adjusted to 300 mA. After electrophoresis the slides were flooded with two changes of neutralization buffer (0.4 M Tris, pH 7.5) for 5 min each. This removed any remaining alkali and DNA fragments, which could have interfered with staining. The slides were drained before being stained with 80 μl of 20 μg/ml ethidium bromide (Sigma-Aldrich, Italy).

Coded slides were viewed using an epifluorescence microscope (Nikon Eclipse E800) equipped with a filter for ethidium bromide visualization. Observations were made at a final magnification of 600×. Fifty randomly selected cells (25 cells from each of two replicate slides) per experimental point were evaluated using a semi-automated Komet Image Analysis System, Version 5.0 (Kinetic Imaging Ltd, Liverpool, UK). Results were reported as tail DNA percentage, a parameter describing the number of migrated fragments, represented by the fluorescence intensity in the tail, expressed as the mean of the 50 cells scored.

Enzyme preparation and treatment for detection of oxidative DNA damage

The alkaline version of the Comet assay modified by the use of endo III and fpg was performed to detect oxidized pyrimidines and purines, respectively. The enzymes were kindly provided by Dr A.R.Collins (Aberdeen, UK).

Enzymes were diluted in enzyme buffer (PBS containing 40 mM Na3EDTA, 0.1 M KCl, 0.2 mg/ml BSA, 40 mM HEPES, pH 8.0; Sigma-Aldrich, Italy) and stored at ~80°C. Slides for enzyme treatment were removed from lysis solution and washed three times for 5 min each in enzyme buffer at 4°C. Then aliquots of 100 μl of enzyme solution, or buffer alone as a control, were placed on slides and covered with coverslips. Slides were put into a moist box to prevent desiccation and incubated at 37°C for 45 (endo III) or 30 min (fpg) before alkaline unwinding. The enzymes recognize and cut oxidized bases, producing DNA single-strand breaks (SSBs) that are expressed as fragments which migrate towards the anode during electrophoresis.

Statistical analysis

Multifactor analysis of variance was performed to statistically elaborate data obtained from the micronucleus assay, FISH analysis and the Comet assay in pre-treatment MD patients and healthy controls.

The comparisons between the mean frequencies of micronucleated binucleated cells and of cell migration by the Comet assay in MD patients before and after CoQ10 therapy were evaluated by using paired Student’s t-test. The difference was considered significant at the 95% confidence level (P < 0.05).

Results

Demographic characteristics of the study population

A summary of the demographic characteristics of the study population and of the clinical features of patients is reported in Table I. The MD patient group consisted of eight women and five men, mean age 54.3 ± 15.1 years (age range 27–74 years), while the control group included 12 healthy individuals, six males and six females, with a mean age comparable to that of patients (54.1 ± 15.8). All subjects but two (one patient and one control) were non-smokers.

Cytokinesis block micronucleus assay and FISH analysis

The data presented in Table II indicate that the frequency of micronucleated binucleated lymphocytes ranged between 9.5 and 45.0% in MD patients, whereas healthy individuals showed greatly lower values, between 1.0 and 21.0%.

The average frequency of micronucleated cells in untreated MD patients (25.1 ± 10.3%), after adjusting for confounding factors, was clearly different (P < 0.001) from that of healthy controls (8.8 ± 5.3%). A significant age-related increase in spontaneous MN frequency was observed when considering all samples (P < 0.01). FISH analysis, performed employing pancentromeric DNA probes, revealed a prevalence of centromere-containing MN (C+ MN % = 66.8 ± 7.2%) in patients, derived from loss of entire chromosomes or of centric fragments. Controls presented a lower frequency of C+ MN (61.4 ± 5.9%), but this difference did not reach statistical significance (P = 0.0617).

Table III reports the frequencies of micronucleated cells detected in a subset of 10 patients who had undergone a 2 week therapy with CoQ10, compared with the frequencies recorded in the same individuals at the time of first blood sampling. The frequencies measured after treatment ranged from 7.5 to 34.0%. A significant reduction (P < 0.05) in micronuclei occurrence (16.8 ± 9.8%) in comparison with the baseline condition (27.0 ± 10.9%) was observed.

