Case Report

The Genetic Puzzle of a SOD1-Patient with Ocular Ptosis and a Motor Neuron Disease: How Many Variants Contribute to the Phenotype?

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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with a complex genetic architecture, showing monogenic, oligogenic, and polygenic inheritance. In this study, we describe the case of a 71 years-old man diagnosed with ALS with an atypical phenotype, due to the presence of a progressive ocular ptosis. Genetic analyses revealed two variants in *SOD1* and *TBK1* genes respectively and the LHON-associated m.14484T>C variant in the mitochondrial DNA (mtDNA). We discuss how all these variants may synergically impinge on mitochondrial function, contributing to the pathogenic mechanisms which might ultimately lead to the neurodegenerative process, possibly influencing the clinical ALS phenotype.

Keywords: *SOD1*; amyotrophic lateral sclerosis; *TBK1;* mitochondrial DNA; oligogenic inheritance

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease conventionally classified as familial or sporadic. However, besides this simple subdivision, the genetic architecture underlying the disease's pathogenesis is highly complex, showing monogenic, oligogenic, and polygenic inheritance, with variable gene penetrance and heritability [1].

Monogenic familial amyotrophic lateral sclerosis accounts for 10–15% of affected individuals, albeit with incomplete penetrance [2]. In the remaining 85%, large genome-wide association studies (GWAS) have been useful to identify rare or private variants that might act as risk factors and/or disease modifiers, thus modulating phenotypic presentation [2].

Nowadays, ALS' pathogenesis is considered as a multi-step disease process, where multiple hits, both genetic and environmental, are needed to develop the disease [3]. Furthermore, several observations proved that the burden of multiple genetic rare variants might trigger the degenerative process, modulating also the clinical phenotype [4].

Recently, a large study on ALS [5] pointed to the burden of multiple risk factors disclosed in the nuclear genome but missed to consider the impact of mitochondrial DNA (mtDNA) variation, which is frequently neglected but may contribute to the pathogenesis or modulate the phenotype also in ALS. As an example, we reported the unique association of ALS and Leber's hereditary optic neuropathy (LHON) in two unrelated patients who had a late onset ALS with rapid diseases course, speculating that mtDNA might have contributed as a possible risk factor or disease modifier in ALS [6].

Here we report a new case of a *SOD1* patient carrying an additional variant in *TBK1* gene and the LHON-associated m.14484T>C variant in the mtDNA. This case supports the oligogenic nature of ALS and further rises questions on the possible contributory role of mtDNA variation in the ALS pathogenesis and clinical expression.

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2. Case presentation

A 71-years-old man came to our attention for the progressive onset of weakness in his legs, started three years before, and worsened in the past year, leading to the use of aids in walking. Since the age of 58, he also reported progressive bilateral eyelid droop, without clear daily fluctuation.

His past history was relevant for hypertension under pharmacological treatment. From the age of 54, he also suffered from a progressive hearing loss, interpreted as mixed (transmissive and sensory) and partially related to otosclerosis, for which he underwent surgery, without any improvement.

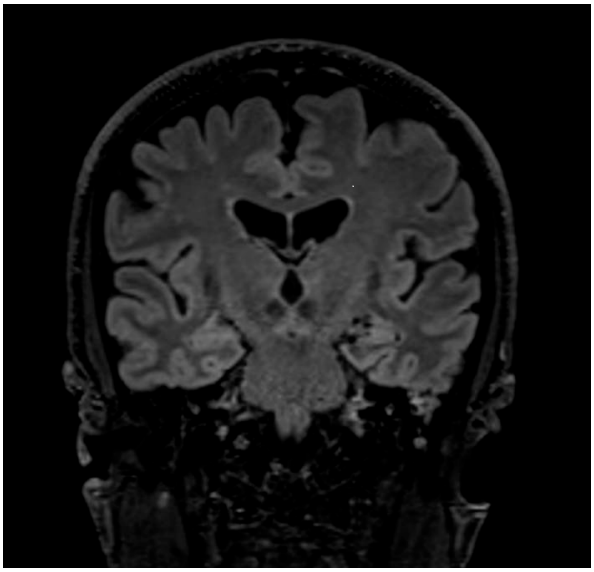
In his family history, he had a brother with progressive hearing loss (started at 55 years), and unspecified gait disturbances associated with increased creatin kinase (CK) levels. The patient did not report any other neurological disease recurring in his family.

