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Complete elimination of a pathogenic homoplasmic mtDNA mutation in one generation.

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Abstract

Mitochondrial DNA (mtDNA) mutations have been implicated in a wide variety of neurological conditions and is maternally inherited through a complex process which is not fully understood. Genetic counselling for mitochondrial conditions secondary to a mtDNA mutation can be challenging as it is not currently possible to accurately predict the mutational load/heteroplasmy of the mutation which could be passed to the offspring. In general, one expects that the higher the level of heteroplasmy the more likely that the same mtDNA mutation will be seen in her offspring. We report here a family which places a caveat on genetic counselling for mtDNA disorders. The proband is a 63 year old woman with m.14459G>A associated dystonia/spasticity/ataxia. The m.14459G>A mutation was detected at homoplasmic/near homoplasmic levels in her muscle tissue and fibroblasts, but does not appear to have been passed on to any of her offspring. To our knowledge this is the first report of complete selection against a homoplasmic variant within maternally transmitted mtDNA. It is not clear if this novel phenomenon occurred by random chance or by another method of mitochondrial selection.

Introduction

The human mitochondrial genome (mtDNA) has a high mutation rate in comparison to nuclear DNA. MtDNA mutations have been implicated in a wide variety of neurological and often multi-systemic diseases. Unlike nuclear DNA, mtDNA is maternally inherited through a complex process of intergenerational selection, referred to as the "mitochondrial bottleneck", which is still not completely understood (Marlow, 2017). This bottleneck likely occurs during postnatal folliculogenesis and can lead to rapid inter-generational shifts in the proportion of mutant mtDNA molecules. These shifts in the level of heteroplasmy can contribute to the wide variability of clinical phenotypes in pedigrees with pathogenic mtDNA mutations. Although it was initially believed that the mitochondrial bottleneck was a random process of genetic drift some recent reports suggest that other factors, such as the location of the mtDNA mutation (Wilson et al., 2016), play a significant role in the enrichment of mutant mitochondria in humans.

Given our limited understanding of the mitochondrial bottleneck, pre-test genetic counselling for mtDNA mutations in family members can be quite challenging (Nesbitt et al., 2014; White et al., 1999; Wilson et al., 2016). In general, we are unable to accurately predict the mutational load/heteroplasmy of a mtDNA mutation that a woman may pass on to her offspring, or the resulting phenotype of that child in relation to the level of heteroplasmy. In general, genetic counselling for such mtDNA mutations is based on the assumption that higher levels of heteroplasmy of a pathogenic mtDNA mutation are statistically more likely to be at a similar or higher level of heteroplasmy in the offspring. With the exception of large mtDNA deletions or mtDNA mutations only seen in the muscle of the proband (Fu et al., 1996; Holt et

al., 1988; Weber et al., 1997), most clinicians would counsel that a woman with a mtDNA mutation at homoplasmic/near-homoplasmic levels in blood derived mtDNA would be most likely to pass on a high to homplasmic mutational load to the offspring.

We report here a patient with a classic presentation of m.14459G>A associated dystonia in whom the mutation appears to have been eliminated in one generation. The m.14459G>A mutation was first described in 1994 in a Hispanic pedigree with Leber hereditary optic neuropathy (LHON), pediatric-onset dystonia, and LHON plus dystonia (Jun et al., 1994a). Since then the m.14459G>A mutation has been reported in several other pedigrees with a high degree of clinical variability at both high heteroplasmic and homoplasmic levels in blood leukocytes (De Vries et al., 1996; Gropman et al., 2004; Shoffner et al., 1995; Tarnopolsky et al., 2004; Wallace and Brown, 1997). There have also been reports of the m.14459G>A mutation associated with Leigh disease in high heteroplasmic or homoplasmic levels (Kirby et al., 2000; Ronchi et al., 2011).

The proband described here (Figure 1 – III.1) was identified to have the m.14459G>A mutation at near homoplasmic levels in both skeletal muscle and skin fibroblasts. Her family history was also suspicious for other members being affected (Figure 1). Genetic testing of two of her biological children (IV.1 and IV.2) did not identify the m.14459G>A variant. The proband's granddaughter (V.1) also tested negative for the m.14459G>A mutation. To our knowledge this is the first documented case of complete elimination of a pathogenic mtDNA mutation from near homoplasmic levels in a single generation.

