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Biochimica et Biophysica Acta 1364 (1998) 261–270

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Human complex I defects in neurodegenerative diseases

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Received 22 January 1998; accepted 29 January 1998

Abstract

Complex I deficiency, either specific or associated with other respiratory chain defects, has been identified in myopathies, encephalomyopathies and in three 'neurodegenerative' disorders: Parkinson's disease, dystonia and Leber's hereditary optic neuropathy. The complex I defect is expressed in blood in all these three but, to date, only in LHON have specific mitochondrial DNA mutations been identified. Recent work with ρ° cybrids indicates that, in a subgroup of patients at least, the complex I deficiency is determined by mtDNA, in contrast to dystonia where a nuclear gene defect or toxic influence appears a more likely cause. The actions of specific toxins, e.g., MPTP continue to play an important role in our understanding of pathogenesis of neurodegeneration, particularly in PD. © 1998 Elsevier Science B.V.

Keywords: Parkinson's disease; Complex I; Mitochondrion; mtDNA; Dystonia; Leber's hereditary optic neuropathy

1. Introduction

NADH ubiquinone reductase (complex I) is the first enzyme protein complex of the mitochondrial respiratory chain. Details of the structure and function of complex I will be described elsewhere in this issue, and this review will focus on neurodegenerative disorders associated with complex I deficiency.

Complex I comprises 41 subunits of which seven (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6) are encoded by mitochondrial DNA (mtDNA). Numerous mutations of mtDNA have now been associated with human disease [1]. In the majority of cases, mutations result in a demonstrable defect of respiratory chain activity. Multiple respiratory chain deficiencies, e.g., decreased activity of complex I and complex IV, are seen more frequently than isolated defects. This is because the majority of mtDNA mutations involve transfer RNAs (tRNA), abnormal functioning of which will inevitably affect those polypeptides with the relevant constituent amino acid. However, three neurodegenerative diseases are associated with isolated or predominant complex I deficiency: Parkinson's disease (PD), focal dystonia and Leber's hereditary optic neuropathy (LHON).

2. Parkinson's disease

PD is characterised clinically by bradykinesia, rigidity and tremor. Onset is usually in the sixth or seventh decades of life and lifetime risk is estimated at 1 in 40. Pathologically, there is severe degenera-

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PII S0005-2728(98)00032-2

tion and cell loss in the substantia nigra pars compacta, predominantly if not exclusively affecting dopaminergic neurones and their projections to the striatum. Other areas of the central nervous system are affected including the locus ceruleus (noradrenergic) and substantia innominata (cholinergic). Ubiquitin-rich intracycloplasmic eosinophilic inclusions (Lewy bodies) are seen in a proportion of surviving neurones. Symptomatic treatment is based on dopamine receptor activation by dopamine replenishment (via L-dopa) or dopamine receptor agonists. However, the disease is progressive and no intervention has been shown unequivocally to alter progression.

The aetiology and pathogenesis of PD are unknown but recent discoveries have provided evidence that both the environment and hereditary may play some role. 1-Methyl-4-phenyl 1,2,3,6 tetrahydropyridine (MPTP) was produced as a synthetic meperidine analogue for use as a 'designer' narcotic, but found to be capable of inducing parkinsonism within 7–14 days of injection [2]. MPTP appears to induce clinical features similar to, but not identical with, idiopathic PD, but which still remain responsive to dopamine receptor activation. Pathological study of a brain from an MPTP addict with parkinsonism showed severe destruction of dopaminergic neurones in the substantia nigra [3]. ^{18}-Fluorodopa positron emission tomography in patients with MPTP parkinsonism indicated progression of nigrostriatal cell loss over 7 years at a rate faster than aging and comparable with idiopathic PD [4]. This implies that the toxicity of this compound continues long after initial exposure and indicates the presence of ongoing biochemical abnormalities causing cell death.