The neurological examination revealed bilateral severe ocular ptosis without deficits in ocular movements, bilateral hypoacusia, hyposthenia and hypotrophy in the lower limbs, especially in the distal compartment on the right side. Deep tendon reflexes were diffusely reduced. Gait showed right drop foot with steppage. Exacerbation of ptosis after effort or repeated closing of the eyes was not noted.

Due to the association of hypoacusia and bilateral ptosis, a mitochondrial disorder was suspected.

Routine blood exams only showed an increase of CK (367 U/L, n.v. < 170). Antibodies against acetylcholine receptor were negative.

Brain Magnetic Resonance revealed bilateral fronto-parietal atrophy and mild signs of microangiopathy. FLAIR-T2-weighted sequence displayed hyperintensity of the cortico-spinal tracts (Figure 1a,b). Spectroscopy did not reveal pathological lactate in ventricles.



(a)

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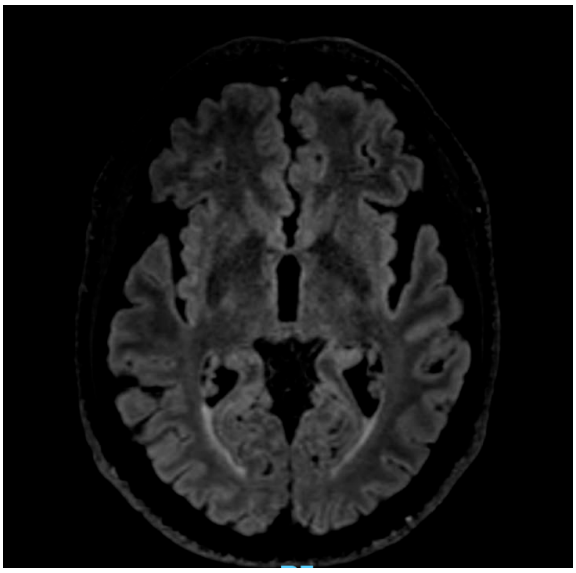


(b)

Figure 1. Brain Magnetic Resonance revealed bilateral fronto-parietal atrophy; coronal (a) and axial (b) FLAIR-T2-weighted sequences displayed hyperintensity of the cortico-spinal tracts (arrows).

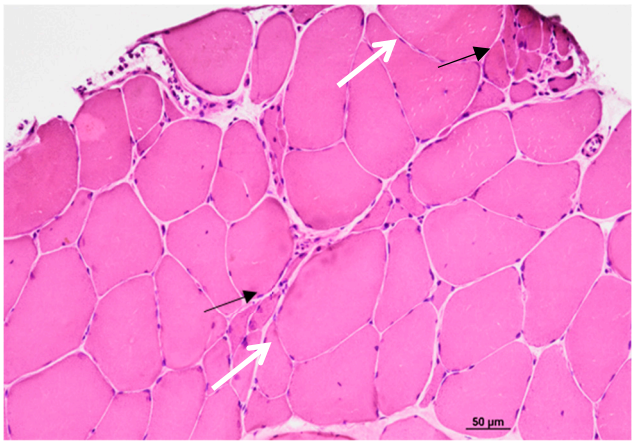
The optic coherence tomography (OCT) showed normal findings.

Similarly, the cerebrospinal fluid analysis revealed normal proteins and cells levels.

Electromyography (EMG) showed the presence of subacute neurogenic changes in the lower limbs (right > left), in particular in right vastus medialis, left gastrocnemius and bilateral tibialis anterior muscles. Genioglossus, right biceps brachialis and left first dorsal interosseous were normal.

These findings raised the suspicion of a motor neuron disease.

