

LETTER TO THE EDITOR

The expanding neurological phenotype of *DNM1L*-related disorders

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Sir,

We read with interest the findings of Gerber *et al.* (2017) detailing the observation that three large multi-generational families with dominant optic atrophy appear to have dominant segregating *DNM1L* missense alleles (Gerber *et al.*, 2017). The phenotypic similarity to human subjects with *OPA1* alleles is interesting given the role of both *DNM1L* and *OPA1* in mitochondrial dynamics, although *OPA1* and *DNM1L* are involved in opposed aspects, mitochondrial fusion and fission, respectively. Indeed monogenic examples of additional loci tying altered mitochondrial dynamics with optic atrophy have recently been uncovered, e.g. *ATAD3A* is implicated in optic atrophy as well as in severely altered mitochondrial dynamics (Harel *et al.*, 2016). The observation of dominant optic atrophy and segregating *DNM1L* alleles represents the first observation of a dominant inherited disease due to *DNM1L* mutations as the other dominant cases have been due to *de novo* events or from parents with low-level mosaicism. Optic atrophy is a dramatically milder phenotype than all previously published *DNM1L* related cases. The authors provide a review of 'all cases previously reported', but fail to include an infant with a p.G350R variant, a patient that exhibited an early onset neurodegenerative disease with optic atrophy (Chao *et al.*, 2016). The latter study used model organism studies to demonstrate that the p.G350R corresponds to a dominant negative allele and is one of a handful of heterozygous alleles experimentally demonstrated to be dominant negative (Fig. 1A).

The genetic mechanism as to how these novel *DNM1L* missense alleles reported by Gerber *et al.* cause human disease remains to be explored. The authors propose a 'restricted dominant negative' effect based on fibroblast analyses, and they state that they base this on the observation that the protein levels are not affected by the variants, and the ability of the mutant to polymerize with wild-type or mutant *DNM1L* appears intact. However, it is not obvious to us why these mutations are not loss- or gain-of-function mutations, as the function of the protein can be affected without impacting the levels. Given that there are three general mechanisms to explain dominant inheritance, haploinsufficiency, dominant negative, and gain of function, these three possibilities should be explored further. The parallels between the human subjects and the haploinsufficient mouse model provide support for the haploinsufficiency model rather than the dominant negative model proposed by the authors. If haploinsufficiency is indeed the cause, we would predict that the parents of the reported cases in Nasca *et al.* (2016) and Yoon *et al.* (2016) would be at risk of developing similar optic atrophy and could become symptomatic. Unfortunately, this information is not currently available.

A subtle gain of function could be considered as the phenotypes are consistent with what has been reported with *OPA1*. In the case of *OPA1*, haploinsufficiency of a gene required for mitochondrial fusion would be consistent with a gain of function of *DNM1L* as that would lead to increased fission of mitochondria. However, the observed