MPTP is systemically distributed following intravenous injection but targets the dopaminergic neurones of the substantia nigra through specific uptake and conversion mechanisms (see Ref. [5] for review). MPTP is a protoxin, metabolised to its active derivative 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase B (MAO-B), the distribution of which therefore determines the site of toxicity. MAO-B concentration is highest in the glial cells of the CNS, thereby defining this area as highest in MPP^+ concentration. MPP^+ is a substrate for the dopaminergic re-uptake pathway and so results in high concentration in nigrostriatal dopaminergic neurones. MPP^+ is then accumulated by mitochondria via an energy dependent system for cations. MPP^+ is a specific reversible inhibitor of complex I and results in a fall in ATP levels. MPP^+ probably interacts with complex I at the same site as rotenone and piericidin A [6]. MPP^+ induces more severe and irreversible inhibition of complex I if cytochrome oxidase (complex IV) is inhibited [7]. This inhibition was prevented with free radical scavengers indicating oxidative damage of complex I under these conditions. Complex I inhibition results in increased free radical generation from the respiratory chain and so the MPP^+ model suggests that a self amplifying cycle of complex I deficiency and damage may result in progressive cell damage. Such a situation would fit well with the progressive striatal lesion in MPP^+ exposed patients as determined by ^{18}-fluorodopa PET.

MPTP parkinsonism can be induced in monkeys and other vertebrates [8], although some species, e.g., rats are relatively resistant. In addition, older monkeys and rats seem more sensitive to the effects of MPTP [9,10]. There is increasing evidence that MPP^+ generates free radicals and oxidative damage in addition to causing complex I inhibition [11,12]. For instance, the nitric oxide synthase inhibitor 7-nitroindazole has been shown to protect monkeys [13] and rats [14]. However, there is also evidence that 7-nitroindazole inhibits MAO-B, and this would prevent conversion of MPTP to MPP^+ [15]. Other factors may also influence the effects of MPTP and in this respect it is interesting that only a very small proportion of addicts exposed to MPTP actually developed parkinsonism. This raises the possibility that genetic influences may play some role in determining susceptibility.

Complex I deficiency in PD brain was first described in 1989 [16] and provided a direct biochemical link between MPTP parkinsonism and the idiopathic disease. Numerous studies have followed to understand the cause of the complex I defect and its relation, if any, to the aetiology and pathogenesis of PD. Early work focused on the anatomic and disease specificity of the mitochondrial abnormality (see Ref. [17] for review). Using post mortem brain homogenates, no deficiency was identified in cortex, cerebellum, caudate, putamen, tegmentum, globus pallidus or, subsequently, cingulate cortex or substantia innominata [18]. Interestingly, an identical distri-

bution was found for a significant reduction in reduced glutathione [18,19]. The substantia innominata is predominantly cholinergic but is also involved in degeneration in PD (see Section 1). The findings of complex I activity and GSH concentrations suggest that they do not contribute to either cholinergic cell death or Lewy body formation in this area. The latter proposition is also supported by normal complex I function and GSH levels in the cingulate cortex of patients with 'dementia with Lewy bodies' (DLB)—an area with a particularly high concentration of Lewy bodies. Thus it would seem that mitochondrial dysfunction and oxidative stress are linked both to each other and to the PD substantia nigra within the brain.

Almost all the PD brains studied biochemically have come from patients treated with L-dopa during life. It was therefore important to establish whether this drug could affect complex I activity. In vitro studies, and in vivo studies in rats, indicated that L-dopa could induce a 20–25% reversible inhibition of complex I [20]. Dopamine concentrations are high in the striatum (caudate, putamen) in PD patients and these sites would therefore be expected to demonstrate any drug-related complex I deficiency. However, mitochondrial function in these areas is normal [21,22].

Patients with multiple system atrophy (MSA) have severe dopaminergic cell death in the substantia nigra and present with core clinical features similar to idiopathic PD. MSA patients are given L-dopa for symptomatic relief in doses similar to, or greater than, PD patients. Analyses of mitochondrial function in substantia nigra and other brain areas in MSA have not identified any defect [22,23]. These observations also address the question as to whether the complex I deficiency in PD is simply a result of neuronal degeneration. The absence of any defect in MSA, in the presence of severe neuronal loss, indicates that the complex I abnormality is not simply a reflection of cell death. This is also supported by the observations in other neurodegenerative diseases where analyses of mitochondrial function in brain have demonstrated different defects e.g., complex II/III deficiency in Huntington's disease (HD) caudate nucleus [24] and complex IV deficiency in Alzheimer's disease (AD) brain [25]. Thus isolated complex I deficiency is not a product of neural death.