The tibialis anterior muscle biopsy confirmed the presence of neurogenic changes (Figure 2a), without clear signs of a primary mitochondrial disease, except for a few fibers displaying subsarcolemmal enhancement and a single COX negative fiber observed with the double COX/SDH staining, both probably due to a secondary defect (Figure 2b).



(a)

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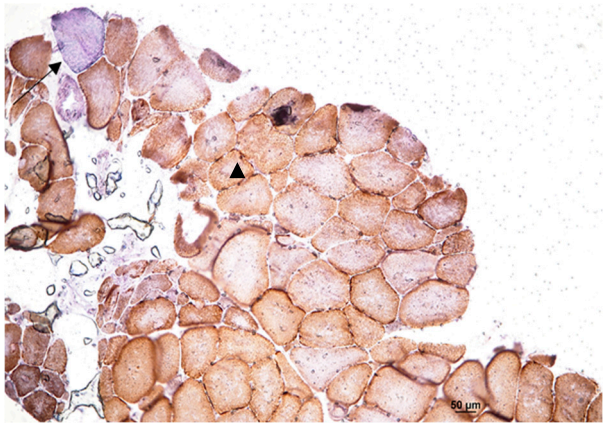

(b)

Figure 2. Hematoxilin Eosin staining (a) showed neurogenic changes with small groups of atrophic fibers (arrows). Cytochrome C Oxidase/Succinate dehydrogenase double staining (b) showed a few fibers displaying subsarcolemmal enhancement (head of arrow) and a single COX negative fiber (arrow).

Genetic analysis, through Whole Exome Sequencing (WES) with an *in silico* panel analysis, was initially focused on the fronto-temporal dementia (FTD)-ALS related genes (*SOD1, FUS, TARBP, SEXT, ANXA11, CHCHD10, DTCN1, FIG4, HNRNPA1, KIF5A, MATR3, NEK1, OPTN, PFN1, SIGMAR1, SPG11, SQSTM1, TUBA4A, UBQLN2, VAPB, VCP*), and revealed the presence of the heterozygous variant c.412A>G (p.Thr138Ala) in the *SOD1* gene (NM_000454.5), and the heterozygous variant c.422T>C (p.Ile141Thr) in the *TBK1* gene (NM_013254.4). The presence of expanded alleles in the *C9Orf72* gene was also excluded.

The heterozygous p.Thr138Ala variant in the *SOD1* gene (OMIM*147450), which was not reported in the gnomAD v.2.1.1 database, was classified as pathogenic (PP5, PP3, PM2, PM5, PM1, PP2 criteria) according to the American College of Medical Genetics (ACMG) guidelines (Richards et al., 2015). Relevantly, the p.Thr138Ala could be a likely founder variant, since it was reported in three other Italian patients with ALS, supporting its pathogenic role [7-9].

The heterozygous p.Ile141Thr in the *TBK1* gene (OMIM*604834) was a very rare variant (1/250,400 alleles in gnomAD) and was classified as hot VUS (PP3, PM2) according to the ACMG guidelines. This gene has been associated to ALS and/or frontotemporal dementia with an autosomal dominant inheritance (MIM#616439).

Therefore, a diagnosis of motor neuron disease with a selective involvement of lower motor neurons (progressive muscular atrophy, PMA) was made.

Due to the progressive bilateral ptosis, atypical for a motor neuron disease, we also screened a panel of genes associated to chronic progressive external ophthalmoplegia (CPEO) (*ABAT, AFG3L2, C10orf2, DGUOK, DNA2, DNM1L, FBXL4, GFER, MFN2, MGME1, MPV17, OPA1, POLG, POLG2, RNASEH1, RRM2B, SLC25A10, SPG7, SUCLA2, SUCLG1, TFAM, TK2, TOP3A, TYMP*) that resulted negative as well as a panel of 176 genes associated with non-syndromic hearing loss and deafness (data not shown).

