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Minireview

Mouse models of dominant optic atrophy: What do they tell us about the pathophysiology of visual loss?

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ABSTRACT

Dominant optic atrophy (DOA) is the most common inherited optic neuropathy affecting one in every 12,000 people. It presents with bilateral visual loss, central visual fields defects, colour vision disturbance and optic disc pallor. *OPA1* has been identified as the responsible gene and its locus mapped to chromosome 3q28-q29. Mutations in this gene are responsible for the clinical phenotype in over 70% of patients with DOA. Histopathological studies in tissues from patients reveal loss of retinal ganglion cells but the paucity of viable human tissue has raised the importance of an animal model to study the pathophysiology of the disease. In the last decade considerable work has gone into the generation of animal, most notably mouse, models of Opa1 DOA. Two murine models of DOA have been published, designated B6;C3-*Opa1*^{Q285STOP} and B6;C3-*Opa1*$^{329\text{-}355del}$ and they provide valuable insights with respect to neurological and visual phenotyping, mitochondrial dysfunction, optic nerve and axonal changes, retinal ganglion cell depletion and dendritic atrophy. Here we summarise the current state of knowledge of the mechanisms of disease based on data from these models of Opa1 DOA.

1. Introduction

Mitochondria are dynamic organelles with an essential bioenergetic function. They continually undergo fusion and fission, in a process that is held in equilibrium by various mitochondrial shaping proteins, for fission; Drp1 and Fis1 (Frank et al., 2001; Lackner & Nunnari, 2009; Liesa, Palacin, & Zorzano, 2009; Yu, Fox, Burwell, & Yoon, 2005) and for fusion; the mitofusins (Mfn1 and Mfn2) and Opa1 (Chen & Chan, 2006; Chen et al., 2003; de Brito & Scorrano, 2009; Song, Ghochani, McCaffery, Frey, & Chan, 2009). Mutations in *OPA1* result in the disease dominant optic atrophy (DOA), which is characterised by a mild to moderate progressive loss of visual acuity, central visual field defects, colour vision defects and temporal optic disc pallor. Although the importance of Opa1 protein in controlling mitochondrial fusion is well known the mechanisms by which *OPA1* mutations cause DOA have yet to be elucidated.

1.1. OPA1 and dominant optic atrophy

Mutations in *OPA1* lead to DOA, (Alexander et al., 2000; Delettre, Lenaers, Pelloquin, Belenguer, & Hamel, 2002) the most common optic neuropathy, with an estimated prevalence of 1:12,000 (Carelli, Ross-Cisneros, & Sadun, 2002) rising to 1:10,000 in certain populations (Delettre et al., 2002). DOA typically presents in the first decade of life as bilateral visual loss with pallor of the optic disc, centrocecal visual field scotoma and tritanopia (Delettre et al., 2002; Votruba, Moore, & Bhattacharya, 1998). Visual loss may be slowly progressive. There is considerable intra- and interfamilial variability in severity of visual loss ranging from legally blind to asymptomatic carriers. Some pedigrees have associated clinical features such as ptosis, myopathy and progressive external ophthalmoplegia. Histological assessment from donor eyes shows thinning of the retinal ganglion cell layer suggesting degeneration of retinal ganglion cells (RGCs). Demyelination has been observed in the optic nerve, chiasm and tract (Kjer, Jensen, & Klinken, 1983; Milea et al., 2010). The shape of the optic nerve has been reported to be characteristic (Fournier, Damji, Epstein, & Pollock, 2001; Votruba, Thiselton, & Bhattacharya, 2003) and the size of the optic nerve head is reduced (Barboni et al., 2010).

Over 200 different *OPA1* mutations have been reported to date (Ferré, Amati-Bonneau, Tourmen, Malthiery, & Reynier, 2005; Olichon et al., 2006). Isolated mutations in the *OPA1* gene have also been shown to cause a 'DOA plus syndrome', in which optic atrophy is accompanied by sensorineural deafness, ataxia, axonal sensory-motor polyneuropathy, chronic progressive external ophthalmoplegia and mitochondrial myopathy with cytochrome c oxidase negative and Ragged Red Fibres (Amati-Bonneau et al., 2009; Huang, Santarelli, & Starr, 2009; Milone, Younge, Wang, Zhang, & Wong, 2009; Yu-Wai-Man et al., 2010). Remarkably, it has recently emerged that, in rare cases, *OPA1* mutations can be

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associated with hearing loss, ptosis and oculomotor deficits in the absence of any detectable optic atrophy (Milone et al., 2009).

