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Autophagy, mitochondria and cell death in lysosomal storage diseases

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Abstract

Lysosomal storage diseases (LSDs) are debilitating genetic conditions that frequently manifest as neurodegenerative disorders. They severely affect eye, motor and cognitive functions and, in most cases, abbreviate the lifespan. Postmitotic cells such as neurons and mononuclear phagocytes rich in lysosomes are most often affected by the accumulation of undegraded material. Cell death is well documented in parts of the brain and in other cells of LSD patients and animal models, although little is known about mechanisms by which death pathways are activated in these diseases, and not all cells exhibiting increased storage material are affected by cell death. Lysosomes are essential for maturation and completion of autophagy-initiated protein and organelle degradation. Moreover, accumulation of effete mitochondria has been documented in postmitotic cells whose lysosomal function is suppressed or in aging cells with lipofuscin accumulation. Based upon observations in the literature and our own data showing similar mitochondrial abnormalities in several LSDs, we propose a new model of cell death in LSDs. We suggest that the lysosomal deficiencies in LSDs inhibit autophagic maturation, leading to a condition of autophagic stress. The resulting accumulation of dysfunctional mitochondria showing impaired Ca^{2+} buffering increases the vulnerability of the cells to pro-apoptotic signals.

Keywords

autophagy; mitochondrial homeostasis; lysosome; calcium; caspase; programmed cell death; mucolipidosis; neurodegeneration; Niemann-Pick; neuronal ceroid lipofuscinosis

Lysosomal storage diseases (LSDs) comprise >40 rare genetic disorders with cytoplasmic accumulation of lipids, proteins and/or mucopolysaccharides. Some LSDs are caused by inactivating mutations of lysosomal hydrolytic enzymes (e.g. mucopolysaccharidoses) or mutations that affect their delivery to lysosomes (mucolipidoses II-IIIc). Other LSDs involve impaired export of hydrolysis products (cystinoses) [1, 2]. The mechanistic etiologies of several LSDs including Niemann-Pick disease remain unexplained.

Most LSDs involve impaired cognitive and motor functions. Loss of neurons is well documented in LSD patients and mouse models [3-10] supporting the concept that the nervous system is particularly susceptible to altered lysosomal function [11]. Tissue degeneration also occurs in the liver, spleen and thyroid of affected patients and mice [12]. Cell death mechanisms in LSDs may include signaling effects of aberrant lipid/protein composition [6, 13-15], and dysregulation of autophagy [16-18]. Similar degenerative phenotypes are observed in unrelated LSDs suggests involvement of common downstream mechanisms, irrespective of the underlying genetic cause of the particular LSD.

Macroautophagy is the major pathway by which organelles are targeted for lysosomal degradation, and this process is essential for basal health of postmitotic cells such as neurons [7, 8]. Altered autophagolysosomal function is implicated in aging and a wide range of diseases [19-21]. Analysis of disease mechanisms relating to autophagy is complicated by concurrent activation of multiple homeostatic and injury pathways, and growing evidence of regulatory crosstalk between autophagy and apoptosis [22-24]. We have proposed a unifying hypothesis that multiple mechanisms resulting in dysregulated or imbalanced induction, maturation, or degradation converge to create the pathologic condition of "autophagic stress" [25], recognized by sustained increases in autophagic vacuoles. Excessive organelle damage and degradation, relative impairments in autophagolysosomal maturation, and differences in co-activated pathways/cellular context may determine whether activation of autophagy plays a pro-survival or pro-death role [25-27], as recently shown for endoplasmic reticulum stress [28]. Rapamycin, a drug that inhibits mTOR and stimulates autophagy, shows anti-apoptotic effects [29]. Some cell types may be intrinsically less tolerant of autophagic stress than other cell types. While basal autophagy is essential for cellular health, autophagic stress resulting from disruption of finely balanced regulatory mechanisms serves to promote cell death and disease [25].

Mucolipidosis type IV (MLIV) is caused by mutations in the lysosomal ion channel mucolipin 1 (TRP-ML1). TRP-ML1 has been implicated in endosome-lysosome interactions on the basis of lipid traffic delays in TRP-ML1-deficient cells [30]. However, lipid trafficking deficits are also observed in LSDs that are clearly linked to hydrolytic defects [31-33]. TRP-ML1 could also regulate lysosomal acidity [34]. We found that MLIV skin fibroblast cells exhibit accumulation of fragmented mitochondria with impaired Ca^{2+} buffering capacity, increasing susceptibility to caspase 8-dependent cell death [18]. Moreover, similar alterations were observed not only in cells from four different LSDs, but also in normal cells treated with inhibitors of autophagy or lysosomal function [18].

