Genetics inMedicine | CORRESPONDENCE

Comment on the criteria for interpretation of mitochondrial tRNA variants

The authors of the article "Interpretation of mitochondrial tRNA variants" developed a set of criteria modeled after the 2015 American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines for nuclear variant evaluation[1] for the classification and interpretation of mitochondrial transfer RNA (mt-tRNA) variants. Although their proposed criteria strive to create a similar objective framework, they fail to account for the complexity of mitochondrial DNA (mtDNA)-related disorders and heavily rely on expert opinion rather than discrete criteria. According to our experience in mitochondrial genetic testing for over 70,000 individuals, including concurrent next-generation sequencing (NGS) based whole mitochondrial genome sequencing and exome sequencing (trio, with the patient and both parents) for over 30,000 patients with suspected mitochondrial diseases, and evaluation of the criteria, many of these criteria are ambiguous, inappropriate, or not suitable for wide application. Therefore, the proposed criteria would lead to further inconsistency in variant classification and thus require significant refinement before clinicians and diagnostic laboratories can consider using them.

For PS2, a strong criterion defined as "present at ≥5% heteroplasmy, in >2 different tissues of the affected individual but 0% in asymptomatic mother," the 5% heteroplasmy cutoff, which is also used for PM2, P8, PM9, PP1, PP6, and PP7, is too low. A cutoff of <25% heteroplasmy in the affected tissue might only be suitable for the extremely rare and most deleterious mt-tRNA variants that alter the anticodon triplet that have such a low threshold,[2] but would not be suitable for the vast majority of mt-tRNA variants that usually have a diseasecausing threshold of 40% or higher in affected tissues.[3] For some mt-tRNA variants, the level of heteroplasmy could be <5% in the blood, an unaffected tissue, of affected individuals; for many other mt-tRNA variants, the heteroplasmy in blood of affected individuals could range from 17% to 99%.[3–7] Therefore, 5% heteroplasmy of any mt-tRNA variant in tissue does not guarantee it is clinically relevant, and it alone is not sufficient to provide support for or against the pathogenicity of a novel mt-tRNA variant. Instead, difference in level of heteroplasmy between affected and unaffected tissue of an individual, or between the same type of tissue of an affected individual and an unaffected relative, provides evidence for evaluation of pathogenicity, although it requires a higher heteroplasmy level than 5% to be clinically relevant. Besides, "0% in asymptomatic mother" is not accurate, as even for NGS, the claimed detection limit in general is 1.5% and above;[8] it would be more accurate if modified to "absent in the asymptomatic mother tested by NGS." Furthermore, requiring testing of more than two different tissues of an individual to apply PS2 is neither feasible nor necessary.

PS3 mentioned different functional assays for mt-tRNA. To be considered as a well-established functional study for a genetic variant, it should be reproducible, robust, and specific; electron transport chain (ETC) enzymology, oxygen consumption rate (OCR), mtDNA copy number, and morphology on samples collected from patients directly do not meet these criteria. Transmitochondrial cybrid studies evaluating one mtDNA variant at a time are one of the only types of study specific enough to establish that a functional defect is attributed to a mtDNA variant.[9] With well-established functional studies the level of functional defect caused by a variant can be quantified and thus the level of pathogenic criteria (strong, moderate, or supporting) can be applied based on the extent of functional deficiency. Additionally, when the extent of functional defect does not correlate with heteroplasmy, this is lack of segregation should be evidence supporting that a variant is benign rather than supporting pathogenicity as in PM10.

The use of PS4 requires case–control studies with statistically significant odds ratios. However, pathogenic mt-tRNA variants are usually associated with early-onset severe diseases and are mostly reported in clinical case studies. Case–control studies are usually lacking. Therefore, PS4 is usually not applicable to mt-tRNA variants, although a moderate criterion may be applied based on prior observation of a variant in multiple unrelated patients with the same phenotype, and its absence in controls (note 2 for PS4).[1] The PS4 criterion was not used for the evaluation of any mt-tRNA variant in this article.

Regarding PS5 and BS1, the authors defined PS5 as "Rare variants previously reported as pathogenic" and BS1 as "Reported in public databases (e.g., MITOMAP or mtDB) or literature as polymorphism." The defining strategy for these criteria is very similar to that for the PP5 and BP6 criteria in the ACMG/Association for Molecular Pathology (AMP) guideline.[1] The ACMG guidelines explicitly state that PP5 and BP6 should not be used if other criteria can be applied for evidence in publication. The use of PS5 and BS1 creates strong criteria based solely on the use of expert opinion, which is not itself objective evidence for or against a variant's pathogenicity and could lead to discrepant variant classification across laboratories.

PM2, an "absent from controls" based criterion in the ACMG/AMP guideline, was defined in this article as "Absent

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from databases, e.g., mtDB and MITOMAP, and absent or low heteroplasmy (<5%) in the asymptomatic mother of a proband exhibiting ≥5% heteroplasmy" and "PP7 will be applied if mother's sample is unavailable." The mtDB[10] database, which has not been updated since 2007, with multiple known disease-causing mtDNA variants listed as polymorphisms, is apparently not a valid population frequency database for evaluating mtDNA variants. Additionally, the use of segregation data (absence in mother) considered in the application of PS2 confounds the use of population frequency.

Phenotypes associated with mtDNA variants are broad and overlap significantly with disorders caused by variants in the nuclear genome. Therefore, PP4 should only be applied to individuals who have had comprehensive genetic testing including both mtDNA and nuclear genes, such as entire mitochondrial genome sequencing in addition to a large nuclear gene panel, or exome/genome sequencing.

In conclusion, the criteria developed by authors of this article do not fully capture the breadth of variability in mt-tRNA-related disorders and thus are inadequate for precise classification and interpretation of mitochondrial tRNA variants by clinicians and diagnostic laboratories.

DISCLOSURE

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REFERENCES

1. Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424.
2. Sacconi S, et al. A functionally dominant mitochondrial DNA mutation. Hum Mol Genet. 2008;17:1814–1820.
3. Rossignol R, et al. Mitochondrial threshold effects. Biochem J. 2003;370:751–762.
4. Altmann J, et al. Expanded phenotypic spectrum of the m.8344A>G "MERRF" mutation: data from the German mitoNET registry. J Neurol. 2016;263:961–972.
5. Hanna MG, et al. Congenital encephalomyopathy and adult-onset myopathy and diabetes mellitus: different phenotypic associations of a new heteroplasmic mtDNA tRNA glutamic acid mutation. Am J Hum Genet. 1995;56:1026–1033.
6. Karadimas CL, et al. Mitochondrial myopathy and ophthalmoplegia in a sporadic patient with the G12315A mutation in mitochondrial DNA. Neuromuscul Disord. 2002;12:865–868.
7. Mancuso M, et al. Congenital or late-onset myopathy in patients with the T14709C mtDNA mutation. J Neurol Sci. 2005;228:93–97.
8. Cui H, et al. Comprehensive next-generation sequence analyses of the entire mitochondrial genome reveal new insights into the molecular diagnosis of mitochondrial DNA disorders. Genet Med. 2013;15:388–394.
9. Pye D, et al. Production of transmitochondrial cybrids containing naturally occurring pathogenic mtDNA variants. Nucleic Acids Res. 2006;34:e95.
10. Human Mitochondrial Genome Database. http://www.mtdb.igp.uu.se/. Accessed 6 February 2020.

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