Finally, to further investigate the CPEO phenotype, we analyzed the mtDNA extracted from skeletal muscle. Quantitative testing for pathologic accumulation of single/multiple macrodeletions was normal and the mtDNA copy number assessment showed an increase of around 30% compared to controls. The complete mtDNA sequencing revealed the presence of the Leber's hereditary optic neuropathy (LHON) associated homoplasmic variant m.14484T>C/*MT-ND6* on a H1a3c haplogroup background.

Neurofilament light chain levels in the CSF were also investigated and were significantly increased (5822 pg/ml, v.n. 340-650).

The patient's clinical conditions continued to progress over the years: at a 2-years follow-up he was not able to stand or walk autonomously anymore, and he also showed a moderate hypotrophy and weakness in the upper limbs with functional deficits; no bulbar involvement was detected.

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3. Discussion

Ocular ptosis is not a classic symptom of ALS, although it has been rarely reported [10-12]. Intriguingly, De Marchi et al. reported two siblings with a history of progressive ptosis without ocular movement impairment, diagnosed with a bulbar ALS with rapid progression [12]. Thus, notwithstanding the rarity, our case is not unprecedented. To disclose the possible genetic cause, we first aimed at screening the major genes associated with ALS and FTD, as well as searching for expansion in *C9Orf72*, revealing a combination of two different genetic variants of interest.

To further refine the genetic investigation, in consideration of the ptosis and sensorineural deafness affecting our patient pointing to a possible mitochondrial disease, we also screened mtDNA extracted from the muscle biopsy. Analyses for mtDNA single/multiple deletions was within normal range. Sequencing the entire mtDNA revealed as *incidental* finding the homoplasmic pathogenic variant m.14484T>C/*MT-ND6*, on a haplotype H1a3c background. The copy number was higher than control range, in agreement with the compensatory mechanism already described in patients carrying LHON pathogenic variants [13] and congruently to the occasional subsarcolemmal increase of COX/SDH staining. The m.14484T>C/*MT-ND6* variant is causative of LHON, but was never described in CPEO phenotypes, and therefore cannot explain by itself the ocular ptosis of our patient. Furthermore, our patient did not show any signs of optic neuropathy, and his family history was negative for cases suggestive of LHON on the maternal lineage. This is not surprising, as is well established that the m.14484T>C/*MT-ND6* variant expresses the LHON phenotype principally in the context of the haplogroup J [14] and has been frequently found in population screenings without being linked to LHON [15]. In fact, as recently highlighted, the LHON m.11778G>A/*MT-ND4* and m.14484T>C/*MT-ND6* variants have been both found to be present in 1 every 800-1000 individuals from normal populations, associated with low penetrance mtDNA backgrounds such as haplogroups H and U [16,17], thus reinforcing the idea that deleterious mtDNA variants may be incidentally found when investigating patients for a neurodegenerative disorder.

Overall, the PMA phenotype, classically characterized by a prevalent dysfunction of the lower motor neuron, and the quite long disease duration of our case could be mainly explained by the *SOD1* variant, already found in patients with a spinal onset, absence of bulbar involvement and a slow disease progression, with a variable age at onset (mean 53 years, ranging from 35 to 73 years) [7-9]. On the other hand, the co-occurrence of another variant in an ALS-FTD gene, the *TBK1* gene, is consistent with the oligogenic and complex background underlying both sporadic and familial ALS [1]. *TBK1* has been reported to contribute to around 1.3% ALS, 3%–4% ALS-FTD, and <1% FTD with TDP-43 pathology [18]. Among the patients with *TBK1* mutations, over 50% patients were diagnosed as ALS, as well as 18% FTD, 14% FTD-ALS, and 1.3% AD [19]. The clinical manifestations are highly heterogeneous, and intrafamilial and interfamilial heterogeneity were also reported in patients carrying the same variants [20]. The most common initial symptoms included limb weakness, cognitive deficits, and bulbar signs. This extreme heterogeneity makes genetic-phenotype correlations quite challenging.