We propose that chronic disruption in lysosomal function, whether due to genetic mutations, drug treatments or aging, promotes cell death through accumulation of mitochondria with impaired abilities to buffer pro-death stresses. In support of this are observations of: *i)* mitochondrial fragmentation in aged myocytes whose lysosomal function has been impaired by lipofuscin buildup [35]; *ii)* similarities between LSDs and neurodegeneration in mice deficient in central autophagy genes [7, 8 3, 4, 36], and *iii)* our demonstrations that common patterns of mitochondrial fragmentation and dysfunction in four unrelated LSDs can be recapitulated by multiple distinct pharmacologic inhibitors of autophagy or lysosomal function [18].

In this model, impaired lysosome function results in disrupted autophagy regulation, and accumulation of fragmented, partially dysfunctional mitochondria (Fig 1). As mitochondria balance survival-death stimuli, we hypothesize that increased content of even subtly dysfunctional mitochondria accentuates susceptibility of cells to pro-apoptotic stimuli. Several questions remain to be resolved, including the mechanisms promoting mitochondrial fragmentation. While it is possible that dysregulated fission-fusion due to lipid handling deficits contributes to this LSD phenotype, inhibition of autophagy produces similar fragmentation in control fibroblasts, suggesting inefficient autophagy of mitochondria undergoing fission to facilitate turnover (Fig 1). This interpretation is consistent with the lysosomal-mitochondrial theory of aging [37, 38]. It should be noted, however, that mitochondrial morphology is dynamic and mitochondrial fragmentation precedes apoptosis in several systems [39, 40]. As LSD cells are more sensitive to apoptosis [18], the possibility remains that mitochondrial fragmentation in our system reflects pre-apoptotic changes.

A second question regards the mechanism by which mitochondrial Ca^{2+} buffering is altered. Using two different assays we showed that uptake of Ca^{2+} by mitochondria during agonist-induced Ca^{2+} spikes is deficient in LSD cells. Mitochondrial Ca^{2+} uptake occurs through

mitochondrial Ca^{2+} uniporters driven by negative mitochondrial membrane potential [41-43]. If the shortened mitochondria with dilated cristae are depolarized, this would reduce the driving force for Ca^{2+} movement. It is also possible that impaired mitochondrial Ca^{2+} uptake is the result of mitochondrial transition pore opening that accompanies apoptosis, which will also drop mitochondrial membrane potential and induce Ca^{2+} leakage. Further studies are needed to address these alternatives.

What is the mechanism of cell death in LSDs? While basal apoptosis in MLIV cells are no higher than those of control cells, the former are more sensitive to pro-apoptotic effects of cytoplasmic Ca^{2+} 18. The commonly accepted Ca^{2+} excitotoxicity hypothesis links mitochondrial Ca^{2+} *overload* and destabilization of mitochondrial membranes [44]. Our data show that cell death can occur as a result of inefficient mitochondrial Ca^{2+} buffering in the absence of “overload”, as mitochondria from LSD cells took up less Ca^{2+}, rather than more Ca^{2+} as required by the common theory. Thus, it is unlikely that this death pathway is associated with Ca^{2+}-induced permeability transition pore opening, and we did not detect cytochrome c release. Notably, the amounts of Ca^{2+} accumulated by mitochondria in control cells were relatively small and changes in global Ca^{2+} signaling associated with LSD or with suppression of autophagy were negligible. Based on these data, we propose that local perimitochondrial changes in Ca^{2+} handling, rather than global Ca^{2+} perturbations, are involved in stimulation-induced cell death in LSDs.

Increased caspase 8 activity appeared to be more important than caspase 9 during cell death in Ca^{2+} stimulated MLIV fibroblasts, and inhibition of caspase 8 conferred protection. Notably, caspase 8 upregulation has been shown in a mouse model Niemann-Pick disease [45] and increased caspase 8 activity is observed in vitro model of Niemann-Pick [46]. The mechanisms translating inefficient mitochondrial Ca^{2+} uptake into caspase-8 activity require further elucidation. It is possible that a population of mitochondria-associated calpains may be involved [47]. We have now found that pre-treatment of cells with μ-calpain inhibitors inhibits induced-cell death in MLIV fibroblasts (Fig 2). We hypothesize that during Ca^{2+} stimulation, perimitochondrial Ca^{2+} is rapidly cleared by Ca^{2+} efflux into mitochondria (Fig 3). In LSD cells, reduced mitochondrial buffering results in sufficient perimitochondrial Ca^{2+} to activate μ-calpains, which could cleave caspase-8 and trigger apoptosis.

