Genetics inMedicine | CORRESPONDENCE

Response to Bai et al.

Dear Editor:

The coauthors of "Interpretation of mitochondrial tRNA variants" wish to respond to the critique of Bai et al.[1] Several points raised by Bai et al. have been reviewed and discussed in our paper,[2] including the exceptional complexities of mitochondrial DNA (mtDNA)-related disorders. These complexities are the very reason why one should not expect completely deterministic rules.[2]

Ideally, classifications would use multivariate Bayesian analyses, but since necessary statistics are unavailable, we emulate the approach of Richards et al.,[3] which assigns and tallies scores for meeting defined conditions. This basic approach has proven useful for the classification of nuclear variants and we offer evidence that similar rules can be extended to mitochondrial transfer RNA (mt-tRNA) variants. Heteroplasmy, variation among tissues, high variant rate, selection, and phenotypic variation even within families are the major complications in interpreting mtDNA variants. We have derived a set of data-based rules that mitigate but do not invariantly solve these complexities. Such rules are needed to overcome biases in the early literature and to improve variant classification standards.

Just as the heuristics published by Richards et al.[3] often do not produce definitive determinations, the heuristics that we propose will leave a number of variants subject to reclassification as knowledge accrues. It strikes us as unreasonable to reject our schema based on the expectation that the aforementioned complexities will result in some classification differences among users. This is the case for the rules created by Richards et al.; user differences do not, however, render that model inapplicable or unsuitable.

Bai et al. state that the ≥5% heteroplasmy threshold is set too low. While it is true that phenotypic thresholds are generally higher, we selected the 5% threshold in response to biological selection against some variants and to heteroplasmy variation among tissues. A 5% threshold does not guarantee clinical relevance, but it does prevent the rejection of some significant variants. It is important to keep in mind that classification is not based solely on this threshold; other criteria may argue for or against pathogenicity. Both the 5% value in our paper and the 25% value proposed by Bai et al. are arbitrary. We believe that the 25% cutoff is too high since it may cause the rejection of clinically significant variants. In fact, there are numerous reports of pathogenic mtDNA variants in blood at <25% heteroplasmy, which were present at much higher levels (50–90%) in muscle, liver, or urine.[4–6]

Bai et al. mention that functional study methods must be reproducible, robust, and specific. That is exactly how a diagnostic laboratory operates. A diagnostic laboratory must use carefully validated assays including age and tissue matched controls. It is important to recognize, however, that some assays, such as electron transport chain (ETC) enzymology, can produce similar evidence for various genetic etiologies. Contrary to the assertion of Bai et al., this fact does not abrogate the utility of that assay. Due to sampling issues, we argue that when assays do not correlate with heteroplasmy measurements, the variant should receive only moderate weight (PM10).

It is correct that PS2 requires correlation in ≥2 tissues of the proband and not detected in the asymptomatic mother. If these criteria are not met, then there is no PS2. Bai et al. argue that this requirement is neither feasible nor necessary. As we have shown in a variety of publications, the measurements are technically feasible. With regard to necessity, comparisons among tissues and family members can provide data that are extremely useful for upgrading or downgrading a variant.[7,8]

It is correct that many variants in the public databases are incorrectly classified. We have stated that not all reports are reliable, especially studies published before the application of deep next-generation sequencing (NGS). For this reason, we have reviewed and re-evaluated all published variants. PS5 applies to previously reported pathogenic variants with evidential literature review supporting the pathogenic classification.

Bai et al. assert that mtDB is apparently not a valid population database.[9] While some ethnicities are overrepresented relative to others, most variants in this nondynamic database are present in excess of 3000 healthy individuals. The disease-causing variants listed as polymorphisms are a consequence of a lack of penetrance. These variants are well-known exceptions that do indeed reach polymorphic frequencies. These exceptions aside, the mtDB database does provide frequency data useful to variant classification.

PS4 is rarely used for mt-tRNA variants. It is mostly used for Leber hereditary optic neuropathy (LHON) where unaffected can be homoplasmic. We have not seen an mt-tRNA variant meeting this criterion, but we leave it there just in case.

PP4 is used when patient's phenotype or family history is highly specific for a mitochondrial disease with a single genetic etiology. For example, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS); myoclonic epilepsy with ragged red fibers (MERRF); maternal inherited hearing loss; etc. Thus, we take exception to the claim that PP4 requires comprehensive genetic testing, e.g., exome sequencing. Since a patient may have more than one disease, patients will need additional testing when the mtDNA variant does not fully explain the patient's clinical phenotype.[10,11]

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There are several misstatements by Bai et al. For example "pathogenic mt-tRNA variants are usually associated with early-onset severe diseases." In fact, plenty of evidence disputes this statement. The majority of mtDNA disorders, MELAS, MERRF, diabetes/hearing loss, Kearns–Sayre syndrome (KSS), mitochondrial myopathy, progressive external ophthalmoplegia (PEO), etc., are often adolescent or adult-onset diseases. Early-onset severe diseases are more commonly due to pathogenic variants in the nuclear genome.

The statement that cybrid assay is the only method to directly assay tRNA function is incorrect. The most direct way to assay tRNA function is to measure its ability to serve as a substrate for mitochondrial amino acyl tRNA synthetase.

In summary, we agree that our schema does not fully capture the breadth of variability in mt-tRNA variants, and we assert that such a claim can be levied on any classification model. Our article provides detailed mt-tRNA variant classification strategies based on our experience of over 25 years in the comprehensive mitochondrial diagnostic testing. The heuristics distilled from this experience enable the extrapolation of the American College of Medical Genetics and Genomics (ACMG) guidelines for the classification of nuclear genes to those of the mitochondrial genome. This schema will be beneficial to clinicians and diagnostic laboratories alike.

DISCLOSURE

The authors declare no conflicts of interest.

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