K320E-twinkle^{skin} Mice Are Genetically Heterogeneous for Secondary mtDNA Deletions Impairing Comparison With Controls

With interest we read the article by Oexner et al.[1] about a study of five pairs of extraocular eye muscles (EOMs) by means of histochemistry in a mouse model of progressive external ophthalmoplegia (PEO) caused by the twinkle mutation K320E (K320-twinkle^{skin}) secondarily inducing mtDNA deletions. It was found that intrinsic factors or molecular mechanisms in type IIb fibers predispose for increased generation, clonal expansion, and detrimental effects of single mtDNA deletions.[1] We have the following comments and concerns.

The main shortcoming of the study is that the same twinkle mutation triggered mtDNA deletions of different sizes (deletion 1, deletion 3, deletion 13, deletion 17) with variable breakpoints, implying that the investigated cohort was genotypically heterogeneous with regard to mtDNA size and breakpoints. Because even the same twinkle mutation may additionally have a variable effect on the amount and number of mtDNA deletions over time, phenotypic heterogeneity may further increase. Thus we need to know whether breakpoints and extension of the mtDNA deletions were the same or different between mutant mice. If each individual carried an individual mtDNA deletion, it is quite likely that the phenotype differed between the animals. Genotypic and phenotypic heterogeneity caused by variable size of secondary mtDNA deletions with different breakpoints impair comparison between mutated mice and controls and may produce nonreliable results.

A further shortcoming is that heteroplasmy rates of the mtDNA deletions were not provided. Knowing breakpoints, extension of mtDNA deletions, and heteroplasmy rates is crucial for establishing a genotype/phenotype correlation. We should also know whether secondary mtDNA deletions caused by mutations in the twinkle gene were single deletions or multiple deletions.

Because the amount of mtDNA deletions increased during a 12-month observational period, we should know whether these increase correlated with worsening of clinical manifestations. From humans it is known that the phenotype of PEO is usually slowly progressive and that in addition to the EOMs, other organs or tissues can also be affected, including the brain, ears, endocrine organs, gastrointestinal tract, myocardium, blood cells, or the nerves (PEO plus).[2] We should know whether mutant mice were systematically investigated for affection of organs or tissues other than the EOMs. Recognizing the multiorgan nature of the disease is crucial because it may strongly determine the disease course and outcome of affected individuals.

How do the authors explain that COX-negative fibers were more prevalent in the global layer of the EOMs compared to the orbital layer of the EOMs? Is this simply due to the variable fiber composition of these two layers, or are they variably stressed during eye movements?

We do not agree with the notion that "COX deficiency alters the fiber type composition of EOMs during aging, with the more susceptible type IIb fibers undergoing a preferential decline."[1] Because mtDNA deletions increase over time,[1] it is more likely that the increasing amount of mtDNA deletions drives the reduction of type IIb fibers. We also do not agree with the notion that early slow saccades in PEO are due to selective damage of type IIb or type IIX fibers. Slow saccades may also have a cerebral component, which is why it is crucial that any cerebral involvement be excluded before attributing this finding solely to muscle fiber composition of EOMs.

Overall, the interesting study has a number of shortcomings that should be addressed before drawing final conclusions. Results would be more reliable if the investigated cohorts were genotypically more homogenous.

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References

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