Finally, while it may be assumed that autophagy defects result simply from “backing up” of the degradative system, the situation is likely to be more complex. We observed delayed clearance of starvation-induced autophagy markers upon re-feeding in MLIV fibroblasts [18], suggesting that inefficient autophagosome maturation contributes to autophagic stress in the LSD context. However, differences in regulation of starvation- and injury-initiated autophagy have been reported [48], and our new electron microscopy data indicate that mitochondrial abnormalities observed in LSD cells are not simply the result of impaired degradation. In contrast to lysosomal storage material, which typically accumulates within enlarged lysosomal structures, the electron microscopy data show abnormal mitochondria in the cytoplasm (Fig 4). Thus, lysosomal defects in LSD results not only in delayed autophagosome clearance, but apparently in reduced autophagic sequestration of abnormal mitochondria in the first place, suggesting the existence of unidentified feedback mechanisms. The accumulation of dysfunctional mitochondria in the cytoplasm, rather than as undigested material within lysosomes, plays a critical role in enhanced susceptibiliy to Ca^{2+} related cell death in MLIV and other LSDs.

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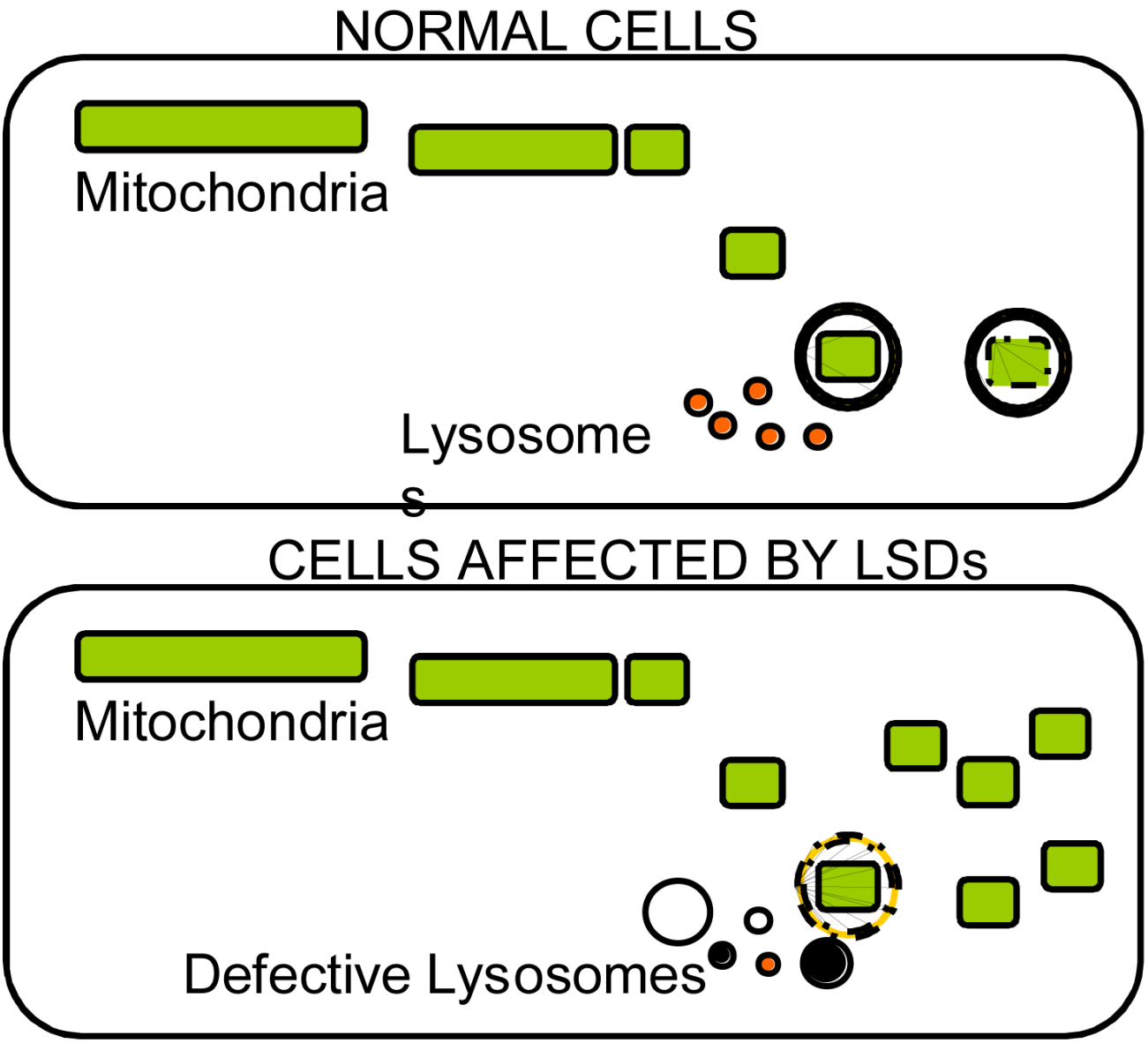