Case Report

The proband (III.1) is a 63 year old female born to non-consanguineous parents of Northern European descent. She initially presented with ataxia around the age of eighteen years and was originally diagnosed with multiple sclerosis and Parkinson disease. Her medical history was also positive for migraine variant headaches. Due to difficulties with mobility she began to use a wheelchair in her forties to travel long distances. An MRI performed at the age of 55 year of age showed high T-2 signal in the basal ganglia and the possibility of an underlying mitochondrial cytopathy was considered and she was referred for further evaluation.

Muscle biopsy from the *vastus lateralis* showed nonspecific changes including fibre atrophy and nuclear clump fibres with an increase in internalized nuclei and no evidence of mitochondrial myopathy with multiple stains (modified Gomori trichrome, NADH-TR, SDH, and cytochrome *c* oxidase). Plasma amino acids, lactate, HbA1C, and CK were all within normal limits.

Sequencing of the mitochondrial genome from muscle tissue identified a homoplasmic pathogenic variant (m.14459G>A) in the MT-ND6 gene. Repeat testing by NGS at a second clinical laboratory (see below) found the m.14459G>A variant at 98.4% heteroplasmy in muscle tissue and 96.8% heteroplasmy in skin fibroblasts.

Cranial nerve examination at 63 y of age showed lateral gaze limitations with horizontal gaze evoked nystagmus. She has very slow dystonic speech and significant dystonia of the neck and hands. She also had spasticity in the upper and lower extremities, clonus at the ankles, and the plantar response was extensor bilaterally. Vibration was moderately reduced at the ankles and she had a marked ataxic gait.

Two of the proband's daughters (IV.1 and IV.2) and her granddaughter (V.1) were seen for genetic counselling and testing at ages 39, 36, and 20 years, respectively. Physical examination of all three women was within normal limits with no signs of ataxia or dystonia. All reported medical histories positive for migraine variant headaches. None of them reported any vision loss. All three were found to be negative for the m.14459G>A mutation in blood. Testing for IV.1 was repeated on a second blood sample as well as at a second clinical laboratory (LHSC). Unfortunately, none of the three returned for further testing (i.e., urine sediment, muscle biopsy) in spite of repeated contact attempts. A list of all polymorphisms can be found in Table 1 of the supplemental data.

Methods

Genetic Testing

Samples were tested at CLIA-certified laboratory between 2011-2014 (Transgenomics). Testing for III.1 and IV.1 were repeated at London Health Sciences Clinical Molecular Genetics Laboratory (LHSC) in 2016. Full mitochondrial genome (mtDNA) sequencing was performed at LHSC using standard laboratory protocols (Kerkhof et al., 2017; Schenkel et al., 2016).

Discussion

We report here a patient with a classic presentation of m.14459G>A associated dystonia, spasticity and ataxia with testing showing the m.14459G>A DNA variant at near homoplasmic levels in both muscle tissue and skin fibroblasts. The m.14459G>A mutation is associated with Leber Hereditary Optic Neuropathy (LHON)(Jun et al., 1994b), LHON plus

dystonia(Jun et al., 1994b), and Leigh disease(Ronchi et al., 2011). Genetic testing in two of her daughters (IV.1 and IV.2) and a granddaughter (V.1) was negative for the m.14459G>A mutation in peripheral blood mononuclear cells (PBMCs), suggesting that this pathogenic variant was not passed on to her offspring. Based on the family history of similar symptoms in the proband's mother (II.2), brother (III.2), and sister (III.5) it is unlikely that the m.14459G>A mutation occurred as a *de novo* mutation in non-germ line tissues in the proband. Her brother and sister declined testing. There was limited information/contact with other family members. Other possibilities, such as sample-mix up, non-maternity, and paternal mtDNA inheritance (only documented in *in vitro* experiments (John and Schatten, 2004)), were considered. Analysis of the mtDNA polymorphisms between the proband (III.1) her two daughters (IV.1 and IV.2) and granddaughter (V.1) were a match (Supplemental Table 1). This strongly suggested that maternal transmission of mtDNA did occur to her daughters (IV.1 and IV.2) and granddaughter (V.1); but without the high level of the m.14459G>A variant. Although the m.14459G>A variant was only assessed in PBMCs in the offspring, we typically find that the heteroplasmy of pathogenic variants in PBMCs is usually no less than 70 and 40 % of urine sediment or muscle biopsy tissue, respectively, in mtDNA disorders (Tarnopolsky, et al., unpublished data, 2018). To our knowledge this is the first report of complete selection against a near homoplasmic variant within maternally transmitted mtDNA.