1.2. The Opa1 protein

The *OPA1* gene codes for the protein OPA1, a dynamin-like mitochondrial related guanosine triphosphatase (GTPase) and a 100 K member of the GTPase superfamily (Hinshaw & Schmid, 1995). OPA1 is homologous with the yeast (*Saccharomyces cerevisae*) gene Mgm1 (Olichon et al., 2002) and is located primarily on the mitochondrial inner membrane. Mgm1/Opa1 has multiple functions, playing a central role in the maintenance of mitochondrial morphology and mitochondrial networks through its effects on endocytosis, vesicular traffic and coated vesicle formation (Hinshaw & Schmid, 1995). It also influences mitochondrial motility (including mitochondrial fission and fusion) to ensure an appropriate distribution of mitochondria and adequate supplies of ATP within the cytosol. OPA1 is thought to play a protective role as an anti-apoptotic GTPase by limiting the detrimental impact of apoptotic stimuli (Davies et al., 2007). The OPA1 protein is expressed ubiquitously throughout the body with high levels in the retina, brain (Aijaz, Erskine, Jeffery, Bhattacharya, & Votruba, 2004; Misaka, Miyashita, & Kubo, 2002) liver and heart. Given the critical importance of OPA1 to so many cellular activities it is interesting that OPA1 mutations manifest primarily as DOA (Davies & Votruba, 2006).

1.3. Mouse models of dominant optic atrophy

Ocular and CNS tissue from patients with DOA is scarce and the published histology of DOA has come from a very small number of elderly patients with severe disease (Johnston, Gaster, Smith, & Tripathi, 1979; Kjer et al., 1983). This limitation has created a pressing need for an animal model of DOA. Such a model must combine the genetic and clinical characteristics of DOA in animals that are suitable for genetic analysis. Mice are widely used as genetic disease models due to the relative ease of genetic manipulation and high homology to the human genome. The murine retina shows relatively good homology to the human retina rendering the mouse suitable for modelling a wide range of human visual diseases with a genetic basis (Smith, John, Nishina, & Sundberg, 2002). However, there is a range of anatomical limitations, and it should be recognised that although the murine eye is a good model for human eye disease it is by no means perfect. Despite this, much has been learnt from mouse models of human genetic eye disease. In the last 5 years two mouse models of Opa1 DOA (based on OPA1 haploinsufficiency) have been published: the B6;C3-*Opa1*^{Q285STOP} Opa1 mutant mouse (Davies et al., 2007) and the B6;C3-*Opa1*$^{329\text{-}355del}$ Opa1 mutant mouse (Alavi et al., 2007). Both display a broad correlation with the human DOA phenotype. (The two models are compared and contrasted in Table 1.)

2. Mouse models of dominant optic atrophy

2.1. Generation of the B6;C3-*Opa1*^{Q285STOP} and B6;C3-*Opa1*$^{329\text{-}355del}$ *Opa1 mutant mice*

Both the B6;C3-*Opa1*^{Q285STOP} mutant mouse (Davies et al., 2007) and the B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse (Alavi et al., 2007) were generated after screening an ENU-mutagenized DNA library of mouse DNA (Ingenium, Martinsried, Germany) for mutants with sequence changes in *Opa1*.

The B6;C3-*Opa1*^{Q285STOP} *Opa1* mutant mouse was generated by screening an ENU-mutagenized DNA archive from C3HeB/FeJ males for point mutations in *Opa1* exons 1, 8, 9, 10, 12 and 28, selecting a heterozygous nonsense mutation in exon 8, which codes for a C–T transition at 1051 bp (Q285STOP). This mutation causes protein truncation at the beginning of the dynamin GTPase, close to the location of a number of human disease mutations (c.868C>T (R290 W) and c.869G>T (R290Q) (Ferré et al., 2005)). The Opa1 mutant mouse line (B6;C3-*Opa1*^{Q285STOP}) was produced through *in vitro* fertilization with mutant sperm and C57Bl/6 J females to produce a heterozygous, *Opa1*±, mouse. The founder (F1) generation was then systematically outcrossed to C57Bl/6 J up to at least G4. The pdeβ (RD1 mutation), carried by the C3H line, was excluded by systematic genotyping and breeding. Heterozygous *Opa1*± mice were intercrossed to generate generation cohorts.