Fig 1. Mitochondrial fragmentation in LSDs

In the normal cells, mitochondrial fragments are cleared from the cytoplasm by autophagy followed by lysosomal degradation (Top). In LSD-affected cells, autophagy is suppressed through unknown mechanisms; as a result, mitochondrial fragments with altered functional capacities accumulate (Bottom).

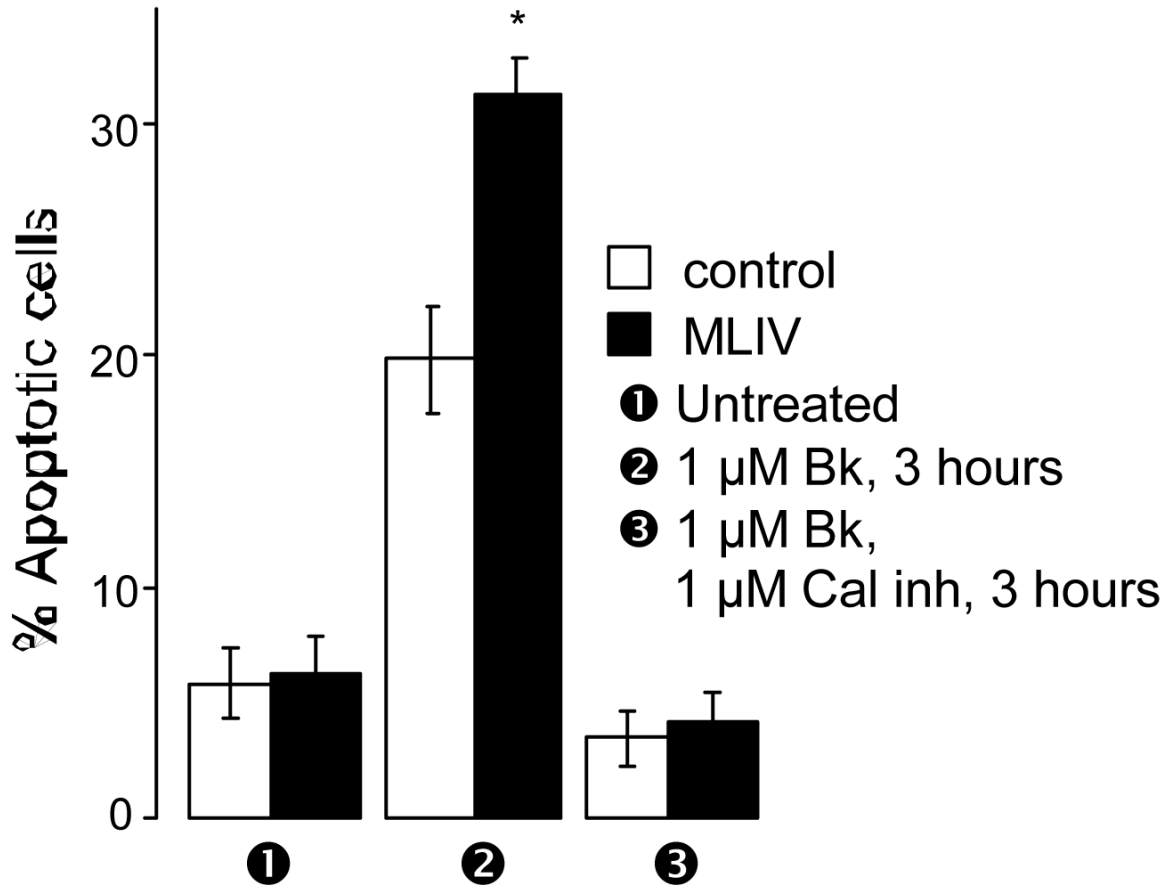

Fig 2. Inhibition of μ-calpain suppresses Ca^{2+}-dependent cell death in MLIV fibroblasts

Cells were pretreated with 1 μM of calpain II (μ-calpain) inhibitor (Calbiochem) for 15 min before experiments and apoptosis was induced as in [18]. Note that apoptosis rates of pretreated cells are significantly lower than those of untreated cells. The results represent 3 separate experiments; more than 20 cells were analyzed in each experiment under each condition.

