LETTER TO THE EDITOR

Reply: Parsing the differences in affected with LHON: genetic versus environmental triggers of disease conversion

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Sir,

Carelli and colleagues (2016) draw attention to an interesting feature of a figure that we published in a Brain article on the role of environmental factors in precipitating visual loss in Leber’s hereditary optic neuropathy (LHON), which is an important cause of inherited mitochondrial blindness (Kirkman et al., 2009a). Our original study involved 125 LHON families. One hundred and ninety-six affected and 206 unaffected LHON carriers harbouring one of three common mitochondrial DNA (mtDNA) mutations (m.11778A>G, m.3460A>G, and m.14484T>C) were recruited. All the participants took part in a standardized phone-based interview to collect information on lifestyle habits, in particular smoking and alcohol consumption. Overall, our study showed a strong consistent relationship between smoking and the risk of visual failure in LHON that was independent of gender and alcohol intake. Rather strikingly, the clinical penetrance of the pathogenic LHON mtDNA mutations was 93% in the males who smoked compared within the 50% overall lifetime risk generally quoted in the literature (Newman et al., 1991).

Figure 3 in our original study (Kirkman et al., 2009b) showed the proportion of LHON mutation carriers harbouring one of three common mitochondrial DNA (mtDNA) mutations (m.11778A>G, m.3460A>G, and m.14484T>C) were recruited. All the participants took part in a standardized phone-based interview to collect information on lifestyle habits, in particular smoking and alcohol consumption. Overall, our study showed a strong consistent relationship between smoking and the risk of visual failure in LHON that was independent of gender and alcohol intake. Rather strikingly, the clinical penetrance of the pathogenic LHON mtDNA mutations was 93% in the males who smoked compared within the 50% overall lifetime risk generally quoted in the literature (Newman et al., 1991).

Figure 3 in our original study (Kirkman et al., 2009b) showed the proportion of LHON mutation carriers that remained symptom-free as a function of age, stratified by their tobacco consumption, both cumulative (Fig. 3A) and the maximum intensity (Fig. 3B). Figure 3A showed an unexpected and unexplained feature that was noticed by reviewers of the manuscript, and subsequently highlighted in the accompanying Scientific Commentary (Newman, 2009). Curiously, for individuals under 50 years of age, the clinical penetrance of the pathogenic LHON mtDNA mutations appeared to be lower in those individuals who have had the highest cumulative smoking exposure up to that age. However, all other statistical analyses gave a consistent message that smoking was a major risk factor for precipitating visual failure in LHON. Of note, when considering maximum intensity of smoking (quantified in terms of the maximum number of cigarettes consumed in a single day), there was no difference between ‘light’ and ‘heavy’ smokers. Both groups of smokers had a significantly higher risk of visual loss compared with non-smokers, and no protective effect was noted in terms of a delay in the age of onset (Fig. 3B). At the time, we were somewhat reluctant to speculate on this isolated observation that ‘bucked the trend’, particularly given the inherent difficulties of measuring historical environmental exposures. However, Carelli and colleagues (2016) have now observed the same intriguing finding in a totally independent replication cohort of 134 affected and 126 unaffected LHON mutation carriers from 75 Italian families using the same study protocol detailed in our original study (Kirkman et al., 2009b). There must therefore be a rational explanation for these concordant observations, and we can no longer disregard the seemingly protective effect of heavy smoking in delaying the onset of visual loss as ‘unexplained noise’.
As mentioned by Nancy Newman (Newman, 2009) in her critical appraisal of our data, the counterintuitive protective benefit of heavy smoking in LHON has been observed previously (Kerrison et al., 2000), albeit to a less prominent extent, raising the possibly of a ‘survivor bias’. LHON has an age-dependent penetrance with a peak age of onset in the second and third decades of life, but with a sizable proportion of patients developing blindness after the age of 50 years. As a consequence, longer-lived individuals are more likely to develop visual failure (in absolute terms), whereas those who die younger are less likely to experience visual failure, even more so if they do not reach the danger zone of 20–30 years of age. Following the same argument, an older LHON mutation carrier will have accumulated a higher total smoking exposure by the time that visual loss eventually ensues. Heavy smoking is strongly associated with a shortened lifespan, thus providing a possible explanation for the peculiar survival trend highlighted in our original Fig. 3A (Kirkman et al., 2009b). In keeping with our hypothesis, no such paradoxical relationship was seen when the LHON mutation carriers were stratified based on maximum smoking intensity (Fig. 3B). For maximum intensity, hierarchy observed makes biological sense, with the risk of visual loss being highest among heavy smokers and intermediate among light smokers, but still significantly higher for both groups when compared against non-smokers. One could even argue that for the same cumulative amount, a higher dose of a particular mitochondrial toxin over a shorter period of time (i.e. maximum intensity) is much more likely to have a more potent detrimental effect on respiratory chain function and retinal ganglion cell survival than a more prolonged chronic exposure.

It should also be noted that we also observed a similar ostensibly protective effect for high cumulative alcohol consumption in our LHON study cohort (see Fig. 4A in Kirkman et al., 2009b), with a later age of onset among individuals who were heavy drinkers prior to disease conversion. As for heavy smoking, heavy alcohol consumption is associated with premature death, and this could have contributed to a similar artefactual survivor bias when analysing our data.