Comet assay

Table IV summarizes the results obtained with the Comet assay in leukocytes of patients and controls, expressed as tail DNA percentage. The multifactor analysis of variance did not reveal...
any significant difference either in primary DNA damage, expressed as SSBs, between patients (8.3 ± 1.9%) and controls (7.0 ± 1.9%) or in SSBs induced by the enzymes at oxidized pyrimidines (8.5 ± 6.3% in affected versus 6.6 ± 4.5% in controls) or purines (6.8 ± 4.8 versus 5.4 ± 3.5%).

Finally, Table V shows Comet assay results for 10 patients before and after CoQ10 administration. Although a slight decrease in primary DNA damage and in enzyme-sensitive oxidized bases was observed, analysis of the data did not detect any statistical significance.

Discussion
The present study provides the first evidence of elevated baseline nDNA damage in peripheral blood lymphocytes of patients with MD. The CBMN assay detected a higher level of cytogenetic damage, expressed as frequency of micronucleated binucleated cells, in patients than in healthy matched controls. Moreover, multifactor analysis of variance showed that the micronuclei frequencies significantly increased with chronological age of both patients and controls. This fact is consistent with the large age range of our study population. Several studies reported age as a relevant factor determining variations in spontaneous chromosomal instability. The increase in micronuclei frequencies with age may be explained by an accumulation of DNA damage as a consequence of a progressive decline in DNA repair capacity (Barale et al., 1998; Bolognesi et al., 1999; Bonassi et al., 2001).

Micronuclei arise as a consequence of chromosome breakage and chromosome loss, triggered for example by oxidative stress and exposure to genotoxic agents. The use of FISH with probes specific for the centromere sequences of all human chromosomes allowed the evaluation of the origin of micronuclei in the study population. In patients a prevalence of micronuclei with a centromere signal was observed compared with healthy controls, however, this was not statistically significant. Our data were in substantial agreement with other studies reporting percentages of ~60% of micronuclei containing whole chromosomes lagging behind in anaphase in the general population (Catalán et al., 1995; Scarpato et al., 1996).

Endogenous oxidative stress is believed to play a key role in the onset and progression of MDs: it was found that intracellular H₂O₂ and oxidative damage to DNA and lipids are significantly higher in skin fibroblasts from patients with MD as compared with those of age-matched controls (Wei et al., 2001). ROS are mainly released as a byproduct of electron transport during mitochondrial respiration (Turrens et al., 1985; Sohal and Sohal, 1991). Furthermore, the mtDNA mutations found in MD may produce deficient respiratory chain holoenzymes that block the correct flow of electrons, which may be preferentially transferred to molecular oxygen, thus enhancing ROS production during respiratory activity (Robinson, 1998). Under ordinary conditions free radicals are removed by the action of scavenging enzymes and antioxidants before they can damage DNA, lipids and proteins. However, if the ratio of prooxidants to antioxidants increases, significant damage occurs and may persist despite cellular repair pathways. At the molecular level the DNA damage introduced by ROS includes SSBs, double-strand breaks, abasic sites, formation of micronuclei and oxidized bases (von Sonntag, 1997, 2001). ROS are mainly released as a byproduct of electron transport during mitochondrial respiration (Turrens et al., 1985; Sohal and Sohal, 1991). Furthermore, the mtDNA mutations found in MD may produce deficient respiratory chain holoenzymes that block the correct flow of electrons, which may be preferentially transferred to molecular oxygen, thus enhancing ROS production during respiratory activity (Robinson, 1998). Under ordinary conditions free radicals are removed by the action of scavenging enzymes and antioxidants before they can damage DNA, lipids and proteins. However, if the ratio of prooxidants to antioxidants increases, significant damage occurs and may persist despite cellular repair pathways. At the molecular level the DNA damage introduced by ROS includes SSBs, double-strand breaks, abasic sites, formation of micronuclei and oxidized bases (von Sonntag, 1987; Lindahl, 1993; Demple and Harrison, 1994; Yakes and Van Houten, 1997).

In order to evaluate oxidative DNA damage levels, in recent years a modified Comet assay procedure, based on the use of lesion-specific enzymes that recognize and cut oxidized bases producing SSBs, was successfully introduced. In particular, endo III is specific for oxidized pyrimidines while fpg

<table>
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<th>Table II. Results of the CBMN assay and FISH analysis with a pancentromeric DNA probe in peripheral blood lymphocytes of untreated MD patients and healthy controls</th>
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¹Frequency of micronucleated cells per 1000 binucleated cells. 2000 binucleated lymphocytes per subject were scored.
²Binucleated cells/total cells.
³Characterization of 100 MN scored for the presence of the centromere signal.
⁴P < 0.001, MD patients versus controls. Age and sex effect controlled.

