

What does phenotype have to do with it?

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A recurring theme in this column and in articles published in *Neurology® Genetics* is the problem of interpreting DNA variants, ever more present as next-generation sequencing invariably leads to the identification of multiple rare variants in a given individual. This is discussed in this issue in 2 articles and an accompanying editorial.^{1–3}

The Berkovic group critically examines reported variants in autosomal dominant epilepsies.¹ They make use of the ExAC database and in a 2nd analysis of the expanded ExAC V2 plus gnomAD databases with whole-exome and whole-genome sequencing data on 123,136 individuals and 15,496 individuals, respectively. They find that occurrence of a variant more than once in this database greatly reduces the probability that the respective variant is disease causing with high penetrance. Their analysis also reflects on the potentially erroneous assignments of DNA variants in *EFHC1* and *SRPX2* as disease causing in familial epilepsy syndromes.

The report by McNeil and colleagues² describes a molecular genetic case study of a patient presenting with an unusual leukodystrophy. The authors initially identify compound heterozygous mutations in the *EARS2* gene that led to reduction in protein levels by 70%. But as Massimo Pandolfo points out in the accompanying editorial,³ the typical clinical and imaging phenotypes associated with mutations in *EARS2* did not fit the phenotype of the patient. McNeill et al. were aided by a prior study of this patient that had also shown compound heterozygous mutations in *SNORD118*, encoding a small nucleolar RNA involved in ribosome biogenesis. In addition, the phenotype associated with *SNORD118* mutations actually fit the patient much better! We need to realize that the clinical or imaging subtleties of leukoencephalopathy, brain calcifications, and cysts vs leukoencephalopathy with thalamus and brainstem involvement and high lactate may escape the non-subspecialist.

Several years ago, we came across a similar scenario.⁴ By screening patients with autosomal dominant ataxias for mutations in *KCNC3*, the gene encoding the Kv3.3

potassium channel, we identified a Gly263Asp amino acid substitution. This variant occurred in an amino acid that was evolutionarily conserved, but not nearly to the same degree as amino acids changed by other verified *KCNC3* mutations. Mutation prediction programs predicted this amino acid substitution to be deleterious and indeed, on introducing the variant Kv3.3 channel into frog oocytes, the biophysical characteristics were altered. What saved the day for us was the fact that the patient had a concomitant repeat expansion mutation in *CACNA1A*, and the *KCNC3* variant did not segregate with other affected family members. In retrospect, a tip-off might have been that the biophysical changes in channel function, although significant, were relatively mild compared with the gain-of-function Phe448Leu mutation associated with early onset ataxia.⁵ Thus, significant may not always equal relevant.

As Pandolfo points out, it is important to examine the phenotype in its relationship to the gene variant and established phenotypes. Our colleagues in neuroradiology know this well and frequently add to their reports “clinical correlation is needed.” The same applies to the interpretation of in vitro assays. The compound heterozygous variants in *EARS2* described by McNeill et al. reduced protein levels to only 30% in the patient’s skin fibroblasts, probably not a sufficient reduction to be functionally important. The next challenge will be understanding the implications of changes in functional assays that may be significant in the context of in vitro studies, but may not be significant at the organismal level. Maybe biochemists and neurophysiologist need to append “clinical correlation required!”

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Sciences; has received license fee payments from Cedars-Sinai Medical Center; holds patents for Nucleic acids encoding ataxin-2 binding proteins, Nucleic acid encoding Schwannomin-binding proteins, Transgenic mouse expressing a polynucleotide encoding a human ataxin-2 polypeptide, Methods of detecting spinocerebellar ataxia-2 nucleic acids, Nucleic acid encoding spinocerebellar ataxia-2 to, Schwannomin-binding proteins, and Compositions and methods for spinocerebellar ataxia; and receives an honorarium from the AAN as the Editor of *Neurology: Genetics*. Go to Neurology.org/ng for full disclosure forms.

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