The B6;C3-*Opa1*$^{329\text{-}355del}$ Opa1 mutant mouse was also generated by screening an ENU-mutagenized DNA library of mouse DNA and this time identifying a splice site mutation in murine *Opa1* intron 10*:* c.1065+5G → A. Using a purebred C3HeB/FeJ outcross on C57Bl/6 a mouse model for DOA carrying this splice site mutation in the *Opa1* gene was created. The mutation is close to three reported human mutations (c.1065+2T>C, c1065+2T>G and c.1065+3A>C (Ferré et al., 2005)) and results in skipping of exon 10 in the OPA1 gene causing an in-frame deletion of 27 amino acid residues in the dynamin GTPase domain.

Both models show ∼50% reduction in *Opa1* transcript in retinal tissue and a ∼50% reduction in Opa1 protein across a range of tissues, suggesting that haploinsufficiency underlies the pathophysiological mechanism. Both the B6;C3-*Opa1*^{Q285STOP} and B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse are embryonic lethal when homozygous; at <E13.5 in the B6;C3-*Opa1*^{Q285STOP} mutant mouse (Davies et al., 2007) and ca. E8.5 (between E3.5 and E12) in the B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse (Alavi et al., 2007).

2.2. Visual, neurological and neuromuscular abnormalities

Visual function in the B6;C3-*Opa1*^{Q285STOP} mutant mouse has been assessed with a rotating optokinetic drum (OKN) using high (2°, corresponding to 0.25 cycles/degree) to low (4° and 8°, 0.125 and 0.0625 cycles/degree) resolution gratings. Two studies (Davies et al., 2007; Yu-Wai-Man et al., 2009) have looked at visual function at 6, 12, 13 and 18 month old mice. Significantly decreased mean tracking frequencies from 12 months in *Opa1*± mice were detected at high and low spatial frequencies. Furthermore, reduced detection of the low resolution gratings was documented from 18 months onwards in *Opa1*± mice.

Given the ubiquitous expression of Opa1 (Alexander et al., 2000) an Opa1 deficiency may be expected to adversely affect other organ systems, especially those with high levels of mitochondria and high metabolic demands. Detailed (non ocular) phenotyping of the B6;C3-*Opa1*^{Q285STOP} mouse model by SHIRPA neurological testing has revealed subtle systemic neurological and neuromuscular abnormalities (Davies et al., 2007), such as decreased locomotor activity.

There are several reported neurological and metabolic abnormalities in the B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse phenotype (Alavi et al., 2009). SHIRPA testing showed that over a half of the Opa1 mutant mice had an abnormal clutching reflex with a third (11 males and 2 females) suffering a tremor by 22 months of age. Opa1 mutant mice also performed significantly worse than controls on the Rotarod; a rotating rod used to test the physical performance of rodents. Although Opa1 mutant mice maintained a normal food intake they were significantly lighter than controls regardless of sex. Post-mortem examination revealed significantly less body fat than controls though the muscle fibre morphology was unaffected. The extra-ocular phenotype reported in the B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse has recently been supported by findings on Rotarod in the B6;C3-*Opa1*^{Q285STOP}. This also applies to the tendency for B6;C3-*Opa1*^{Q285STOP} mutants to have lower

Table 1
A comparison of the B6;C3-*Opa1*Q285STOP and B6;C3-*Opa1*$^{329\text{-}355del}$ Opa1 mouse models.