<table>
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<tr>
<th>Table III. Results of the CBMN assay in peripheral blood lymphocytes of a subset of MD patients before and after CoQ10 therapy</th>
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Mean ± SD 27.0 ± 10.9⁶ 47.6 ± 7.5 16.8 ± 9.8⁸ 44.3 ± 9.8⁸

¹Frequency of micronucleated cells per 1000 binucleated cells. 2000 binucleated lymphocytes per subject were scored.
²Binucleated cells/total cells.
⁶P < 0.05, MD patients before therapy versus after therapy.
⁸P < 0.001, MD patients before therapy versus after therapy.
recognizes damaged purines, including 8-oxodG, a typical indicator of oxidative damage. In the present study this technique detected only slightly higher levels of SSB expression of both primary and oxidative DNA damage in affected patients compared with matched healthy controls. This finding is reinforced by data derived from the FISH analysis, showing a prevalence of MN including whole chromosomes and so not deriving from chromosome fragments generated by unrepaired primary DNA lesions. Furthermore, as fpg and endo III are not the only enzymes involved in the excision repair of oxidative damage and the detection of cut sites by these enzymes represents an indirect marker of oxidative damage, our results may represent an underestimation of the real levels of oxidized bases (Collins, 1999). Another possibility is that fpg and endo III might cut only a fraction of the oxidized DNA sites, because of steric interference. It has been argued that free radicals may attack DNA producing clusters of oxidized lesions, which may be considered by the enzymes as a single cut site (Gedik et al., 1998).

A further aim of our work was the assessment of the effects that a 2 week therapy with CoQ10 may have at the cellular level on the variation in nDNA damage in patients exposed to elevated levels of endogenous oxidative stress. Despite great strides in our understanding of the biochemical and molecular causes of MD, therapy for these devastating disorders remains inadequate: the goals of treatment are, at present, alleviation of the symptoms and slowing down of the progression of the disease. Several attempts have been made by administering drugs either to increase respiratory chain efficiency or to minimize deleterious consequences of the altered cell metabolism, such as excessive cytoplasmic acidification or membrane lipid peroxidation. To counteract the effects of oxidative stress, several oxygen radical scavengers have been utilized, most commonly CoQ10. CoQ10 is an endogenously synthesized provitamin located in the inner mitochondrial membrane where, realizing an interaction between specific enzyme complexes, it acts as an essential cofactor of the mitochondrial respiratory chain (Crane et al., 1993). CoQ10 seems to have membrane-stabilizing properties and, in its reduced form (ubiquinol 10), an efficient free radical scavenging action (Beyer et al., 1987; Ernster and Dallner, 1995). A recent study, using the alkaline Comet assay, showed that lymphocytes treated in vitro with CoQ10 were protected from DNA damage, in terms of SSBs and alkali-labile sites (Tomasetti et al., 1999). These data seem to indicate that CoQ10 is somehow involved in the reduction in primary DNA damage.

### Table IV. Results of the Comet assay in patients and controls

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<td>4.4</td>
<td>3.6</td>
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</tr>
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<td>6.2</td>
<td>11.9</td>
<td>6.0</td>
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<td>1.4</td>
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<td>6.1</td>
<td>6.8</td>
<td>6.8</td>
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<tr>
<td>MD 8</td>
<td>9.5</td>
<td>6.2</td>
<td>1.4</td>
</tr>
<tr>
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<td>5.9</td>
<td>1.3</td>
<td>9.1</td>
</tr>
<tr>
<td>MD 10</td>
<td>9.2</td>
<td>22.8</td>
<td>11.1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>8.3</td>
<td>6.6</td>
<td>6.8 ± 4.8</td>
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**Controls**

<table>
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<tr>
<th>Patients</th>
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<th>tail DNA (%)</th>
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<td>6.9</td>
<td>3.2</td>
<td>2.6</td>
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<td>0.3</td>
<td>7.8</td>
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<td>1.9</td>
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<tr>
<td>Mean ± SD</td>
<td>7.0</td>
<td>6.6</td>
<td>5.4 ± 3.5</td>
</tr>
</tbody>
</table>

Net enzyme-sensitive sites were obtained by subtracting single-strand breaks detected with buffer alone from those measured with the enzyme.

*Single-strand breaks.