There are dozens of case reports in the literature about *de novo* mtDNA mutations which appear at homoplasmic or near-homoplasmic levels in the proband but absent of low in the parent (Blakely et al., 2006; Degoul et al., 1997; Götz et al., 2012; Tarnopolsky et al., 2013; Taylor et al., 2002; Wray et al.), but we believe that this is the first report of a complete

elimination (near homoplasmic to non-detectable) of a pathogenic mtDNA variant in one generation. Most *de novo* events occur only once in one individual in a family. The observation of the complete disappearance of the m.14459G>A mtDNA mutation in the proband's two daughters (IV.1 and IV.2) suggested that a mechanism of selection against the near homoplasmic m.14459G>A mutation, by luck or biological intention, may have occurred in the proband's ovarian tissue/oocyte progenitor cells early in postnatal folliculogenesis.

There has been a significant amount of research focused on better understanding the maternal inheritance of mitochondria and mitochondrial selection in oocytes (Marlow, 2017). It is currently accepted that mechanism of mitochondria purification occurs sometime in oogenesis, where only a small population of mitochondria are selected for transgenerational transmission. Once past this "mitochondrial bottleneck" the small population of mitochondria undergo a step of amplification sometime around the time of oocyte maturity/fertilization. Some studies suggest that the Balbiani body in the primary oocytes of animals such as zebrafish plays a role in an enrichment or selection of the mitochondria with the best activity/respiration, although this is still an area that is being investigated (Tworzydlo et al., 2016; Wilding et al., 2001; Zhang et al., 2008).

Rapid shifts in the level of heteroplasmy seen within a single generation contribute to the wide range in the severity of clinical phenotypes seen in families transmitting mtDNA disease. Although preliminary evidence from human pedigrees initially suggested a random process of genetic drift, some recent reports have describe definite differences in segregation pattern between pedigrees with different mtDNA mutations (Wilson et al., 2016). Wilson et al. recently published data which supported anecdotal reports of rapid shifts in heteroplasmy

between single generations with certain mtDNA mutations (White et al., 1999). This data showed that mitochondrial mutation m.8993T>G/C showed significantly faster segregation within pedigrees than other mtDNA mutations (m.3243A>G, m.8344A>G, m.11778G>A) (Wilson et al., 2016). This suggested that the mitochondrial bottleneck may be, at least partially, influenced by the underlying mtDNA mutation (Wilson et al., 2016).

Other studies in mouse models suggest that a process of selective elimination occurs against pathogenic mtDNA mutations to remove them from the female germline over multiple generations (Fan et al., 2008). In this mouse model it is hypothesized that highly deleterious mutations are less likely to reach homoplasmic levels because the proto-oocytes with higher heteroplasmy of the mutant mtDNA are eliminated by selection before ovulation. Pathogenic mtDNA mutations which are "less damaging", such as LHON mutation m.11778G>A, may not undergo the same selective elimination process and may partially explain why common LHON mutations are typically seen and inherited at homoplasmic or near homoplasmic levels whereas mutations such as m.3243A>G associated with MELAS syndrome are typically heteroplasmic within a pedigree (Wallace et al., 2007). Common LHON mutation such as m.3460G>A, m.11778G>A, and m.14484T>C are typically always seen at homoplasmic or near-homoplasmic levels in symptomatic males (Taylor et al., 2003). Unlike these LHON mutations, m.14459G>A is often associated with extra-ocular manifestations of spasticity/dystonia in both males and females and has often been described to cause symptoms at both homoplasmic and heteroplasmic levels (De Vries et al., 1996; Gropman et al., 2004; Jun et al., 1994a; Shoffner et al., 1995; Tarnopolsky et al., 2004; Wallace and Brown, 1997). It is currently unclear if the

pathogenicity/severity of the m.14459G>A mutation played a role in its elimination from the mitochondrial genome of this family.

In summary, we have evidence for the complete elimination of a near homplasmic mtDNA m.14459G>A variant within one generation in two offspring. This suggests that elimination of the m.14459G>A variant likely occurred in at least two of the probands oocytes early in embryogenesis (Taylor et al., 2003). It is not clear if this occurred by random chance or by another method of mitochondrial selection. This data does place a caveat on genetic counselling for mtDNA disorders.