	Clinical phenotype *OPA1*DOA	B6;C3-Opa1^{Q285STOP}		B6:C3-Opa1$^{329\text{-}355del}$	
Mutation	All exons except exon 4, 4b and 5. Substitutions, deletions and insertions (Ferré et al., 2005)	Exon 8 Nonsense mutation		Intron 10 Splice site mutation	
	Evidences for haploinsufficiency (Pesch et al., 2001)	At DNA level: c. 1051C > T		At DNA Level: c. 1065+5G>A	
		At protein level: p.Q285× 50% reduction in protein levels (Davies et al., 2007)		At protein level: p.329-355del 50% reduction in protein levels (Alavi et al., 2007)	
Mitochondria	Loss of mtDNA in 'plus' mutations (Amati-Bonneau et al., 2009) Morphology fragmentation in patient fibroblasts (Lenaers et al., 2009; Carelli et al., 2002)	mtDNA copy number: no significant difference compared to *wt* counterparts Morphology: powdered appearance		mtDNA copy number: no significant difference compared to *wt* counterparts Morphology: disorganised cristae (Alavi et al., 2007)	
		Increased mitophagy (Davies et al., 2007)			
Mouse	N/A	Strain: C3H:C57Bl/6 J Generation: >G4 (Davies et al., 2007; Yu-Wai-Man et al., 2009)		Strain: C_3H:C57Bl/6 Generation: inter-crosses of F1 (Alavi et al., 2007)	
Homozygous mutant	Presumed embryonic lethal	Embryonic lethality <E13.5 (Davies et al., 2007)		Embryonic lethality ca. E8.5 (Alavi et al., 2007)	
	Phenotype				
	Age observed (months, ∗ = most significant)				
Visual function and electrophysiology	Acuity range from 6/6 to registered blind (Kjer et al., 1996) Optic atrophy with disc pallor (Votruba et al., 1998)	Decreased visual function assessed by OKN (Davies et al., 2007)	6, 12∗	Decrease in VEP amplitude	20–24∗
				No significant change in ERG even in aged mice (Heiduschka et al., 2010)	2, 9, 24
	VEP decreased amplitude ± increased latency, PERG P50:N95 ratio decreased (Holder et al., 1998)				
'Plus 'phenotype	Sensorineural deafness, ataxia, axonal sensory-motor polyneuropathy, chronic progressive external opthalmoplegia and mitochondrial myopathy with cytochrome c oxidase negative and ragged red fibres (Yu-Wai-Man et al., 2009)	Increased transfer arousal Longer freezing periods Decreased locomotor activity (Davies et al., 2007)	6∗ for all	Abnormal clutching reflex Tremor	22∗ 22∗
		No COX-SDH ragged red fibres (Yu-Wai-Man et al., 2009)		Decreased locomotor activity	22∗
				Lighter than wt counterparts	21∗
				Less body fat than wt counterparts	21∗
				No COX-SDH ragged red fibres (Alavi et al., 2009)	
RGC population and morphology	RGC loss (Kjer et al., 1983; Johnston et al., 1979)	No significant difference in population	<10; 10–15, >20	Reduction with age starting in peripheral retina confirmed by retrograde labelling (Heiduschka et al., 2009)	13, 17, 20∗
		Dendritic atrophy with age limited to sublamina b Williams et al., 2010	10–15, >20∗		
Optic nerve	Demyelination Ascending optic neuropathy (Kjer et al., 1983; Johnston et al.,1979)	Demyelination	24∗	Demyelination	8∗
		Myelin clumping	18; 24∗	Disorganisation	For all
		Watery degeneration	9, 24∗	Swollen and distorted axons	
		Dark degeneration	9, 24∗	Complete loss of large axons	
		No axon loss (Davies et al., 2007; White et al., 2009)	6, 9, 24∗	Significant loss of small axons (Alavi et al., 2007)	

body weight (Taylor, Davies, Powell, Davies, & Votruba, 2010). Of significance is the fact that neither model displayed hearing loss.

Both models have been aged up to 24+ months but no robust quantitative data on life expectancy has been published.

2.3. Retinal ganglion cell populations

Both models show normal clinical fundal appearances on dilated ophthalmoscopy. Pattern electroretinogram (PERG) data from patients with DOA show a consistent reduction in the retinal ganglion cell specific P50:N95 ratio (Holder, 2004; Holder et al., 1998). Visually evoked potential (VEP) data in DOA patients show variable reduction in amplitude, suggestive of RGC loss, with occasional delay documented in some patients, consistent with axon dysfunction/damage.

Electroretinography (ERG) in the B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse at 2 and 9 months was normal (Alavi et al., 2007). Reductions in the ERG could not be detected at 24 months of age when the disease might be regarded as 'end-stage' (Heiduschka et al., 2010). (Slight reductions were seen in the scotopic a-wave (photoreceptors) and b-wave (rod bipolar cells (Pinto, Invergo, Shimomura, Takahashi, & Troy, 2007)) and photopic b-wave amplitudes in the Opa1 mice, but these changes did not reach statistical significance.) However, assessment of the visual pathway by visual

evoked potential (VEP) (Heiduschka et al., 2010) showed a significant reduction in the amplitude of scotopic VEPs in Opa1 mice and a non-significant reduction in the amplitude of photopic VEPs, but in neither case was the VEP delayed. This was interpreted as a loss of RGCs, which could be confirmed by retrograde labelling.

