Milder Phenotype of Homoplasmic Versus Heteroplasmic m.8344A>G Variant in the Same Family: A Case Report

Josef Finsterer 1, Sounira Mehri 2

1. Neurology, Neurology and Neurophysiology Center, Vienna, AUT 2. Laboratory of Nutrition and Vascular Health, Faculty of Medicine, Monastir, TUN

Abstract
A myoclonic epilepsy with ragged-red fibers (MERRF) patient who carried the m.8344A>G variant in the homoplasmic form manifested a milder phenotype than his sister who carried the same variant in the heteroplasmic form, which has not yet been reported. The 27-year-old male, with an uneventful history, presented at age 19 with fatigue and persistent tremor in both hands. When he talked for a long time, his speech would slow down, and he would stutter. Although electroencephalography showed spike-wave complexes in both occipital projections with generalization, no anti-seizure drugs were given. At age 20, the patient suffered a fall due to muscle weakness. From age 21, generalized myocloni occurred. Because the sister had been diagnosed with MERRF-plus syndrome, the patient underwent genetic testing, which revealed the m.8344A>G variant in homoplasy. L-carnitine was started. At age 27, the patient experienced a first “syncope” after a long walk, which subsequently recurred up to 2-3 times per day. EEG showed low-amplitude spikes, slow-spike waves at the posterior vertex, and generalized slow-spike waves. Clonazepam was recommended but declined by the patient. In conclusion, the m.8344A>G variant may manifest milder and with a later onset in the homoplasmic as compared to the heteroplasmic form. Further, the homoplasmy of the m.8344A>G variant appears to be more beneficial than harmful.

Keywords: Neurology, homoplasy, merrf, myoclonic epilepsy, respiratory chain, mitochondrial

Introduction
Myoclonic epilepsy with ragged-red fibers (MERRF) is a rare syndromic mitochondrial disorder that presents as either pure MERRF, characterized by four canonical features - epilepsy, myocloni, ataxia, and myopathy - or as MERRF–plus, which manifests, in addition to the brain, in the peripheral nerves, eyes, ears, heart, gastrointestinal tract, or endocrine organs [1,2]. MERRF is currently understood to be due to 26 mutations in 15 different genes [2]. The most common of these mutations is the m.8344A>G variant in MT-TK (tRNA[Lys]), which accounts for 80% of MERRF cases [2]. In the vast majority of cases, the m.8344A>G variant occurs in its heteroplasmic form. Although nearly homoplasmic m.8344A>G variants have occasionally been described [3,4], a homoplasmic patient carrying the m.8344A>G variant is unique, as in the following case.

Case Presentation
The patient is a 27-year-old Caucasian male with uncomplicated early development, who only became conspicuous at age 19 due to fatigue and slight persistent tremor of both hands. When the patient spoke for a long time, his speech would slow down, and he would stutter. Work-up for tremor by electroencephalography (EEG) showed epileptiform discharges in the form of spike-wave activity in both occipital projections with a tendency to generalize in the form of bilateral synchronous discharges. Surprisingly, no treatment with anti-seizure drugs (ASDs) was started at that time. A year later, after a long walk without eating, the patient’s legs buckled and he fell to his knees without losing consciousness. The patient recovered after resting for a week and eating well. After that, he was able to cycle after a long walk without eating, the patient’s legs buckled and he fell to his knees without losing consciousness. The patient recovered after resting for a week and eating well. After that, he was able to cycle

The family history was positive for MERRF–plus syndrome in the sister [5]. The mother presented with easy
fatigability and exercise intolerance at age 30, myocloni from age 52, photosensitivity (i.e., uncomfortable feeling with flickering light and when looking at numerous stones, leaves, or dirty snow on the ground), hypothyroidism, arterial hypertension, and ischemic heart disease.

A clinical neurological exam at age 27 showed an impaired convergence response, permanent left eyeball abduction, positional tremor of both hands (more pronounced in the morning than in the evening), occasional myocloni, and static-locomotor and dynamic ataxia. The patient had difficulty standing on one leg, and his tandem gait was impaired. Serum lactate was increased to 3.2 mmol/L (n, 0.00-2.2 mmol/L). EEG revealed low-amplitude spikes, slow spike-wave complexes in the posterior vertex region, and recurrent, generalized slow-spike wave complexes (Figure 1).