Studies on the tissue expression of the mitochondrial defect in muscle and platelets in PD produced conflicting results. Both in vivo spectroscopic [26,27] and in vitro biochemical analysis (see [17] for review) suggested either no defect or multiple defects. However, the majority of patients in these studies showed no mitochondrial abnormality. The reasons for the discrepancies are not clear but may lie in patient sampling, different experimental methods and, perhaps most importantly, different aetiological factors.

In contrast, almost all studies on platelet mitochondrial function in PD have demonstrated a defect of complex I function (see Refs. [17,28]). The severity of the deficiency did vary between studies, with most showing a $\sim$25% reduction, whilst some showed defects up to 50%. On plotting PD patients against matched controls, however, it was clear that there was overlap between control and PD complex I activity, and the assay was too insensitive to use as a 'diagnostic test' [29]. Nevertheless, the presence of a mitochondrial defect in a site distant from the substantia nigra has important implications for our understanding of the cause of this abnormality. Platelets do have some pharmacological (they contain MAO-B) and uptake (they concentrate MPP^+) mechanisms which are similar to dopaminergic neurones [30] and these could render platelets and dopaminergic neurones susceptible to the same or similar toxins which may cause the mitochondrial defect in both. Candidate toxins have included the isoquinolines and the β-carbolines. 1,2,3,4-Tetrahydroisoquinoline (TIQ) and 2-methyl-TIQ are both structurally related to MPTP and both have been found in human brain, including a patient with PD in whom the level of TIQ was greater than controls [31,32]. TIQ can be toxic to dopaminergic neurones and induce motor deficits in monkeys. Interestingly, TIQ is also a complex I inhibitor [33]. *N*-methyl TIQ is a methylation product of TIQ and is a substrate for MAO-B, producing *n*-methyl-isoquinolinium ($NMIQ^+$). $NMIQ^+$ inhibits tyrosine hydroxylase and MAO. It is found in certain foodstuffs and can cross the blood brain barrier, but can also be formed by condensation reaction in the brain. β-carbolines are also structurally related to MPTP and can also be formed in the brain by condensation reaction. They are only poorly concentrated in mitochondria and induce inhibition of com-

plexes I–III [34]. β-carbolines are not readily taken up into dopaminergic neurones and are weak nigral toxins even after intrastriatal infusion.

An alternative explanation to an environmental/toxic cause for the complex I defect in both substantia nigra and platelets is that both are caused by a genetically determined abnormality. As both nuclear and mitochondrial genomes contribute to complex I polypeptides, the mutation(s) could be in either one. A method recently described, using mtDNA-less (ρ^0) cells as a recipient for donor mtDNA, offers an opportunity to dissect out the cause of the platelet complex I deficiency in PD [35]. ρ^0 cells are derived from a cell line following exposure to ethidium bromide over several passages. The ρ^0 cells have mitochondria but no mtDNA and no functioning respiratory chain and they require a medium enriched in pyruvate and uridine to survive and grow. Platelets are derived from megakaryocytes in the bone marrow and so their mitochondrial proteins are derived from the host nucleus and mitochondrial genomes in the normal fashion. Platelets themselves are budded off from the megakaryocyte and so enter the circulation with mitochondria but no nucleus. Thus they represent an ideal unit for fusion with ρ^0 cells. Platelets act as donors of mtDNA to the resulting fusion cybrid, whilst the ρ^0 cell provides the nuclear environment. Subsequent growth and cloning of the fusion cybrid produces daughter cells derived from the ρ^0 nuclear background but with the donor mtDNA. Using this system, it can be predicted that, if the complex I deficiency present in PD platelet mitochondria is retained in fusion cybrids after growth and passage, the complex I defect must be the result of a mutation or mutations in PD platelet mtDNA. This is because the only remnant of the PD platelets in the daughter fusion cybrids is the mtDNA. Conversely, if the complex I defect is lost then the cause must be either in PD nuclear DNA or due to a circulating toxin.