Retrograde labelling from both superior colliculi using hydroxystilbamidine and by Haematoxylin and Eosin staining was undertaken to quantify any changes in RGC populations (Alavi et al., 2007). RGC counts were unchanged in 2 and 4 month old Opa1 mutant mice but showed a slight reduction by 13 months. These changes were first seen at 9 months, appearing first in the peripheral and mid peripheral retina. By 23 months Opa1 mutant mice showed a marked reduction in RGC layer counts which was confirmed by retrograde RGC labelling. RGCs were reported to be phagocytosed by retinal microglia (Heiduschka et al., 2010). Histology of the retina confirms that there is no abnormality in the photoreceptor or other retinal layers.

Conversely, histological examination of B6;C3-*Opa1*^{Q285STOP} mutant mouse retinas by Haematoxylin and Eosin, Hoechst 33258 staining and TUNEL staining reveals no significant cell loss in the retinal ganglion cell layer or death across all age groups, even at 24 months of age (Davies et al., 2007; Williams, Morgan, & Votruba, 2010). Retinal architecture and morphology is normal on histology with no defect in photoreceptors, however, there was a significant increase in the number of autophagosomes in the RGCs in the retina and surrounding the axons in the 24 month old *Opa1*± mice (White et al., 2009) compared to age matched wild type littermate controls. Since Opa1 is downregulated by ca. 50%, it is hypothesised that there is a reduction in mitochondrial membrane potential forming dysfunctional depolarized mitochondria, which must be eliminated by autophagosomes, or recovered by mitochondria fusion. Since mitochondrial fusion is impaired in this mouse model of DOA, autophagy of mitochondria (mitophagy) is a likely outcome. These data suggest that RGC dysfunction rather than significant RGC loss is the early driver for visual dysfunction in this mouse model.

2.4. Changes in retinal ganglion cell morphology

Specific changes in RGC morphology have been recorded in the B6;C3-*Opa1*^{Q285STOP} mutant mouse. In the absence of significant levels of RGC loss Williams et al. (2010) sought an anatomical basis for the evident retinal ganglion cell dysfunction and pathological changes visible in the optic nerve by analysing the connections between the retinal ganglion cells and the outer retinal layer. The dendritic morphology of retinal ganglion cells was quantified at different ages (4–23 months) using DiOlistic labelling of the retinal ganglion cell layer (Gan, Grutzendler, Wong, Wong, & Lichtman, 2000; Pignatelli & Strettoi, 2004). Quantitative analysis of dendrite length, tree area and complexity indicated atrophic changes that increased with age and were more marked in *Opa1*± mice. Interestingly, these changes were localised to sublamina b (ON layer) of the inner plexiform layer, with dendrites synapsing in the outer OFF layer relatively unaffected. These observations suggest a selective effect of the mutation on ON-centre compared with OFF-centre cells. A possible pathophysiological basis for this selectivity may rest on the different energy demands of ON- and OFF- centre RGCs and on the different neurotransmitter requirements of these pathways (Marc, 2008; Xu & Tian, 2007). It is important to note, that changes in the architecture of the dendritic trees were seen as early as 10 months and preceded optic nerve defects and vision loss in this model. The present data suggest that in the B6;C3-*Opa1*^{Q285STOP} model vision loss results from RGC dysfunction and axon and optic nerve abnormalities (Davies et al., 2007; Williams et al., 2010) rather than ascending RGC death as seen in the B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse model.

2.5. Optic nerve pathology

Although optic nerve pallor is a cardinal feature of human DOA, clinical optic atrophy is hard to categorise in the mouse eye and it has not been a robust phenotypic marker in either model. In the B6;C3-*Opa1*^{Q285STOP} mouse model transmission electron microscopic analyses have indicated little change relative to controls in the optic nerves of 6 month old *Opa1*± mice. However, from 9 months to 18 months there were significant abnormalities in the optic nerve fascicles and myelin bundles, which appeared as large abnormal whirls of myelin as well as the appearance of noticeable demyelination (Davies et al., 2007) associated with significant intra-axonal changes (White et al., 2009). From 9 months of age these were evident as early signs of watery and dark axonal degeneration in the *Opa1*± mice, and reached statistical significance by 24 months, by which time axons counts were significantly reduced. Since axon loss was evident in both the 24 month *Opa1*± mice and age matched wild-type controls, it is likely that the changes reflected age related axon loss and not just the effects of Opa1 deficiency.