Video oculography showed a violation of smooth downward tracking, a violation of vestibulococular reflex suppression, indicating cerebellar dysfunction, and moderately impaired saccade initiation. Nerve conduction studies revealed mixed axonal and demyelinating sensory neuropathy. Visually evoked potentials were non-informative. Genetic workup for suspected MERRF syndrome, which had been diagnosed in his sister [5], revealed the mitochondrial DNA (mtDNA) variant m.8344A>G in homoplasmy in blood lymphocytes (Table 1). At age 27, the patient started taking L-carnitine. His current medication included L-carnitine (3000 mg/d), vitamin-E (600 mg/d), hydroxypyridine (375 mg/d), coenzyme-Q (300 mg/d), citrulline, and threonyl-lysyl-prolyl-arginyl-prolyl-glycyl-proline diacetate (four drops per day). He refused to take the prescribed ASDs.
|                  | Index patient | Sister | Mother |
|------------------|---------------|--------|--------|
| Age (years)      | 27            | 34     | 50     |
| mtDNA variant    | m.8344A>G     | m.8344A>G | m.8344A>G |
| mutation load    | homoplasmic   | heteroplasmic | heteroplasmic |
| heteroplasmy rate| 100%          | 50%    | 40%    |
| Onset age (years)| 19            | 7      | ?      |
| Fatigue          | +             | +      | +      |
| Exercise intolerance | +       | +      | +      |
| Photosensitivity | +             | +      | +      |
| Myocloni         | +             | +      | -      |
| Epilepsy         | +             | +      | -      |
| Hypothyroidism   | -             | -      | +      |
| PCOS             | -             | +      | -      |
| Neuropathy       | +             | +      | -      |
| Ataxia           | +             | +      | -      |

**TABLE 1: Comparison of genotype and phenotype between the heteroplasmic sister and mother and the homoplasmic index patient**

+: present, -: absent. mtDNA: Mitochondrial DNA; PCOS: Polycystic ovary syndrome

**Discussion**

The presented patient was interesting in the following ways: First, the patient had MERRF, which presented with ophthalmoparesis, myocloni, ataxia, tremor, sensory neuropathy, and seizures; Second, MERRF was due to the m.8344A>G variant in homoplasmy, which has not been previously described; and Third, the index patient manifested with a milder phenotype than his sister, who manifested with MERRF-plus and also carried the m.8344A>G variant but with a heteroplasmy of 50% in blood lymphocytes.

The patient’s phenotype met the diagnostic criteria for classical MERRF, but the late onset and the relatively mild phenotype were striking given the homoplastic distribution of the m.8344A>G variant. Usually, homoplasmy is associated with a more severe phenotype compared to heteroplasmy [6,7], but there is increasing evidence that heteroplasmy often correlates poorly with disease severity [8,9], or that there is no correlation at all [10]. A good correlation between heteroplasmy and severity of the phenotype has been reported especially for MT-ATP6 variants [6]. Homoplasmy of the m.8993T>G variant was associated with severe Leigh syndrome [11], while heteroplasmic variants are associated with less severe neuropathy, ataxia, and retinitis pigmentosa syndrome. In a study of three Chinese families carrying the m.616T>C variant in mt-tRNA(Phe) and uniformly manifesting with isolated chronic kidney disease and hyperuricemia without proteinuria, hematuria, or renal cyst formation, patients carrying the variant in homoplasmy manifested equally to those in heteroplasmy [12].

There are a number of reports of m.8344A>G carriers manifesting with a more severe phenotype when the heteroplasmy rate was high. In a 25-year-old male with exercise intolerance since age 6, stroke-like episodes since age 10, and psychomotor regression and myoclonic seizures since age 12, the m.8344A>G variant, with a heteroplasmy rate of 90%, manifested as mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)/MERRF/Leigh overlap syndrome [4]. There are also reports showing that the phenotype can vary between family members carrying the m.8344A>G variant despite a uniform intrapersonal distribution of the mutation load, suggesting that the phenotype is also determined by influences other than the heteroplasmy rate and that the mutation does not change post-natally [13]. For example, a 62-year-old female with MERRF due to the m.8344A>G variant had adult onset despite having a heteroplasmy rate of >90 [14].

The reason why the index patient manifested with a milder phenotype than his sister who carried the same variant with a heteroplasmy rate of 50% remains unclear, but it can be speculated that compensatory...
mechanisms, including anti-oxidative capacity, were better preserved in the index patient than in his sister. It is also conceivable that the mtDNA copy number varied between the two siblings, or that both carried different polymorphisms that modified the phenotype. It can also be speculated that the tissue distribution of homoplasmic variants differs from heteroplasmic m.8344A>G variants, suggesting that homoplasmia prevents broad tissue distribution. Whether the fusion and fission capacity [15] or the capacity of mitophagy differed between the two siblings and thus contributed to the variable phenotypic expression remains speculative. In one large five-generation family, heteroplasy rates of the m.8344A>G variant correlated with the level of F2-isoprostanes, a specific and reliable marker of oxidative damage, suggesting that the level of oxidative stress contributes to the phenotypic heterogeneity of the variant within a family [16].

