# Clinical Significance of mRNA Nonstop Decay in Rare Disease Diagnosis

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## INTRODUCTION

- Nonstop decay (NSD) is a cellular surveillance mechanism that targets and degrades mRNAs lacking stop codons, preventing the translation of potentially harmful truncated proteins.[1,2]
- Stop-loss variants, such as stop codon single nucleotide variants or frameshift variants upstream of the natural stop codon, can trigger NSD if no downstream compensatory stop codon is present before the poly-A tail (**Figure 1**).
- Despite the well-established functional importance of the NSD pathway, these variants currently are not included in the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) variant interpretation guidelines nor the Clinical Genome Resource (ClinGen) Sequence Variant Interpretation (SVI) Workgroup's PVS1 criterion recommendations, potentially leading to the underestimation of these pathogenic variants in the diagnosis of rare diseases.[3,4]

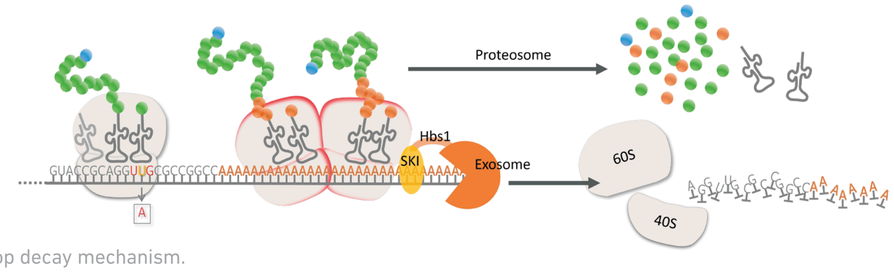


**Figure 1.** Nonstop decay mechanism.

## METHODS

- We developed a computational pipeline to assess genomic positions for missense and small indel variants that can trigger NSD for disease-related human genes.
- We retrieved OMIM's list of 4,954 disease-related protein coding genes, gathered sequences and genetic coordinates for each canonical transcript's exons from Ensembl, and determined the start and stop codon by aligning full mRNA sequences with the corresponding coding sequence.
- We then developed scripts to analyze all mRNA sequences and extracted genetic loci that can bury NSD variants (**Figure 2**). Variants located in the destinate genetic loci were extracted from 2.439 genome sequencing (GS) VCF, and the NSD candidate variants were evaluated with post-screening analysis.


**Figure 2.** Sequence analysis to identify NSD bury genomic regions. For each gene, the mRNA is analyzed for all three frames. For each frame, the start codon is highlighted in blue, the last stop codon is highlighted in red, and regions identified that can bury NSD variants are highlighted in yellow.

## RESULTS

- We identified 333 genes with potential for NSD considering all three reading frames, representing **6.72%** of all the 4,954 known disease-related genes from OMIM. From these 333 NSD-potential genes, we identified 546 exonic regions capable of harboring NSD variants. These loci are scattered throughout the genome (**Figure 3**).