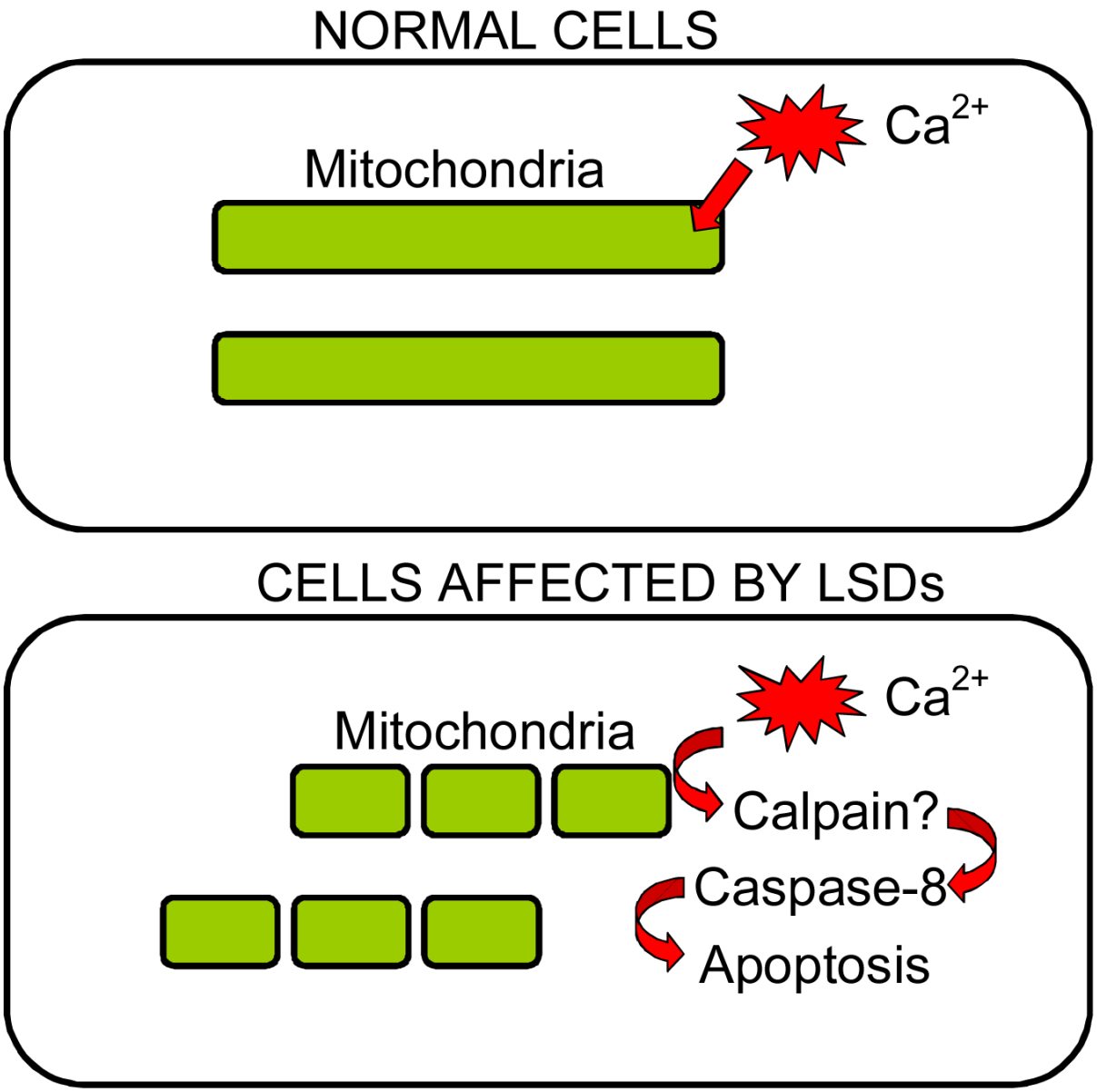

Fig 3. A model of mitochondrial involvement in cell death in LSDs

In a normal cells during Ca^{2+} stimulation, perimitochondrial Ca^{2+} is rapidly cleared into mitochondria. Mitochondria in LSDs-affected cells fail to buffer intracellular increases in Ca^{2+}, potentiating activation of μ-calpain and triggering cell death through caspse-8-dependent mechanisms.