Conclusions

This case demonstrates that the m.8344A>G variant can present with a milder phenotype and with a later onset in the homoplasmic compared to the heteroplasmic form. Contrary to what one would expect, the homoplasmia of the m.8344A>G variant appears beneficial rather than harmful. However, heteroplasm may not be the only factor determining the phenotypic expression of the m.8344A>G variant.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

References

1. Velez-Bartolomei F, Lee C, Enns G: Myoclonic epilepsy with ragged-red fibers (MERRF) . GeneReviews®. Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean L, Gripp KW, Amemiya A (ed): University of Washington, Seattle (WA); 2005.
2. Finsterer J, Zarrour-Mahjoub S, Shoffner JM: MERRF classification: implications for diagnosis and clinical trials. Pediatr Neurol. 2018, 80:8-23. 10.1016/j.pediatrneurol.2017.12.005
3. Russo SN, Goldstein A, Karza A, Koenig NK, Walker M: Leigh syndrome as a phenotype of near-homoplasmic m.8344A>G variant in children. Child Neurol Open. 2021, 8:2329048X21991382. 10.1177/2329048X21991382
4. Hou Y, Zhao XT, Xie ZY, Yuan Y, Wang ZX: Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes/myoclonus epilepsy with ragged-red fibers./Leigh overlap syndrome caused by mitochondrial DNA 8344A>G mutation (Article in Chinese). Beijing Da Xue Xue Bao Yi Xue Ban. 2020, 52:851-5.
5. Finsterer J: Photosensitive epilepsy and polycystic ovary syndrome as manifestations of MERRF . Case Rep Neurol Med. 2020, 2020:8876272. 10.1155/2020/8876272
6. Licchetta L, Ferri L, La Morgia C, et al.: Epilepsy in MT-ATP6 - related mils/NARP: correlation of eletroclinical features with heteroplasm. Ann Clin Transl Neurol. 2021, 8:704-10. 10.1002/acn3.51259
7. Zhao J, Zhao DH, Zhang W, Liu H, Yuan Y, Qi Y, Wang ZX: Clinical heterogeneity associated with mitochondrial DNA A8344G point mutation (Article in Chinese). Zhonghua Yi Xue Za Zhi. 2012, 92:2835-8.
8. de Laat P, Rodenburg RR, Rovelevd N, Koene S, Smeitink JA, Janssen MC: Six-year prospective follow-up study in 151 carriers of the mitochondrial DNA A5243 A>G variant. J Med Genet. 2021, 58:48-55. 10.1136/jmedgenet-2019-106800
9. Stendel C, Neuhofer C, Floride E, et al.: Delineating MT-ATP6-associated disease: From isolated neurupathy to early onset neurodegeneration. Neurol Genet. 2020, 6:e595. 10.1212/NXG.0000000000000595
10. Chinnery PF, Howell N, Lightowers RN, Turnbull DM: Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes. Brain. 1997, 120 ( Pt 10):1713-21. 10.1093/brain/120.10.1715
11. Balasubramaniam S, Lewis B, Mock D, et al.: Erratum: Leigh-Like Syndrome Due to Homoplasmic m.8993T>G mutation (Article in Chinese) J Biol Chem. 2005, 38:678-83.
12. Xu C, Tong L, Rao J, et al.: Heteroplasmic and homoplasmic m.616T>C in mitochondria tRNAPhe promote isolated chronic kidney disease and hyperuricemia. JCI Insight. 2022, 7: 10.1172/jci.insight.157418
13. Jeppesen TD, Al-Hashimi N, Duno M, Wilbrand F, Andersen G, Vissing J: Mitochondrial DNA mutation load in a family with the m.8344A>G point mutation and lipomas: a case study. Clin Case Rep. 2017, 5:2054-9. 10.1002/ect.15096
14. Fekete A, Hadzsiev K, Bene J, Naszai A, Matyas P, Till A, Melegh B: A8344G mitochondrial DNA mutation observed in two generations (Article in Hungarian). Orv Hetil. 2017, 158:468-71. 10.1556/650.2017.50634
15. Jannsen MC, Byun Y, Saeed S, et al.: Mitochondrial DNA mutation load in a family with the m.8344A>G point mutation and lipomas: a case study. Clin Case Rep. 2017, 5:2054-9. 10.1002/ect.15096
16. Canter JA, Shagabian A, Fessel J, et al.: Degree of heteroplasmia reflects oxidative damage in a large family with the mitochondrial DNA A8344G mutation. Free Radic Biol Med. 2005, 38:678-83. 10.1016/j.freeradbiomed.2004.11.051