### Table V. Results of the Comet assay in a subset of patients before and after CoQ10 therapy

<table>
<thead>
<tr>
<th>Patients</th>
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<th>After therapy</th>
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<tbody>
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<td>SSB</td>
<td>endo II</td>
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<td>MD 1</td>
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<td>10.1</td>
</tr>
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<td>18.8</td>
</tr>
<tr>
<td>MD 3</td>
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<tr>
<td>MD 5</td>
<td>9.8</td>
<td>7.1</td>
</tr>
<tr>
<td>MD 6</td>
<td>6.2</td>
<td>11.9</td>
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<tr>
<td>MD 7</td>
<td>9.5</td>
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<tr>
<td>MD 8</td>
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<td>3.4</td>
</tr>
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<td>MD 10</td>
<td>6.1</td>
<td>6.8</td>
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<tr>
<td>MD 14</td>
<td>9.2</td>
<td>22.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>8.4</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Net enzyme-sensitive sites were obtained by subtracting single strand breaks detected with buffer alone from those measured with the enzyme.

*Single-strand breaks.

*Endonuclease III-sensitive sites.

*Formamidopyrimidine-DNA glycosylase-sensitive sites.

Effects of coenzyme Q10 therapy
Cytogenetic damage was assessed in the peripheral blood lymphocytes of a subset of 10 patients with different forms of MD before the onset of CoQ10 therapy and after treatment completion (post-treatment). Supplementation of CoQ10 for 2 weeks resulted in a consistent decrease in the mean frequency of damaged cells. This finding suggests a protective effect of CoQ10 with respect to DNA damage. On the other hand, the Comet assay detected only a slightly different distribution between patients and controls of spontaneous SSBs and of SSBs induced by enzymes at oxidized bases. A non-significant tendency for a reduction in the levels of spontaneous or induced SSBs was measured in patients after CoQ10 administration. Moreover, remarkable inter-individual differences in therapy response were observed with this technique. These observations probably reflect differences in the individual characteristics of patients, severity of the disease, regimen and nutritional status and possibly in the rates of absorption and/or clearance of CoQ10 from the blood circulation. Indeed, in general, treatment may be beneficial and immediately recorded in some individuals or may take a few months to be noticed. In other cases the benefits of treatment may never be seen, but the treatment may be effective in delaying or stopping progression of the disease, whereas some patients may not benefit from the therapy. Several reports found both clinical and biochemical improvements after treatment with CoQ10 in patients with known MDs (Bresolin et al., 1988; Tarnopolsky and Beal, 2001). CoQ10 improved lactate/pyruvate in some patients with MD and oxygen consumption in a patient with MELAS. In contrast, no rigorously controlled therapeutic trial has yet been conducted and one of the larger trials on MDs found that CoQ10 plus several antioxidant vitamins resulted in a 3-fold increase in serum CoQ10 concentration, with no measurable improvements in oxidative metabolism (Matthews et al., 1993).

It cannot be ruled out that the negative results obtained with the Comet assay in our study may be due to a limited sensitivity of our experimental system. Other studies performed using both the MN test and Comet assay did not find correlating results (Gutierrez et al., 1998; Vijayalaxmi et al., 1998). Some authors explained this by remarking that the CBMN procedure deals with lymphocytes, which represent only a minor fraction of white blood cells, while all leukocytes are analyzed in the Comet assay. Indeed, a certain variability among leukocyte populations and individual cells belonging to the same population exists in ROS release within the cell and in their repair capacity (Collins et al., 1995). Thus, the negative results of the Comet assay reported in the present work may be due to a variation not only among individual subjects, but also among white blood cell types in the liberation of free radicals within the cell or in their ability to attack DNA and in cellular antioxidant protection (Green et al., 1994; Collins et al., 1995; Vijayalaxmi et al., 1998).

In conclusion, our work suggests a possible contribution of oxidative stress to elevated levels of chromosome damage found in MD patients. We are aware that the small number of cases included in our study may represent a weakness, but this reflects the limited availability of patients, due to the low frequency of MDs observed in the general population (1 case in every 8500 individuals, as reported by Chinnery and Turnbull, 2001). Furthermore, the protective effect of CoQ10 seems to indicate an involvement of free radicals in cytogenetic alterations. Whether this protection is due to the antioxidant function of CoQ10 or to its action as a cofactor in the respiratory chain remains to be investigated by further studies.

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References


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