Lastly, Carelli and colleagues discuss the results of a follow-up article that we published on 20 patients with ‘late-onset’ LHON defined on the basis that they experienced visual loss after the age of 50 years (Dimitriadis et al., 2014). As expected, these 20 patients had significantly higher mean cumulative tobacco consumption compared with unaffected carriers. However, there was no significant difference between late-onset and more typical patients with onset of visual loss before the age of 50 years with regards to tobacco consumption. We can confirm that this cohort of 20 late-onset LHON patients was selected from the main study database detailed in our earlier Brain report (Kirkman et al., 2009b).

Based on the results of their replication cohort on the effects of smoking exposure in LHON, Carelli and colleagues (2016) provide an alternative explanation for the apparently later age of onset among LHON carriers who are heavy smokers. To set the scene for their arguments, they draw upon a series of clinical observations made on three affected brothers who were part of one branch of a large Brazilian pedigree harbouring the m.11778G>A LHON mutation. They argue cogently that some LHON mutation carriers possess additional genetic risk factors that cause a highly penetrant (pure genetic) disease presenting at a young age, irrespective of any external insult such as exposure to cigarette smoke (which they call ‘LHON type I’). On the other hand, LHON mutation carriers who do not harbour these as yet unidentified visual loss susceptibility genes have a lower absolute lifetime risk of visual failure at birth, but they are more susceptible to environmental triggers, such as smoking, leading to visual failure late in life (which they call ‘LHON type II’). Thus, the apparent protective effect of heavy smoking could be the result of a weaker genetic risk background that is insufficient to cause visual loss, but instead, depending on lifestyle choices, the cumulative exposure to several years of smoking is needed to tip the balance and trigger the onset of visual failure. Is such a categorical classification justified based on what we know about LHON? Although plausible, with obvious important implications for the identification of modifying genetic risk factors in LHON, a number of other possible explanations need to be considered.

First, most children do not smoke, but ~20% of LHON patients develop visual symptoms below the age of 10 years (Man et al., 2003). Secondly, despite being given the best lifestyle advice as part of genetic familial counselling, a good proportion of LHON mutation carriers continue to smoke throughout life, but many do stop after having gone through the catastrophic experience of severe visual loss in both eyes (Kirkman et al., 2009a). These two factors could have introduced unforeseen bias in both our study (Kirkman et al., 2009b), and the current work of Carelli and colleagues. A priori, affected LHON patients who smoke will inevitably be older than those who do not, and late-onset cases will probably have smoked for longer than those who have experienced visual loss earlier in life. This explanation provides an explanation for the overlapping age-of-onset distribution curves for the ‘type I’ and ‘type II’ patient groups (see Fig. 3A in Carelli et al., 2016). Third, we need to put into context the three affected brothers from the Brazilian LHON family. These three cases illustrate contrasting clinical patterns, including highly atypical features such as a more protracted period of visual loss extending over years, and visual recovery occurring nearly two decades after disease onset. While thought-provoking, this Brazilian family live in a specific microenvironment with exposure to various toxins, a more restricted diet, and seemingly a propensity to premature cardiac death (Sadun et al., 2003). Some care must therefore be taken when generalizing observations made on this family to the more typical LHON patient
population and natural history seen in the developed world (Riordan-Eva et al., 1995).

At present, we favour the more conservative explanation and our suspicion is that the apparent protective effect of heavy smoking (and drinking) is likely to be a statistical artefact. However, we cannot be sure, and we fully endorse the experimental approach proposed by Carelli and colleagues (2016) to test this hypothesis more rigorously. Studies in patient-derived LHON cell lines (Ghelli et al., 2009), and in a recently established animal model of LHON (Lin et al., 2012), will hopefully determine whether heavy smoking is protective or not, and crucially, clarify the disease mechanisms involved. These findings will have broader relevance given the well-established link between cigarette smoking and a reduced risk of Parkinson’s disease (Allam et al., 2004). Like LHON, Parkinson’s disease has also been associated with a defect of mitochondrial respiratory chain complex I, opening up avenues for therapeutic interventions (Mortiboy et al., 2015).

A pathogenic LHON mtDNA mutation is found in 1 of 300 healthy population controls (Elliott et al., 2008), and additional genetic and/or environmental factors must invariably contribute to the pathogenesis of retinal ganglion cell death and optic nerve degeneration. As with other complex human traits, ‘extreme phenotypes’—such as very early age at onset—may well have a stronger genetic basis. However, a strict distinction between ‘type I’ and ‘type II’ LHON remains arbitrary at present, and this classification is unlikely to be helpful in the clinic, not least because the categorization can only be done after the onset of symptoms. There is also no evidence to suggest that ‘type I’ and ‘type II’ patients with LHON differ in terms of their disease progression and level of visual impairment (Kirkman et al., 2009a; Dimitriadis et al., 2014). As Carelli and colleagues illustrate beautifully with these three affected Brazilian brothers, the possible role of external precipitating factors can appear to vary considerably within a small family, and more detailed epidemiological studies on larger patient cohorts are required to resolve these issues. Putting aside these gaps in our knowledge, for all LHON mutation carriers, affected or unaffected, the message remains the same: stop smoking.

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**References**