Similar electron microscopic analysis on the B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse (Alavi et al., 2007) has shown a more severe phenotype to that of the B6;C3-*Opa1*^{Q285STOP} mutant mouse showing a complete loss of large axons and a significant loss of small axons by 8 months of age indicative of RGC degeneration. Optic nerve axons were swollen and distorted with associated demyelination and the presence of membranous whorls. The neurofibrillary and collagen contents were both reduced in the optic nerve.

2.6. Clinical relevance of the B6;C3-*Opa1*^{Q285STOP} and B6;C3-*Opa1*$^{329\text{-}355del}$ mouse models

The B6;C3-*Opa1*^{Q285STOP} mouse model is a useful resource for the study of DOA disease mechanisms since it shows late onset and subtle changes in RGCs and optic nerve which are associated with slow progression of the disease. The precise mechanisms linking defects in mitochondrial fusion to visual dysfunction remain unknown. The B6;C3-*Opa1*^{Q285STOP} model shows evidence of mitochondrial dysfunction with *Opa1*± mouse muscle fibroblast culture displaying punctuated and dispersed mitochondria, giving an abnormal 'powdered' appearance (Davies et al., 2007). Apoptosis of RGCs has been proposed as a pathophysiological mechanism in DOA, but cell counts and TUNEL labelling in this mouse model show that visual dysfunction must exist prior to widespread RGC loss. Thus, this model supports dysfunction of RGCs as an early stage in DOA pathophysiology. From the data presented it appears that dendritic pruning of RGCs precedes visual deficit as well as changes in RGC axons and the optic nerve giving a potential timescale for the progression of the disease. The B6;C3-*Opa1*^{Q285STOP} mutant mouse harbours no mtDNA deletions associated with the DOA 'plus' phenotype in patients yet there are subtle extra-ocular neurological abnormalities present suggesting that even those OPA1 mutations not associated with mtDNA loss in patients can be associated with neuromuscular defects. This highlights the importance of conducting full neurological assessments in all DOA patients (Aijaz et al., 2004; Amati-Bonneau et al., 2009; Yu-Wai-Man et al., 2009).

The B6;C3-*Opa1*$^{329\text{-}355del}$ mutant mouse provides a good model of DOA since it retains the key clinical features documented in patients with similar mutation in OPA1 close to intron 10 c.1065 (c.1065+2T>C, c1065+2T>G and c.1065+3A>C (Ferré et al., 2005; Pesch et al., 2001; Puomila et al., 2005)). The B6;C3-*Opa1*$^{329\text{-}355del}$ mouse model of DOA displays no mtDNA deletions (Alavi et al., 2009). Changes in the optic nerve, RGC populations and abnormal mitochondria (Alavi et al., 2007) are all present and these follow clinical observations in patients with DOA. The Opa1 mutant

mouse also has reduced VEP amplitude strongly suggesting RGC loss, a finding supported by retrograde labelling of RGCs.

The evidence for retinal ganglion cell loss as the only mechanism of disease in clinical DOA is partly based on histological data conducted over 30 years ago on a small cohort of two very elderly patients with severe visual loss/'end-stage' disease (Johnston et al., 1979; Kjer et al., 1983). This histology shows retinal thinning and widespread retinal ganglion cell loss. Recent OCT data (Milea et al., 2010) confirm the retinal nerve fibre layer thinning. Based on this data widespread retinal ganglion cell loss in DOA must be a relevant mechanism, but it may not be the earliest change and not necessarily even the only one (see Table 1).

VEP data from patients, with reduced amplitude, supports retinal ganglion cell loss; however, some patients' VEP readings show an increased VEP latency (Holder et al., 1998) indicative of retinal ganglion cell dysfunction, in association with cell loss. With over 200 mutations recorded (Ferré et al., 2005) and electrophysiology available for only a proportion of these, it may be possible that retinal ganglion cell dysfunction precedes loss as the driver for visual dysfunction, and this effect may be mutation specific. Unfortunately, due to the constraints of human retinal phenotyping in patients with early disease it may prove difficult to explore this hypothesis fully.

