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LETTER

Comment on 'Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease'

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In a recent publication in the *European Journal of Human Genetics*, Khajavi *et al*[1] reviewed the physiological role of nonsense-mediated mRNA decay (NMD), its implications for human disease, and why knowledge of NMD is important to understand genotype–phenotype correlations in various genetic disorders. These authors stated that if a transcript carries a premature translation termination codon located more than 50–54 nucleotides upstream the last exon–exon junction, then NMD is triggered to prevent translation, thereby reducing the dominant-negative effect of the C-terminally truncated protein that could be produced.

However, it must be noted that if a premature translation termination codon is sufficiently AUG-proximal, it does not follow the '50–54 nucleotides boundary rule'.[2,3] For example, mutant human β-globin mRNAs with AUG-proximal nonsense mutations escape NMD.[4] Nevertheless, the thalassemic phenotypes of heterozygotes and homozygotes for AUG-proximal nonsense mutations do not appear to be any more or less severe than in patients carrying more distal nonsense mutations that are effectively targeted to NMD. We have demonstrated that the observed NMD inhibition is determined by the proximity of the nonsense codon to the initiation AUG.[2] In addition, the AUG-proximity effect may also operate in parallel with other modifying influences to inhibit NMD.[3] Specifically, the occurrence of translation re-initiation 3′ to the short open reading frame may also contribute to the alleviation of the NMD effect.[3,5] These two parameters can independently contribute to the net overall NMD resistance of a nonsense-containing mRNA with implications for genotype–phenotype correlations in various human genetic disorders. Specifically, if translation re-initiation occurs in-frame downstream to the nonsense codon, it produces an N-terminally truncated protein that may or may not be functional. Some results have been reported, in which translation reinitiation at internal AUG codons modulates disease phenotypes.[6–12] As a recent example, Sanchez-Sanchez *et al*[6] have reported a novel mutation detected in the retinoblastoma 1 (*RB1*) gene in 10 individuals of an extended family, but only three of whom are affected by retinoblastoma. The mutation comprises a 23-basepair duplication in the *RB1* first exon, producing a premature translation termination in exon 2, and no appreciable NMD is induced. Instead, transcription–translation *in vitro* assays revealed that alternative in-frame translation start sites involving methionine (Met) 113 and possibly Met233 are used to generate N-terminally truncated *RB1* products, known and suspected to exhibit tumor suppressor activity. These results strongly suggest that modulation of disease penetrance in this family is achieved by translation re-initiation downstream to a nonsense codon.[6]

In conclusion, when analyzing the genotype–phenotype correlations in genetic disorders owing to nonsense codons, besides the fact that NMD may modulate the corresponding clinical outcome, it is also important to consider that some nonsense codons, although located more than 50–54 nucleotides upstream the 3′-most exon–exon junction of a transcript, do not induce NMD. Instead, translation re-initiation at downstream internal AUG codons may also modulate human disease phenotypes and should therefore be considered whenever unexpected phenotypes resulting from severe mutations located early in the coding region are observed.

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Reply to Inácio *et al*

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We have recently reviewed the physiological role of nonsense-mediated decay (NMD) and its implications for human genetic disorders. The goal of our review paper was to make the medical community aware that mRNA processing steps must be taken into consideration when attempting to elucidate genotype–phenotype relationships.[1] Although the exact mechanism for NMD remains to be determined, previous studies in model organisms provide insight into how transcripts containing premature termination codons (PTCs) are recognized by NMD factors and rapidly degraded resulting in loss-of-function alleles.[2] Evidence from a number of studies suggests a model for the mechanism of NMD surveillance system in which a PTC in the last exon, or within less than ~55 base pairs from the final intron in penultimate exon (also known as '55 nt boundary rule'), is not recognized and may cause expression of large amounts of aberrant truncated proteins with potential harmful effects in cells. We also review and cite the observations that several genes appear to show exception to this rule and thus, mRNAs with nonsense codons present in such positions are subject to the NMD pathway. This suggests either additional or entirely different *cis*-acting signals may exist to initiate NMD for select genes.[3]

NMD mechanistic models will require refinement with additional experimental observations as more genes (eg *HBB* for human β-globin and *BRCA1*) are examined. Recent studies have shown that premature nonsense mutations located proximal to an internal AUG in some genes may escape NMD and result in synthesis of N-terminally truncated proteins by a 'translation re-initiation' mechanism.[4–6] The extent of the effect of escaping NMD by this mechanism and its implications for genotype–phenotype correlations in human disease may also vary depending on both the toxicity of truncated proteins and the nature of traits encoded by different genes. These observations define another factor that can contribute to the complexity and efficiency of the NMD surveillance system in regulating gene expression.

Nevertheless, these findings further support the thesis of our review that one must recognize the role of mRNA and protein processing steps (ie information transfer downstream from the DNA mutation) in conveying the ultimate clinical outcome. It also illustrates the view that understanding of the molecular mechanism of NMD is far from complete and there are no 'universal rules' that have been defined for all genes. Thus, it is essential to carefully verify the potential role of NMD experimentally to enable accurate conclusions regarding genotype–phenotype correlations underlying genetic disorders.

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