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References

Blakely, E.L., Rennie, K.J., Jones, L., Elstner, M., Chrzanowska-Lightowlers, Z.M.A., White, C.B., Shield, J.P.H., Pilz, D.T., Turnbull, D.M., Poulton, J., 2006. Sporadic intragenic inversion of the mitochondrial DNA MTND1 gene causing fatal infantile lactic acidosis. Pediatr. Res. 59, 440-444.

De Vries, D., Went, L., Bruyn, G., Scholte, H., Hofstra, R., Bolhuis, P., Van Oost, B., 1996. Genetic and biochemical impairment of mitochondrial complex I activity in a family with Leber hereditary optic neuropathy and hereditary spastic dystonia. A. J. Hum. Genet. 58, 703.

Degoul, F., Francois, D., Diry, M., Ponsot, G., Desguerre, I., Heron, B., Marsac, C., Moutard, M.L., 1997. A near homoplasmic T8993G mtDNA mutation in a patient with atypic Leigh syndrome not present in the mother's tissues. J. Inherit. Metab. Dis. 20, 49-53.

Fan, W., Waymire, K.G., Narula, N., Li, P., Rocher, C., Coskun, P.E., Vannan, M.A., Narula, J., Macgregor, G.R., Wallace, D.C., 2008. A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. Science 319, 958-962.

Fu, K., Hartlen, R., Johns, T., Genge, A., Karpati, G., Shoubridge, E.A., 1996. A novel heteroplasmic tRNAleu (CUN) mtDNA point mutation in a sporadic patient with mitochondrial encephalomyopathy segregates rapidly in skeletal muscle and suggests an approach to therapy. Hum. Mol. Genet. 5, 1835-1840.

Götz, A., Isohanni, P., Liljeström, B., Rummukainen, J., Nikolajev, K., Herrgård, E., Marjavaara, S., Suomalainen, A., 2012. Fatal neonatal lactic acidosis caused by a novel de novo mitochondrial G7453A tRNA-Serine (UCN) mutation. Pediatr. Res. 72, 90-94.

Gropman, A., Chen, T.J., Perng, C.L., Krasnewich, D., Chernoff, E., Tifft, C., Wong, L.J.C., 2004. Variable clinical manifestation of homoplasmic G14459A mitochondrial DNA mutation. Am. J. Med. Genet. Part A. 124, 377-382.

Holt, I., Harding, A., Morgan-Hughes, J., 1988. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature 331, 717-719.

John, J.C.S., Schatten, G., 2004. Paternal mitochondrial DNA transmission during nonhuman primate nuclear transfer. Genetics 167, 897-905.

Jun, A.S., Brown, M.D., Wallace, D.C., 1994a. A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia. Proc. Natl. Acad. Sci. U.S.A. Proc. Natl. Acad. Sci. 91, 6206-6210.

Jun, A.S., Brown, M.D., Wallace, D.C., 1994b. A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia. Proc. Natl. Acad. Sci. U.S.A. 91, 6206-6210.

Kerkhof, J., Schenkel, L.C., Reilly, J., McRobbie, S., Aref-Eshghi, E., Stuart, A., Rupar, C.A., Adams, P., Hegele, R.A., Lin, H., 2017. Clinical Validation of Copy Number Variant Detection from Targeted Next-Generation Sequencing Panels. J. Mol. Genet.

Kirby, D.M., Kahler, S.G., Freckmann, M.L., Reddihough, D., Thorburn, D.R., 2000. Leigh disease caused by the mitochondrial DNA G14459A mutation in unrelated families. Ann. Neurol. 48, 102-104.

Marlow, F.L., 2017. Mitochondrial matters: Mitochondrial bottlenecks, self-assembling structures, and entrapment in the female germline. Stem Cell Res. 21, 178-186.

Nesbitt, V., Alston, C.L., Blakely, E.L., Fratter, C., Feeney, C.L., Poulton, J., Brown, G.K., Turnbull, D.M., Taylor, R.W., McFarland, R., 2014. A national perspective on prenatal testing for mitochondrial disease. Eur. J. Hum. Genet. 22, 1255.