Drawing from murine models of other neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's (PD) disease, there is evidence of both dendritic atrophy (AD) (Grutzendler, Helmin, Tsai, & Gan, 2007) and widespread neural cell loss (PD) (Beal, 2001) as drivers for the disease and as the major mouse phenotype. Mitochondrial fusion and fission are essential for proper dendritic morphology and we see rapid dendritic remodelling in terms of dendritic spine morphology when the fusion/fission balance is disturbed (Li, Okamoto, Hayashi, & Sheng, 2004). Retinal ganglion cells do not possess dendritic spines but there is evidence for dendritic remodelling over a longer time frame (Marc, Jones, Watt, & Strettoi, 2003). It is feasible that this process is happening in a subset of DOA patients as an early disease marker (as in the B6;C3-*Opa1*^{Q285STOP} mouse) and that widespread retinal ganglion cell loss (as in the B6;C3-*Opa1*$^{329\text{-}355del}$ mouse) occurs prior to gross visual dysfunction in that same way that there is an 70% neural loss in the substantia nigra in PD patients before the disease is clinically apparent.

3. Conclusions

Here we compare two models, both showing a clinical phenotype compatible with DOA as well as some traits present in the DOA plus phenotype. The B6;C3-*Opa1*$^{329\text{-}355del}$ mouse is hypothesised to develop visual dysfunction as a result of RGC loss and an ascending optic neuropathy, whilst the earliest changes detected in the B6;C3-*Opa1*^{Q285STOP} mouse are RGC dendritic changes leading to dysfunctional RGCs. However, it is not clear why the models should display such a difference in phenotype. The nature of the mutation and its precise effect on protein tertiary structure may be relevant. Both mutations give rise to a ca. 50% reduction in protein levels detected on western blots, suggesting that haploinsufficiency is an element in the pathophysiology. The c.1051C>T mutation is a nonsense change and truncates the protein immediately before the GTPase domain (B6;C3-*Opa1*^{Q285STOP}). It is not known what, if any, effects the truncated protein fragment may have on the overall function of the residual protein. The c.2065+5A>T change is a splice site mutation and gives rise to skipping of exon 10, B6;C3-*Opa1*$^{329\text{-}355del}$, and a polypeptide with an in-frame deletion in the GTPase domain. In addition, there are subtle differences between the two models in terms of genetic background, the degree of congenicity, as well as breeding and environmental factors. In this context it is recognised that there must be similar effect in patients.

There is evidence for both intra-familial as well as inter-familial variability in phenotype, and the same mutation in different families can result in varying degrees of clinical severity. Genotype/phenotype correlations in DOA are relatively weak (Yu-Wai-Man et al., 2010).

Over 200 mutations have so far been reported and some may be associated with 'plus' phenotypes (Yu-Wai-Man et al., 2010). While the Opa1 models display useful variation in the timing of disease onset, severity and progression, their data should be considered separately in view of their different genetic aetiologies. Work still needs to be done to determine the precise course of disease progression in the two models and the identification of early disease markers will be essential in order to allow the development of therapeutic intervention.

The mechanisms directly relating mitochondrial fusion defects and visual dysfunction remain obscure (Amati-Bonneau et al., 2009; Heiduschka et al., 2010; Olichon et al., 2006; Williams et al., 2010; Yarosh, Monserrate, & Tong, 2008). Hypotheses so far include the role of reactive oxygen species and decreased ATP levels and lipid peroxidation leading to ATPase dysfunction, as well as lower rates of Ca^{2+} sequestering, cytochrome c release and problems in mitochondrial transport and shuttling. These mechanisms need to be explored further in detail and their validity as therapeutic targets assessed.

Why are retinal ganglion cells so susceptible to defects in mitochondrial function? It may be related to mitochondrial distribution and mislocalization (Chen & Chan, 2006). Data show the accumulation of mitochondria in varicosities in the soma and unmyelinated, intraretinal axons of RGCs, with a relatively low population in the post-lamina myelinated axons, perhaps partly explaining why some areas of the RGC are affected while others are not (Olichon et al., 2006).

Finally, work is yet to be done on the terminal arbours of the retinal ganglion cells to see whether these also undergo degenerative changes similar to those seen in dendrites and to determine the extent to which these changes contribute to the global reduction in visual sensitivity. The existence of two *bona fide* mouse models for DOA provides a firm foundation for studies into the pathophysiology of this disease.

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