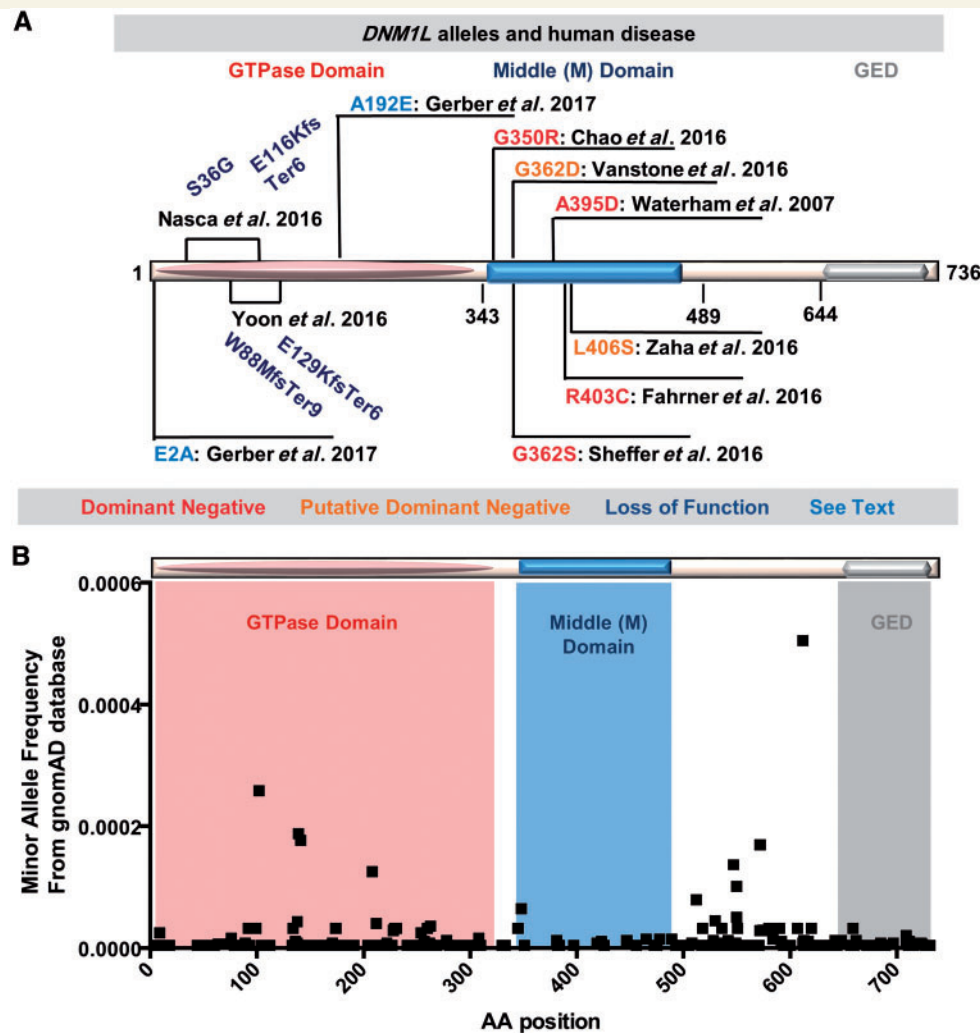


Figure 1 *DNMI1* alleles in humans. (A) *DNMI1* alleles associated with human disease and their effects on gene function. Red: dominant negative heterozygous mutations associated with early onset disease such as encephalopathy due to defective mitochondrial and peroxisomal fission-I (EMPF1) and experimentally validated in cells or animal models as dominant negative alleles. Orange: putative dominant negative heterozygous mutations associated with early disease. Dark blue: Compound heterozygous mutations resulting in loss of function in cases with bi-allelic variants in *DNMI1*. Light blue: heterozygous mutations associated with isolated optic atrophy reported by Gerber *et al.* (B) A graph of minor allele frequency on the y-axis versus the encoded amino acid position in the *DNMI1* protein on the x-axis for all the missense allele calls available in the gnomAD (gnomad.broadinstitute.org) a database of > 120 000 individuals. An approximate overlay of the domains encoded by the amino acids for the GTPase, middle domain and GED (effector domain) are shown in salmon, blue and grey, respectively.

increased mitochondrial network in the patients' fibroblasts from these optic atrophy families would argue against a gain of function mutation.

Third, a dominant negative function is also possible, and as noted this is what the authors favour. However, it should be noted that these cases are much more subtle than the severe human phenotypes that have been observed with the already reported *DNMI1* dominant negative alleles (Fahrner *et al.*, 2016; Sheffer *et al.*, 2016; Vanstone *et al.*, 2016; Zaha *et al.*, 2016) (Fig. 1A). These possibilities have not yet been explored sufficiently to conclude that the mutations described here are subtle dominant negative mutations.

Several genetic mechanisms are currently known for *DNMI1* in human disease. In four reported individuals

from two families, a progressive infantile disorder resulting from biallelic loss-of-function pathogenic variants in *DNMI1* has been observed. In these cases the parents were reportedly heterozygous carriers without known disease at the time of the reports (Nasca *et al.*, 2016; Yoon *et al.*, 2016). In a number of other cases *de novo* variants (Waterham *et al.*, 2007; Fahrner *et al.*, 2016; Sheffer *et al.*, 2016; Vanstone *et al.*, 2016; Zaha *et al.*, 2016), or variants inherited from a mosaic parent (Chao *et al.*, 2016) have been reported in individuals with a range of severe metabolic and neurological disease. The study by Gerber *et al.* is the first report of dominant inherited disease due to *DNMI1*. If the optic atrophy in these individuals were in fact due to *DNMI1* loss-of-function then the implication is that the parents of individuals with biallelic disease could

have milder disease as well. Indeed, the individuals Gerber reports were often asymptomatic when ascertained earlier in life (Gerber *et al.*, 2017).

One possible explanation is that the authors are correct and these missense alleles are all dominant negative but have different functional impact depending on what protein domain is affected. We had previously observed that for *DNM1L* the middle domain appears to have a paucity of missense variation in genomic databases from more general human populations (Chao *et al.*, 2016) suggesting this region is relatively intolerant to missense change. With the availability of genomic data on over 120 000 individuals in the gnomAD database (Lek *et al.*, 2016) a similar picture emerges of rare missense variation with few alleles and in particular the middle domain and GED domain appear intolerant to missense changes in the population at least based on the allele frequencies in comparison to the protein domain where missense changes occur (Fig. 1B). The middle domain is responsible for protein assembly and the molecular mechanism for how missense alleles in the middle domain can produce dominant negative effects has been established and relates to the assembly of *DNM1L* oligomers (Chang *et al.*, 2010).

The impact on mitochondrial and peroxisomal morphology has also been explored *in vivo* and the dominant negative middle domain variants affect these organelles dramatically (Waterham *et al.*, 2007; Chao *et al.*, 2016). In contrast, the GTPase domain is not implicated in oligomerization or assembly *per se* and dominant negative GTPase alleles have not been previously observed in humans. Dominant negative GTPase alleles for *DNM1L* have been generated (Li and Gould, 2003). A simple overexpression of the variants found in the patients in cell culture or in model organisms would resolve the dominant negative versus haploinsufficiency question.

The clarification of the functional impact has implications for the individuals and family members, especially those that carry truncating alleles. In the previous reports of autosomal recessive disease due to *DNM1L* (Nasca *et al.*, 2016; Yoon *et al.*, 2016), healthy unaffected parents have been reported carrying heterozygous loss-of-function alleles. If haploinsufficiency of *DNM1L* can cause optic atrophy as suggested by the mouse model, these parents would be at risk for optic atrophy and should potentially be screened. In addition, a number of potential individuals in the general population may exhibit *DNM1L* heterozygous loss-of-function alleles. However, if the authors' hypothesis of restricted dominant negative mechanisms is correct, there could be therapeutic implications. Advances in oligonucleotide-based therapies for neurodegenerative disease would

theoretically allow the specific alleles to be targeted for degradation (Schoch and Miller, 2017). In either case, we propose that determining genetic mechanisms will require robust cellular and model organism studies that account for genetic mechanisms in the models and the specific variants in all the human cases.

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