Furthermore, this case, in particular the uncertain role of the mtDNA m.14484T>C/*MT-ND6* pathogenic variant, deserves a few more comments. We previously reported the unprecedented occurrence in two unrelated patients with ALS of another LHON homoplasmic mutation, the most common m.11778G>A/*MT-ND4* change, associated with optic atrophy in only one of the two patients. We speculated that the mtDNA could have played a role as disease modifier in these patients, as they were characterized by late-onset ALS and rapid course to death [6]. Our current findings in this other patient remark that mtDNA variants in ALS are probably more frequent than expected and deserve to be systematically explored in conjunction with the nuclear genome analysis, to better evaluate their possible role in the disease pathogenesis, penetrance and clinical evolution. In support, rare cases of ALS patients with causative mtDNA mutations and clear signs of mitochondrial disease have been also reported [21].

Remarkably, all genes found in our patient are known to play an essential role in mitochondrial function. First, the Cu/Zn superoxide dismutase 1 protein (SOD1) is an abundantly expressed antioxidant enzyme that exists as a homodimer and localizes to the cytosol, but also in the

intermembrane mitochondrial space, whereas the manganese superoxide dismutase 2 (SOD2) is in the mitochondrial matrix [22]. Besides the antioxidant properties, it has been implicated in signal transduction, and both its wild-type and mutant forms are prone to misfolding [23,24]. Therefore, dysfunctional SOD1 may lead to increased reactive oxygen species (ROS) levels, but also to the formation of insoluble aggregates, associated with the mitochondrial outer membrane, of both perturbated and wild-type forms. It is already known that in the SOD1-G93A mouse model mitochondria appeared with dilated and disorganized cristae, both in the axons and dendrites of motor neurons at onset of disease [25]. Changes in mitochondrial morphology, such as swelling or enlargement, were also found in soma, proximal axons and motor nerve terminals in tissue from ALS patients [26], which result in an impairment of their axonal transport in motor neurons [27]. Since morphological abnormalities in mice appeared before the onset of symptoms of motor neuron degeneration, it has been postulated that mitochondria impairment may play a key role in starting motor neuron degeneration in ALS, although the exact pathological mechanism remains unclear.

On the other hand, the NF-kappa-B-activating kinase encoded by the *TBK1* gene plays a critical role in several cellular pathways, including selective clearance of mitochondria and regulation of inflammation. Indeed, this kinase binds to and phosphorylates a number of proteins involved in innate immunity and autophagy, including optineurin (OPTN) and p62, both of which have been implicated in ALS. In details, TBK1-OPTN axis targets damaged mitochondria for degradation via PINK1/parkin-mediated mitophagy [28]. Functional studies revealed that mutations disrupting the structure of the protein impair the recruitment of TBK1 to damaged mitochondria, inhibiting the mitophagy process [29]. Moreover, primary neurons expressing TBK1 mutations demonstrated higher baseline levels of mitochondrial stress and an inability to manage induced oxidative damage, both of which may contribute to neurodegeneration [30].

To conclude, as primary mtDNA defects such as the m.14484T>C/*MT-ND6* variant are well known to impair the activity of complex I, leading to a decrease in ATP synthesis and an increasing generation of ROS [31,32], we propose as plausible that all variants identified in this patient may contribute to mitochondrial dysfunction and motor neuron degeneration, possibly leading to the atypical phenotype of our patient. This case illustrates how multiple nuclear and mitochondrial variants may ultimately contribute to the neurodegenerative process, with a leading role for mitochondrial dysfunction in ALS pathogenesis. A systematic parallel analysis of nuclear encoded risk factors for ALS in conjunction with mtDNA sequence analysis is warranted in large cohort studies to fully clarify this possible missing genetic contribution to ALS pathogenesis.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Area Vasta Emilia Romagna (CE-AVEC -17151-17152).

