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CORRESPONDENCE

A commentary on identification of the rare compound heterozygous variants in the *NEB* gene in a Korean family with intellectual disability, epilepsy and early-childhood-onset generalized muscle weakness

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Jin *et al.*[1] reported two compound heterozygous missense variants in the nebulin gene (*NEB*) as the probable cause of intellectual disability, epilepsy and early-childhood-onset generalized muscle weakness in a Korean family with two affected children. The variants, c.2603T>C (p.L868P) in exon 27 and c.21340C>T (p.R7114W) in exon 143, had been identified by whole-exome sequencing (WES). Chromosomal abnormalities, gene copy number variation (CNV) and mitochondrial DNA mutations had been analyzed before WES. We argue, however, that the two reported alterations are highly unlikely to be disease causing.

NEB has 183 exons and encodes one of the largest polypeptides in human, with hundreds of different isoforms generated through alternative splicing.[2] Nebulin is predominantly expressed in skeletal muscles, but a lower expression of nebulin in pyramidal neurons and subcortical endothelial cells in adult human brain has also been reported.[3] The function of nebulin in brain is, however, still unknown.

In a recent mutational update we summarize 212 different pathogenic *NEB* variants causing different clinical subtypes of nemaline myopathy (NM) in 143 families and NM-related myopathies (core-rod myopathy, distal myopathy, fetal akinesia/lethal multiple pterygium syndrome) in 16 families.[4] Intellectual disability, epilepsy or any other brain abnormality were not present in any of the patients in our study, only the skeletal muscles were affected. In our experience, pathogenic missense variants in *NEB* are usually associated with milder disease phenotypes than the more disruptive variants such as frameshift and nonsense mutations.[5]

Exons 27 and 143 encode one simple repeat each in the super repeat region of nebulin. The super repeats bind actin and tropomyosin, and the binding sites on nebulin are conserved.[6] We have shown that missense variants in or close to these binding sites compromise actin and tropomyosin binding.[7] The variants reported by Jin *et al.*[1] are not in or close to actin- or tropomyosin-binding sites on nebulin. Nor do Jin *et al.* present any experimental data confirming that the variants p.L868P and p.R7114W have a negative effect on nebulin function.

Missense variants are very common in *NEB*. The current Exome Variant Server (EVS) release (ESP6500SI-V2, http://evs.gs.washington.edu/EVS/) contains 522 *NEB* missense variants, of which 107 (20%) are predicted to be benign, 98 (19%) to be possible damaging and 295 (56%) are predicted to be probably damaging by PolyPhen2. For the remaining 22 variants no prediction could be made. The EVS data set contains samples of 2203 African-American and 4300 European-American unrelated individuals, totaling 6503 samples. The samples are from healthy controls, as well as from individuals representing the extremes of specific traits (low-density lipoprotein and blood pressure), and specific diseases (early-onset myocardial infarction and early-onset stroke) and lung diseases. Six European-American heterozygous carriers of the p.L868P variant are listed in EVS. The p.R7114W variant is not included in EVS. It seems highly unlikely, that >50% of all missense variants in *NEB* identified in the EVS cohorts would compromise the normal functions of nebulin. A very high false positive rate (75%), that is, damaging predictions for neutral variants, has indeed been reported for PolyPhen2.[8] Both p.L868P and p.R7114W are predicted to be probably damaging by PolyPhen2, but both are predicted to be neutral with the improved prediction tool MEGA-MD.[9]

On the basis of the evidence above, our view is that the variants p.L868P and p.R7114W are highly unlikely to be causative of the complex and quite severe phenotype in the Korean family. It should always be kept in mind that WES does not completely cover the whole coding region of the human genome. Many important regions, such as GC-rich first exons of genes and repetitive exons (for example, *NEB* exons 82–105) are usually not covered in the WES panels. Moreover, the resolution of conventional whole-genome array-CGH is on average 200 kb or lower, which means that CNVs <200 kb are not detected. CNVs of a few kilobases are not readily detected by WES either. Therefore, the true disease-causing variants in the Korean family may well have escaped detection with the methods used.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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