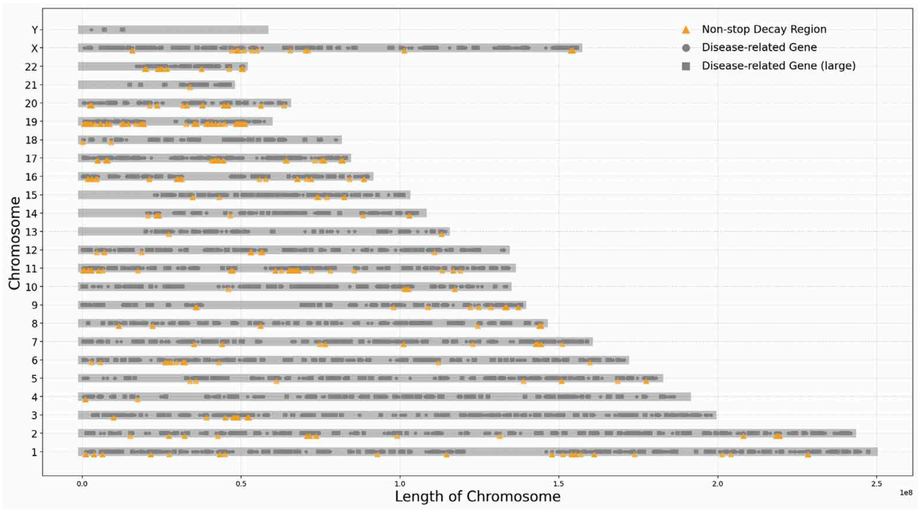


**Figure 3.** Distribution of NSD capable loci throughout the genome.

- A total of 56,908 SNV and small indels were found located within these 546 regions from 2,439 GS VCFs, and 356 out of which are NSD variants filtered with post-screening criteria. 332 out of the 356 identified NSD variants are in blood type related genes, which leaves 24 NSD variants identified in disease related genes (**Figure 4**).

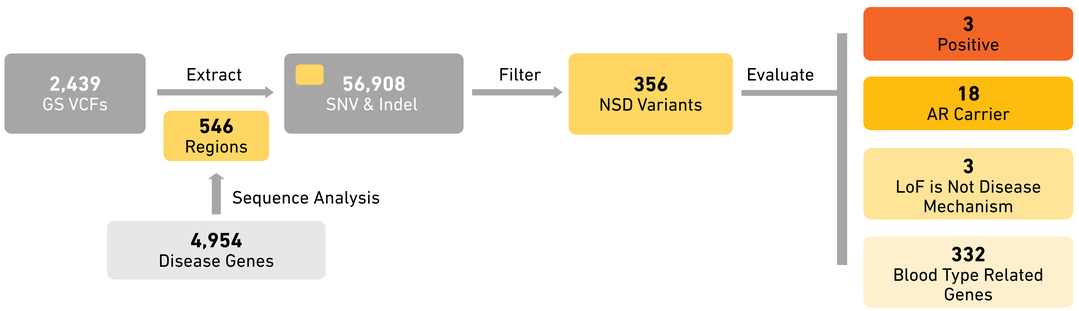


**Figure 4.** NSD variant identification and evaluation yields.

- Notably, we present here three GS cases in which NSD variants were identified in the disease-causing genes that are consistent with the clinical phenotypes of the patients (**Table 1**). **These NSD variants would have been classified as variants of uncertain significance under the current ACMG/AMP variant interpretation guidelines, underscoring the importance of recognizing mRNA nonstop decay in rare disease diagnosis**. Meanwhile, 18 other cases are carriers of NSD variants in autosomal recessive diseases not related to patients' phenotypes.

| Gene | Disease | Inheritance | Variant | Protein Length | Patients' Phenotype | ACMG/AMP/ ClinGen Classification |
|---|---|---|---|---|---|---|
| ***NDUFS7*** | Mitochondrial Complex I Deficiency, Nuclear Type 3 (OMIM: 618224) | AR Comp Het | NM_024407.5: c.610del (p.E204Sfs*?); c.364G>A (p.V122M) | 213 | Respiratory failure, Abnormal brain MRI, Motor developmental delay, Hypotonia, Lethargy, Respiratory arrest | VUS |
| ***ISG15*** | Immunodeficiency 38 (OMIM: 616126) | AR Hom | NM_005101.4: c.463dup (p.R155Pfs*?) | 165 | Skin rash, Recurrent skin infections, Dry skin, Mucosal ulcers | VUS |
| ***HCN2*** | HCN2 -related Epilepsy (OMIM: 602477) | AD De Novo | NM_001194.4: c.2328_2334dup (p.S779Pfs*?) | 889 | Focal -onset seizure, EEG abnormality, Brain imaging abnormality, Hypertonia | VUS |

**Table 1.** Positive GS cases with NSD variants identified in disease-causing genes that are consistent with the clinical phenotypes of the patients.

## CONCLUSIONS

NSD variants are clinically significant in rare disease diagnosis and are prevalent across genes associated with human diseases. These results support applying the PVS1 criterion in the ACMG/AMP variant interpretation guidelines to stop-loss variants in identified NSD susceptible genes to improve diagnostic accuracy and enhance patient care.

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References: 1) Monaghan et al., EMBO J. 2023, PMID: 37605642. 2) Powers et al., Curr Opin Struct Biol. 2020, PMID: 32688260. 3) Richards et al., Genet Med. 2015, PMID: 25741868. 4) Abou Tayoun et al., Hum Mutat. 2018, PMID: 30192042.