Informed Consent Statement: Written informed consent has been obtained from the patient to publish this paper.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article

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References

1. Goutman SA, Hardiman O, Al-Chalabi A, Chió A, Savelieff MG, Kiernan MC, Feldman EL. Emerging insights into the complex genetics and pathophysiology of amyotrophic lateral sclerosis. Lancet Neurol. 2022 May;21(5):465-479. doi: 10.1016/S1474-4422(21)00414-2.
2. Ryan M, Heverin M, McLaughlin RL, Hardiman O. Lifetime Risk and Heritability of Amyotrophic Lateral Sclerosis. JAMA Neurol. 2019 Nov 1;76(11):1367-1374. doi: 10.1001/jamaneurol.2019.2044.
3. Al-Chalabi A, Calvo A, Chio A, Colville S, Ellis CM, Hardiman O, Heverin M, Howard RS, Huisman MHB, Keren N, Leigh PN, Mazzini L, Mora G, Orrell RW, Rooney J, Scott KM, Scotton WJ, Seelen M, Shaw CE, Sidle KS, Swingler R, Tsuda M, Veldink JH, Visser AE, van den Berg LH, Pearce N. Analysis of amyotrophic lateral sclerosis as a multistep process: a population-based modelling study. Lancet Neurol. 2014 Nov;13(11):1108-1113. doi: 10.1016/S1474-4422(14)70219-4.
4. van Blitterswijk M, van Es MA, Hennekam EA, Dooijes D, van Rheenen W, Medic J, Bourque PR, Schelhaas HJ, van der Kooi AJ, de Visser M, de Bakker PI, Veldink JH, van den Berg LH. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. Hum Mol Genet. 2012 Sep 1;21(17):3776-84. doi: 10.1093/hmg/dds199. Epub 2012 May 29.
5. van Rheenen W, van der Spek RAA, Bakker MK et al (2021) Common and rare variant association analyses in amyotrophic lateral sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specifc biology. Nat Genet 53(12):1636–1648. https://doi.org/10.1038/s41588-021-00973-1
6. Amore G, Vacchiano V, La Morgia C, Valentino ML, Caporali L, Fiorini C, Ormanbekova D, Salvi F, Bartoletti-Stella A, Capellari S, Liguori R, Carelli V. Co-occurrence of amyotrophic lateral sclerosis and Leber's hereditary optic neuropathy: is mitochondrial dysfunction a modifier? J Neurol. 2023 Jan;270(1):559-564.
7. Zhang M, Xi Z, Ghani M, Jia P, Pal M, Werynska K, Moreno D, Sato C, Liang Y, Robertson J, Petronis A, Zinman L, Rogaeva E. Genetic and epigenetic study of ALS-discordant identical twins with double mutations in SOD1 and ARHGEF28. J Neurol Neurosurg Psychiatry. 2016 Nov;87(11):1268-1270. doi: 10.1136/jnnp-2016-313592.