Ronchi, D., Cosi, A., Tonduti, D., Orcesi, S., Bordoni, A., Fortunato, F., Rizzuti, M., Sciacco, M., Collotta, M., Cagdas, S., Capovilla, G., Moggio, M., Berardinelli, A., Veggiotti, P., Comi, G.P., 2011. Clinical and molecular features of an infant patient affected by Leigh Disease associated to m.14459G>A mitochondrial DNA mutation: a case report. BMC Neurol. 11, 85.

Schenkel, L.C., Kerkhof, J., Stuart, A., Reilly, J., Eng, B., Woodside, C., Levstik, A., Howlett, C.J., Rupar, A.C., Knoll, J.H., 2016. Clinical next-generation sequencing pipeline outperforms a combined approach using sanger sequencing and multiplex ligation-dependent probe amplification in targeted gene panel analysis. J. Mol Diagnost. 8, 657-667.

Shoffner, J.M., Brown, M.D., Stugard, C., June, A.S., Pollock, S., Haas, R.H., Kaufman, A., Koontz, D., Kim, Y., Graham, J.R., 1995. Leber's hereditary optic neuropathy plus dystonia is caused by a mitochondrial DNA point mutation. Ann. Neurol. 38, 163-169.

Tarnopolsky, M., Meaney, B., Robinson, B., Sheldon, K., Boles, R.G., 2013. Severe infantile Leigh syndrome associated with a rare mitochondrial ND6 mutation, m. 14487T> C. Am. J. Med. Genet Part A. 161, 2020-2023.

Tarnopolsky, M.A., Baker, S.K., Myint, T., Maxner, C., Robitaille, J., Robinson, B.H., 2004. Clinical variability in maternally inherited leber hereditary optic neuropathy with the G14459A mutation. Am. J. Med. Genet. Part A. 124, 372-376.

Taylor, R.W., Jobling, M.S., Turnbull, D.M., Chinnery, P.F., 2003. Frequency of rare mitochondrial DNA mutations in patients with suspected Leber's hereditary optic neuropathy. J. Med. Genet. 40, e85.

Taylor, R.W., Schaefer, A.M., McFarland, R., Maddison, P., Turnbull, D.M., 2002. A novel mitochondrial DNA tRNAIle (A4267G) mutation in a sporadic patient with mitochondrial myopathy. Neuromus. Disord. 12, 659-664.

Tworzydlo, W., Kisiel, E., Jankowska, W., Witwicka, A., Bilinski, S.M., 2016. Exclusion of dysfunctional mitochondria from Balbiani body during early oogenesis of Thermobia. Cell Tiss. Res. 366, 191-201.

Wallace, D., Lott, M., Connor, J.M., Pyeritz, R.E., Korf, B.R., 2007. Emery and Rimoin's principles and practice of medical genetics, 5 ed. Churchill Livingstone Elsevier.

Wallace, D.C., Brown, M.D., 1997. Detection of mitochondrial DNA mutation 14459 associated with dystonia and/or Leber's hereditary optic neuropathy. Google Patents.

Weber, K., Wilson, J., Taylor, L., Brierley, E., Johnson, M., Turnbull, D., Bindoff, L., 1997. A new mtDNA mutation showing accumulation with time and restriction to skeletal muscle. Am. J. Hum. Genet. 60, 373.

White, S.L., Collins, V.R., Wolfe, R., Cleary, M.A., Shanske, S., DiMauro, S., Dahl, H.-H.M., Thorburn, D.R., 1999. Genetic counseling and prenatal diagnosis for the mitochondrial DNA mutations at nucleotide 8993. Am. J. Hum. Genet. 65, 474-482.

Wilding, M., Carotenuto, R., Infante, V., Dale, B., Marino, M., Di Matteo, L., Campanella, C., 2001. Confocal microscopy analysis of the activity of mitochondria contained within the 'mitochondrial cloud' during oogenesis in Xenopus laevis. Zygote 9, 347-352.

Wilson, I.J., Carling, P.J., Alston, C.L., Floros, V.I., Pyle, A., Hudson, G., Sallevelt, S.C., Lamperti, C., Carelli, V., Bindoff, L.A., Samuels, D.C., Wonnapinij, P., Zeviani, M., Taylor, R.W., Smeets, H.J., Horvath, R., Chinnery, P.F., 2016. Mitochondrial DNA sequence characteristics modulate the size of the genetic bottleneck. Hum. Mol. Genet. 25, 1031-1041.