A putative mtDNA defect is unlikely to be the cause of all PD and so we have suggested that those patients with the lower platelet complex I activity might be those most likely to carry such a mutation [29]. A mtDNA mutation might be expected to result in observable maternal inheritance of a disease. Whilst occasional families with maternally inherited PD do exist [36], the majority occur as sporadic cases or in autosomal dominant families [37]. However, the majority of patients with mtDNA mutations associated with disease have no family history, including 40% of LHON patients with the 11778 mutation (see below). Thus, the lack of maternal inheritance in PD does not exclude a role for mtDNA, at least in a proportion of cases.

We selected eight patients with complex I activity >1 S.D. below control mean and showed that this defect was consistent over time [38]. Platelets from these patients were then individually fused with A549ρ^0 cells. Interestingly, the fusion cybrids grew more slowly than the matched control cybrids. Analysis of the PD fusions provided evidence for a mtDNA defect underlying the complex I defect in these patients.

(1) The PD fusion clones had a specific 25% decrease in citrate synthase corrected complex I activity ($p = 0.007$). There was a significant correlation between an individual control or PD patient's complex I activity and their cybrid complex I activity. This implies that the donor mtDNA (control or PD) was a major factor determining complex I function. Furthermore, correlation of platelet and cybrid complex I enabled a more clear and sensitive distinction between PD and controls.

(2) MtDNA mutations are invariably heteroplasmic and are randomly distributed to daughter cells during cloning. We hypothesised that if the PD cybrids carried a heteroplasmic mtDNA mutation, cloning would produce cells with variable respiratory chain activity, depending upon the mutant load in each clone. This hypothesis was proved correct.

(a) Cloning of one of the PD patients produced a series of clones with a range of respiratory chain activity. Eight of 16 PD clones had complex I/citrate synthase ratios >1 S.D. below the control mean, and ten of 16 clones had complex IV activities >1 S.D. below control mean. Overall, there was a mean 25% reduction in complex I and 20% decrease in complex IV.

(b) Cytochrome oxidase (COX, complex IV) histochemistry in clonal cells with the 3243 'MELAS' mutation demonstrated significant intercellular variability. Some cells stained strongly, whilst others stained weakly or not at all. This is thought to be a reflection of the variation in the 3243 mutant load between cells, which if $\geqslant 95\%$ results in complex IV

deficiency in A549ρ^0 cells. Similar staining of the PD clones produced an identical pattern, some cells staining normally whilst others were COX negative. Control clonal cells all stained positively for COX.

(c) Double fluorescent staining with Mitotracker—a mitochondrial stain—and a monoclonal antibody to the mtDNA encoded COI subunit of COX showed that all PD clones had mitochondria and, whilst some had normal COX I binding, other cells had none or very little. The loss of COI staining in these cells implied defective mtDNA COI translation. This was also a pattern identical to that seen in 3243 'MELAS' clones. Control clonal cells all stained with both Mitotracker and COI.

(d) Finally, using JC-1, a fluoroprobe sensitive to mitochondrial membrane ($\Delta\Psi_m$) potential and an indirect measure of respiratory chain activity, produced a heterogeneous intercellular pattern in the PD clones indicating some had normal ($\Delta\Psi_m$) but others had low ($\Delta\Psi_m$) implying low respiratory chain function. Again, all control clones produced a normal and uniform ($\Delta\Psi_m$) staining pattern.

These results produce strong but as yet indirect evidence for a mtDNA mutation in PD platelets and are in agreement with a previous study of PD mixed cybrids [39]. Direct sequencing of small numbers of unselected PD patients has been undertaken but without positive results [40–43]. We would suggest that informative mtDNA sequences are more likely to be derived from PD patients selected for their low complex I activity, as these patients probably constitute the subgroup most likely to carry relevant mtDNA mutations.

A putative mtDNA mutation capable of producing a platelet mitochondrial complex I defect would be the most likely cause of the complex I deficiency in PD substantia nigra. MtDNA mutations are usually systemically distributed, although the proportion of mutation load will vary from one tissue to another. To some extent this may explain the variable results seen in skeletal muscle in PD patients, some with, and others without, a mtDNA defect. Even in those who do have a mtDNA mutation, a variable mutation load may result in a defect in some but not others. If a mtDNA defect results in a complex I defect in substantia nigra, it is likely that other factors in the nigra will exacerbate such a defect. There is clear evidence of oxidative stress and damage in the PD substantia nigra (see Ref. [44] for review). Already outlined above is the reciprocal relationship between a respiratory chain defect and free radical generation. In addition, auto-oxidation of neuromelanin and the presence of increased, possibly 'free' iron [45,46] will further enhance the toxic environment for complex I. Furthermore, the use of L-dopa may, as discussed above, exacerbate the complex I deficiency. Thus, it is our proposition that any inborn complex I defect in PD is enhanced by the biochemistry and pharmacology of the substantia nigra.