8. Visani M, de Biase D, Bartolomei I, Plasmati R, Morandi L, Cenacchi G, Salvi F, Pession A. A novel T137A SOD1 mutation in an Italian family with two subjects affected by amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2011 Sep;12(5):385-8. doi: 10.3109/17482968.2011.582648.
9. Origone P, Caponnetto C, Verdiani S, Mantero V, Cichero E, Fossa P, Bellone E, Mancardi G, Mandich P. T137A variant is a pathogenetic SOD1 mutation associated with a slowly progressive ALS phenotype. Amyotroph Lateral Scler. 2012 Jun;13(4):398-9. doi: 10.3109/17482968.2012.684216. PMID: 22632445.
10. Pinto S, de Carvalho M. Amyotrophic lateral sclerosis patients and ocular ptosis. Clin Neurol Neurosurg. 2008 Feb;110(2):168-70. doi: 10.1016/j.clineuro.2007.08.022.
11. Shindo K, Satake A, Fukao T, Ichinose Y, Takiyama Y. Palpebral ptosis as the initial symptom of amyotrophic lateral sclerosis. Neurol Sci. 2020 Jan;41(1):211-212. doi: 10.1007/s10072-019-04030-y.
12. De Marchi F, Corrado L, Bersano E, Sarnelli MF, Solara V, D'Alfonso S, Cantello R, Mazzini L. Ptosis and bulbar onset: an unusual phenotype of familial ALS? Neurol Sci. 2018 Feb;39(2):377-378. doi: 10.1007/s10072-017-3186-0. Epub 2017 Nov 13.
13. Giordano C, Iommarini L, Giordano L, Maresca A, Pisano A, Valentino ML, Caporali L, Liguori R, Deceglie S, Roberti M, Fanelli F, Fracasso F, Ross-Cisneros FN, D'Adamo P, Hudson G, Pyle A, Yu-Wai-Man P, Chinnery PF, Zeviani M, Salomao SR, Berezovsky A, Belfort R Jr, Ventura DF, Moraes M, Moraes Filho M, Barboni P, Sadun F, De Negri A, Sadun AA, Tancredi A, Mancini M, d'Amati G, Loguercio Polosa P, Cantatore P, Carelli V. Efficient mitochondrial biogenesis drives incomplete penetrance in Leber's hereditary optic neuropathy. Brain. 2014 Feb;137(Pt 2):335-53. doi: 10.1093/brain/awt343.
14. Hudson G, Carelli V, Spruijt L, Gerards M, Mowbray C, Achilli A, Pyle A, Elson J, Howell N, La Morgia C, Valentino ML, Huoponen K, Savontaus ML, Nikoskelainen E, Sadun AA, Salomao SR, Belfort R Jr, Griffiths P, Yu-Wai-Man P, de Coo RF, Horvath R, Zeviani M, Smeets HJ, Torroni A, Chinnery PF. Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. Am J Hum Genet. 2007 Aug;81(2):228-33. doi: 10.1086/519394.
15. Bolze A., Mendez F., White S., Tanudjaja F., Isaksson M., Rashkin M., Bowes J. er al. Selective constraints and pathogenicity of mitochondrial DNA variants inferred from a novel database of 196, 554 unrelated individuals. bioRxiv. 2019; (Preprint at) https://doi.org/10.1101/798264