Wray, C.D., Friederich, M.W., du Sart, D., Pantaleo, S., Smet, J.l., Kucera, C., Fenton, L., Scharer, G., Van Coster, R., Van Hove, J.L.K., A new mutation in MT-ND1 m. 3928G> C p. V208L causes Leigh disease with infantile spasms. Mitochondrion 13, 656-661.

Zhang, Y.Z., Ouyang, Y.C., Hou, Y., Schatten, H., Chen, D.Y., Sun, Q.Y., 2008. Mitochondrial behavior during oogenesis in zebrafish: a confocal microscopy analysis. Dev. Growth Diff. 50, 189-201.

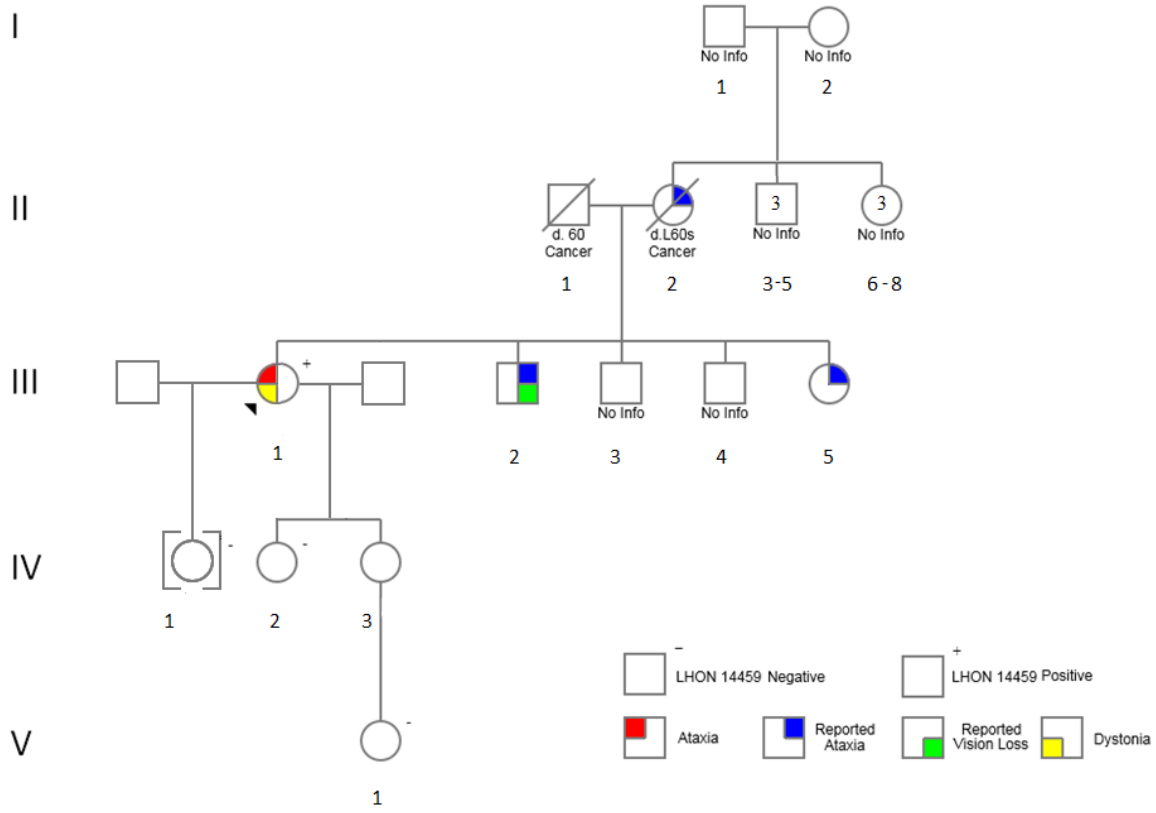

Figure. 1

Highlights

- Family with female proband with m.14459G>A associated dystonia/spasticity/ataxia.
- m.14459G>A mutation detected at homoplasmic/near homoplasmic levels in her muscle tissue and fibroblasts
- m.14459G>A mutation not passed to her offspring
- To our knowledge this is the first report of complete selection against a homoplasmic variant within maternally transmitted mtDNA in a single generation