If a mtDNA mutation is present in a proportion of PD patients, how does it induce dopaminergic cell death? Firstly, neurones are particularly dependent on oxidative phosphorylation as an energy source—thus a defect in the respiratory chain may have a disproportionate effect on survival. Secondly, as outlined above, other factors may exacerbate the mitochondrial deficiency in the nigra. Thirdly, the proportion of mtDNA mutations may vary according to anatomical location and only those above a specific threshold may suffer sufficient nigral neuronal death to induce PD. Given these factors, the most likely direct result of the mtDNA mutation and complex I deficiency will be a fall in ATP levels. The results of homogenate studies indicate that there is a 30–40% complex I defect in PD substantia nigra, such an abnormality being likely to involve both neurones and glia but more severely affecting neurones. The simplest explanation is therefore that the dopaminergic neurones die from a fall in ATP levels. However it is unlikely that this is the only cause, oxidative damage, calcium release and probably additional as yet unknown factors also contributing.

Cells may die by necrosis or apoptosis. Apoptosis is characterised by certain morphological features including chromatin condensation and the formation of apoptotic bodies but with the retention of organeller structure. A number of factors have been shown to induce apoptotic cell death including free radicals [47], L-dopa [48], MPP^+ [49] and TIQ [50]. Recent evidence suggests that MPP^+, rotenone and TIQ^+ induce apoptosis via a free radical related mechanism [50]. Thus the biochemical defects already identified in PD substantia nigra, or compounds known to induce complex I deficiency and parkinsonism, are also know to cause apoptosis. There is some evidence that apoptotic cell death may also occur in

PD [51] although others have found no apoptotic changes [52]. Apoptosis occurs rapidly and so may be missed when a single time point (time of death) is selected for study. Furthermore, apoptotic cell death may occur more frequently in the early stage of PD than in late end stage disease [49]. Against these factors must be balanced the possibility that agonal state may itself increase the proportion of cells undergoing apoptosis. Nevertheless, the presence of so many proapoptotic factors in PD makes this mode of cell death a possible, if not probable, contributor to pathology. If so, this would allow the development of novel forms of neuroprotection.

Recent research has highlighted the important role that mitochondria play in apoptosis. ρ^0 cells, despite the lack of a respiratory chain, still generate sufficient energy to maintain ($\Delta\Psi_m$) [53]. Both ρ^0 and ρ^+ (normal) cells lose their ($\Delta\Psi_m$) and generate free radicals in response to agents that stimulate apoptosis. These events precede nuclear DNA fragmentation in both ρ^0 and ρ^+ cells, although they take place at a slower rate in ρ^0 cells. The loss of ($\Delta\Psi_m$) is the result of the opening of permeability transition pores, or mega-channels [54]. Permeability transition and ($\Delta\Psi_m$) collapse result in the release of molecules 10–15 kDa in size capable of inducing chromatin condensation in isolated nuclei [55], one of which is cytochrome *c* [56]. These events appear to be important early steps in apoptosis. A mtDNA mutation and complex I defect will adversely affect ATP synthesis and cause a fall in ($\Delta\Psi_m$)—as shown in the PD cybrids with JC-1 staining. Although such a fall in ($\Delta\Psi_m$) may be insufficient to result in apoptosis, it may 'prime' cells such that additional biochemical insults, e.g., free radicals, may cause sufficient collapse of ($\Delta\Psi_m$) to induce apoptotic cell death. If this is shown to be of relevance to PD, new neuroprotective agents directed to maintaining mitochondrial ($\Delta\Psi_m$) could be developed and tested.