16. Watson EC, Davis RL, Ravishankar S, Copty J, Kummerfeld S, Sue CM. Low disease risk and penetrance in Leber hereditary optic neuropathy. Am J Hum Genet. 2023 Jan 5;110(1):166-169. doi: 10.1016/j.ajhg.2022.11.013.
17. Mackey DA, Ong JS, MacGregor S, Whiteman DC, Craig JE, Lopez Sanchez MIG, Kearns LS, Staffieri SE, Clarke L, McGuinness MB, Meteoukki W, Samuel S, Ruddle JB, Chen C, Fraser CL, Harrison J, Howell N, Hewitt AW. Is the disease risk and penetrance in Leber hereditary optic neuropathy actually low? Am J Hum Genet. 2023 Jan 5;110(1):170-176. doi: 10.1016/j.ajhg.2022.11.014.
18. Nguyen HP, Van Broeckhoven C, van der Zee J. ALS Genes in the Genomic Era and their Implications for FTD. Trends Genet. 2018 Jun;34(6):404-423. doi: 10.1016/j.tig.2018.03.001.
19. Yu H, Yu W, Luo SS, Yang YJ, Liu FT, Zhang Y, Chen Y, Sun YM, Wu JJ. Association of the TBK1 mutation p.Ile334Thr with frontotemporal dementia and literature review. Mol Genet Genomic Med. 2019 Mar;7(3):e547. doi: 10.1002/mgg3.547.
20. Freischmidt A, Wieland T, Richter B, Ruf W, Schaeffer V, Müller K, Marroquin N, Nordin F, Hübers A, Weydt P, Pinto S, Press R, Millecamps S, Molko N, Bernard E, Desnuelle C, Soriani MH, Dorst J, Graf E, Nordström U, Feiler MS, Putz S, Boeckers TM, Meyer T, Winkler AS, Winkelman J, de Carvalho M, Thal DR, Otto M, Brännström T, Volk AE, Kursula P, Danzer KM, Lichtner P, Dikic I, Meitinger T, Ludolph AC, Strom TM, Andersen PM, Weishaupt JH. Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. Nat Neurosci. 2015 May;18(5):631-6. doi: 10.1038/nn.4000.
21. Hirano M, Angelini C, Montagna P, Hays AP, Tanji K, Mitsumoto H, Gordon PH, Naini AB, DiMauro S, Rowland LP. Amyotrophic lateral sclerosis with ragged-red fibers. Arch Neurol. 2008 Mar;65(3):403-6. doi: 10.1001/archneurol.2007.65.
22. Vijayvergiya C, Beal MF, Buck J, Manfredi G. Mutant superoxide dismutase 1 forms aggregates in the brain mitochondrial matrix of amyotrophic lateral sclerosis mice. J Neurosci. 2005 Mar 9;25(10):2463-70. doi: 10.1523/JNEUROSCI.4385-04.2005.
23. Pasinelli P, Belford ME, Lennon N, Bacskai BJ, Hyman BT, Trotti D, Brown RH Jr. Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. Neuron. 2004 Jul 8;43(1):19-30. doi: 10.1016/j.neuron.2004.06.021.
24. Bille A, Jónsson SÆ, Akke M, Irbäck A. Local unfolding and aggregation mechanisms of SOD1: a Monte Carlo exploration. J Phys Chem B. 2013 Aug 8;117(31):9194-202. doi: 10.1021/jp404500b.
25. Vande Velde C, McDonald KK, Boukhedimi Y, McAlonis-Downes M, Lobsiger CS, Bel Hadj S, Zandona A, Julien JP, Shah SB, Cleveland DW. Misfolded SOD1 associated with motor neuron mitochondria alters mitochondrial shape and distribution prior to clinical onset. PLoS One. 2011;6(7):e22031. doi: 10.1371/journal.pone.0022031.
26. Sasaki S, Iwata M. Mitochondrial alterations in the spinal cord of patients with sporadic amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. 2007 Jan;66(1):10-6. doi: 10.1097/nen.0b013e31802c396b.
27. De Vos KJ, Chapman AL, Tennant ME, Manser C, Tudor EL, Lau KF, Brownlees J, Ackerley S, Shaw PJ, McLoughlin DM, Shaw CE, Leigh PN, Miller CCJ, Grierson AJ. Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. Hum Mol Genet. 2007 Nov 15;16(22):2720-2728. doi: 10.1093/hmg/ddm226.
28. He L, Chen L, Li L. The TBK1-OPTN Axis Mediates Crosstalk Between Mitophagy and the Innate Immune Response: A Potential Therapeutic Target for Neurodegenerative Diseases. Neurosci Bull. 2017 Jun;33(3):354-356. doi: 10.1007/s12264-017-0116-3.
29. Richter B, Sliter DA, Herhaus L, Stolz A, Wang C, Beli P, Zaffagnini G, Wild P, Martens S, Wagner SA, Youle RJ, Dikic I. Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. Proc Natl Acad Sci U S A. 2016 Apr 12;113(15):4039-44. doi: 10.1073/pnas.1523926113.
30. Harding O, Evans CS, Ye J, Cheung J, Maniatis T, Holzbaur ELF. ALS- and FTD-associated missense mutations in TBK1 differentially disrupt mitophagy. Proc Natl Acad Sci U S A. 2021 Jun 15;118(24):e2025053118. doi: 10.1073/pnas.2025053118.
31. Carelli V, Ghelli A, Bucchi L, Montagna P, De Negri A, Leuzzi V, Carducci C, Lenaz G, Lugaresi E, Degli Esposti M. Biochemical features of mtDNA 14484 (ND6/M64V) point mutation associated with Leber's hereditary optic neuropathy. Ann Neurol. 1999 Mar;45(3):320-8. PMID: 10072046.

32. Baracca A, Solaini G, Sgarbi G, Lenaz G, Baruzzi A, Schapira AH, Martinuzzi A, Carelli V. Severe impairment of complex I-driven adenosine triphosphate synthesis in leber hereditary optic neuropathy cybrids. Arch Neurol. 2005 May;62(5):730-6. doi: 10.1001/archneur.62.5.730. PMID: 15883259.