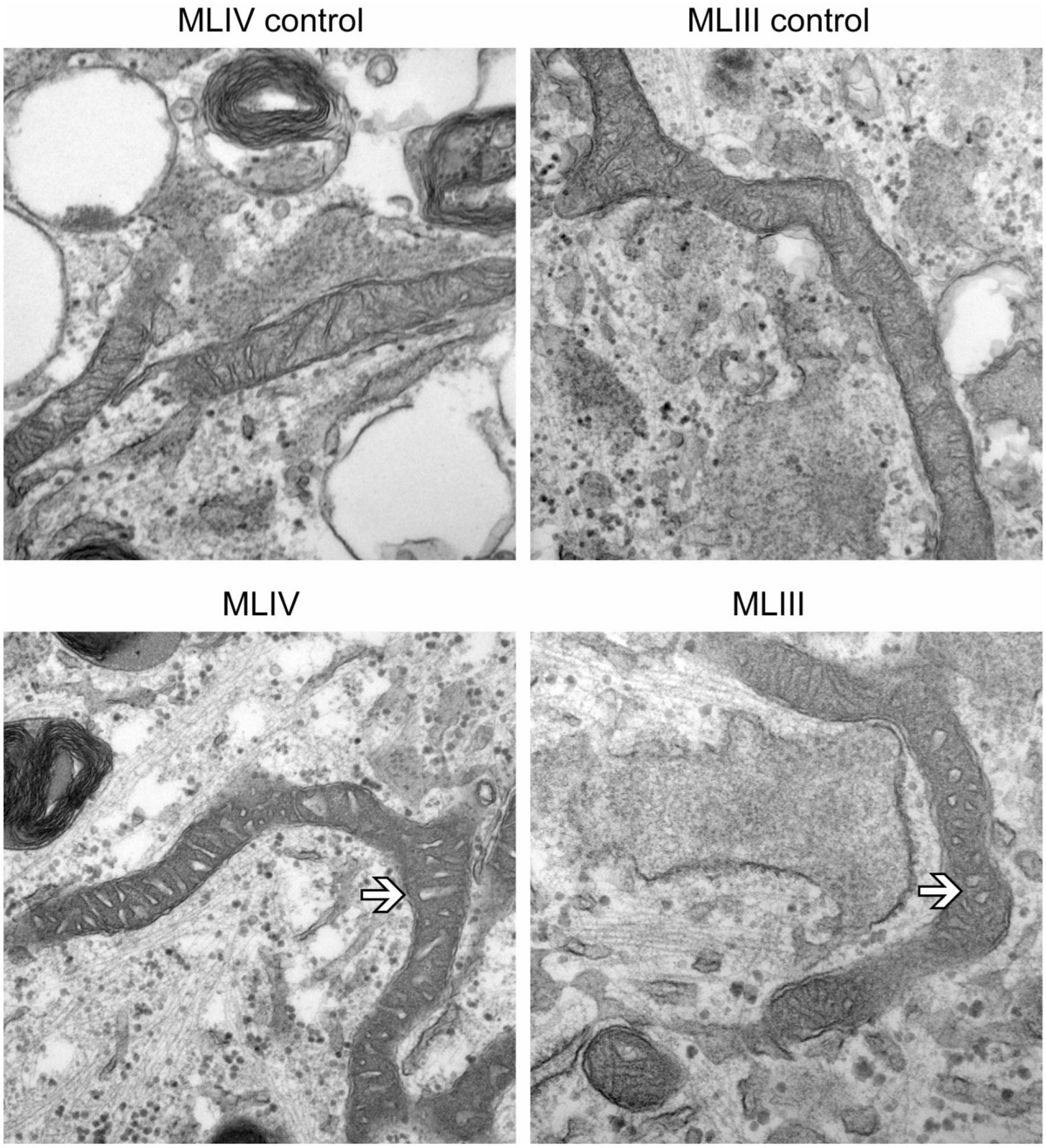

Fig 4. Mitochondrial abnormalities in LSDs

Electron microscopic images of mitochondria in control, MLIV and MLIII fibroblasts. In order to better illustrate the inner mitochondrial structure, large mitochondria were chosen in control and LSDs-affected cells. Please note dilated, distorted cristae (arrows) and dark matrix space in mitochondria from LSDs-affected cells.