In summary, there is now compelling evidence for a complex I defect in PD and that in a proportion of cases at least, this deficiency is caused by a mtDNA abnormality. Further research is now being directed to identifying the relevant mtDNA sequence changes. Once these are defined, their relationship with the complex I deficiency can be investigated along with any interaction such mutation may have with environmental agents. The mitochondrial contribution to PD pathogenesis has also stimulated the search for new agents that might retard or prevent neuronal loss.

3. Dystonia

Idiopathic torsion dystonia (ITD) is the commonest form of primary dystonia. Autosomal dominant genes with reduced penetrance and variable expression probably account for the majority of patients with segmented, multifocal and generalised ITD [57,58]. The DYT1 gene responsible for early onset generalised dystonia has been mapped to 9q34 and recently shown to encode an ATP binding protein [59]. The pathology of dystonia is thought to involve the basal ganglia, although the biochemical abnormalities that may cause dystonia are not understood. Interestingly, dystonia is seen relatively commonly in patients with mitochondrial myopathy [60] including Leigh's syndrome [61], Furthermore several families with LHON-dystonia and specific mtDNA mutations have been described (see below).

In 1992, one study reported a specific defect of complex I activity in platelet mitochondria from patients with segmental, generalised and focal dystonia [62]. This group found that complex I function was reduced by 62% in segmental and generalised dystonia patients, and by 37% in those with focal dystonia, providing some parallel in mitochondrial dysfunction with the severity and extent of the dystonia. However, a subsequent report found no abnormality of respiratory chain activity in patients with focal or generalised dystonia [63]. This area was further investigated by studying patients with generalised dystonia whose linkage to DYT1 was defined, and in patients with sporadic, focal dystonia [64]. Results showed a significant decrease in mean citrate synthase corrected platelet complex I activity (22% decrease, $p = 0.001$) in patients with focal dystonia. However, there was no abnormality in mitochondrial function found in patients with generalised dystonia, whether or not they were linked to DYT1. This study therefore confirmed the previous observation of a specific complex I deficiency in focal dystonia. The lack of any association of DYT1 with a complex I defect suggests that the nuclear mutation in this disorder does not involve mitochondrial dysfunction.

The association of a platelet complex I deficiency with focal dystonia raises similar questions with regard to aetiology and pathogenesis as were discussed in the section on PD. The presence of this abnormality in a site distant from pathology naturally raises the prospect of a genetically determined biochemical defect with variable expression and pathological consequences. As in PD, the complex I defect in the patients with focal dystonia may be encoded by a nuclear or mitochondrial gene. In order to address this issue, the ρ^0 cybrid system was applied to platelets from patients with focal dystonia. In contrast to the PD patients, the dystonia mtDNA transformed A549ρ^0 cells did not perpetuate the complex I deficiency on subsequent passage. This was further examined by undertaking clonal analysis and, again in contrast to the PD patients, there was no significant variation in either complex I or complex IV in the daughter clones [65]. Cell histochemistry, COI immunohistochemistry and JC-1 staining all showed homogeneous intercellular patterns, again contrasting with that seen in PD.

These studies in A549-dystonia cybrids have important implications.

(1) The cybrid analysis in both mixed culture and clonal lines from focal dystonia patients suggests that the cause of the complex I defect is not related to mtDNA but rather to a nuclear defect or circulating toxin.

(2) The 'negative' results obtained with the dystonia patients (who demonstrated a platelet complex I defect of similar severity to PD patients), using the same A549 nuclear background as in the PD fusion studies. serves to emphasise the importance and validity of the data implicating mtDNA as the cause of the complex I defect in the PD patients studies.

Further progress in studying the relationship of the complex I defect in dystonia is limited by accessibility to brain samples—it is not known whether any mitochondrial defect occurs in basal ganglia or other areas of the dystonia brain.

4. Leber's hereditary optic neuropathy

LHON is the commonest cause of blindness in otherwise healthy young men with an incidence of approximately 1 in 50 000. Presentation is usually with subacute painless sequential visual loss (usually within 8 weeks) with early fundoscopic changes of a peripapillary telangiectatic microangiopathy, with subsequent development of optic atrophy. Recovery of vision is variable and may be related to the underlying mutation. The frequency of the presence of additional neurological features is difficult to determine but a multiple sclerosis-like illness [66], cerebella ataxia [67], peripheral neuropathy [68] and dystonia (see below) have all been described in LHON families. whilst one study suggested additional neurological abnormalities in 27 of 46 LHON patients [69], only 7 of 107 LHON patients were found to be similarly affected [70].

Maternal inheritance in LHON was recognised early [71] and made this disorder an ideal subject for mtDNA analysis. Several mtDNA mutations have now been identified in LHON: G11778A [72], G3460A [73] and T14484C [74] in the ND4, ND1 and ND6 complex I genes respectively. These three mutations are considered 'primary' in that no additional mtDNA mutations are required to cause the disease and they are not seen in controls. Sixteen other mutations have also been associated with LHON; these are termed 'secondary' mutations because either the mutations must occur together in some combination or because their pathogenicity is in doubt as they are found in controls and result in base changes of doubtful significance [75].

The G11778A mutation in ND4 is found in excess of 50% of all LHON families whilst the other two primary mutations are found in 10–20% [76]. In the G11778A families, there is 95% penetrance of LHON by age 50 years. The primary mutations are virtually homoplasmic or heteroplasmic with high mutant load in the blood of LHON families. There is little relationship between mutant load and the risk of developing LHON, and both symptomatic and asymptomatic family members may carry identical mutant loads. This, together with the observation that there is a preponderance of affected males (4.3:1 for the G11778A mutation) has lead to the suggestion that additional factors, nuclear or environmental, may be involved in determining LHON. The presence of a visual loss susceptibility locus on the X chromosome might explain some of these observations. However, linkage analysis over 169cM of the X chromosome has excluded involvement [77]. Lyonisation favouring

an X-linked susceptibility locus could also explain the pedigree patterns in LHON, but investigation has failed to identify any skewed inactivation [78].

Functional defects of complex I have been reported in all three primary mutations. Decreased NADH CoQ_1 reductase activity (by ~65%) has been described in platelets from patients with the G3460A mutation [79–81] and the T14484C mutation [82]. Impaired oxygen consumption with complex I linked substrates has been observed in the G11778 mutation [80,83]. These respiratory chain defects appear widespread and have been seen in lymphocytes and skeletal muscle, as well as platelets. Interestingly, although a significant complex I defect was demonstrated polarographically in the G11778A mutation [80]. Only a mild enzymatic deficiency was present—and then observed only when corrected for smoking habit [81]. We have recently shown that the severe enzymatic defect in the G3460A mutation is not associated with a decrease in ATP synthesis. Thus there seems to be little relationship between the presence of the G3460A mutation and its effect on energy metabolism. This, coupled with the lack of discriminatory effect of the presence of the primary mutations in inducing LHON amongst siblings highlights the need for clearer insight into the pathogenicity of these mutations.

We proposed that the nuclear background of a cell might influence the biochemical expression of a primary LHON mutation. This might help explain the apparent tissue specificity (to the optic nerve) in a high proportion of patients. The G3460A mutation was chosen for this analysis as it had a clear biochemical marker in terms of its severe enzymatic complex I defect. When platelets homoplasmic for this mutation were fused with 143B (bone derived) ρ^0 cells, and expanded by clonal analysis, there was a mean ~60% decrease in complex I enzyme activity —paralleling that observed in the parent platelets [84]. When similar experiments using fusion and clonal analysis were undertaken using an A549 (lung derived) nuclear background, however, there was no significant difference in mean complex I activities. These results demonstrate that the nuclear environment can influence the expression of the complex I defect in LHON patients with the A3460G mutation.

Previous reports have identified several families with LHON-dystonia. A ~80% defect in complex I activity was identified in platelets from a family with LHON and CNS disease including dystonia, who carried the T14484C mutation together with a further mutation at base pair 4160 in ND1 [85,86]. A second family was found to have a mutation in ND6 at base pair 14459 [87], with defective complex I activity which persisted following transfer to a ρ^0 cell line [88]. A third family with LHON-dystonia and a mutation at 11696 in ND4, in association with a 'secondary mutation' at 14596 in ND6, has been described [89]. Biochemical analysis of skeletal muscle from a patient of this last family showed a 79% decline in complex I function associated with less severe decreases in the activities of complex II–IV. All the LHON-dystonia families exhibited maternal inheritance. The results from these families are of particular interest, bearing in mind the finding of complex I deficiency in focal dystonia.

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