Variants that cross the line: An internal review of classification evidence for truncations of the C-terminus

Megan Judkins; Jessica Connor; John Castiblanco; Nicole Beier; Matthew Brown; Christine Lo; Eric Evans; H. Peter Kang; Rebecca Mar-Heyming

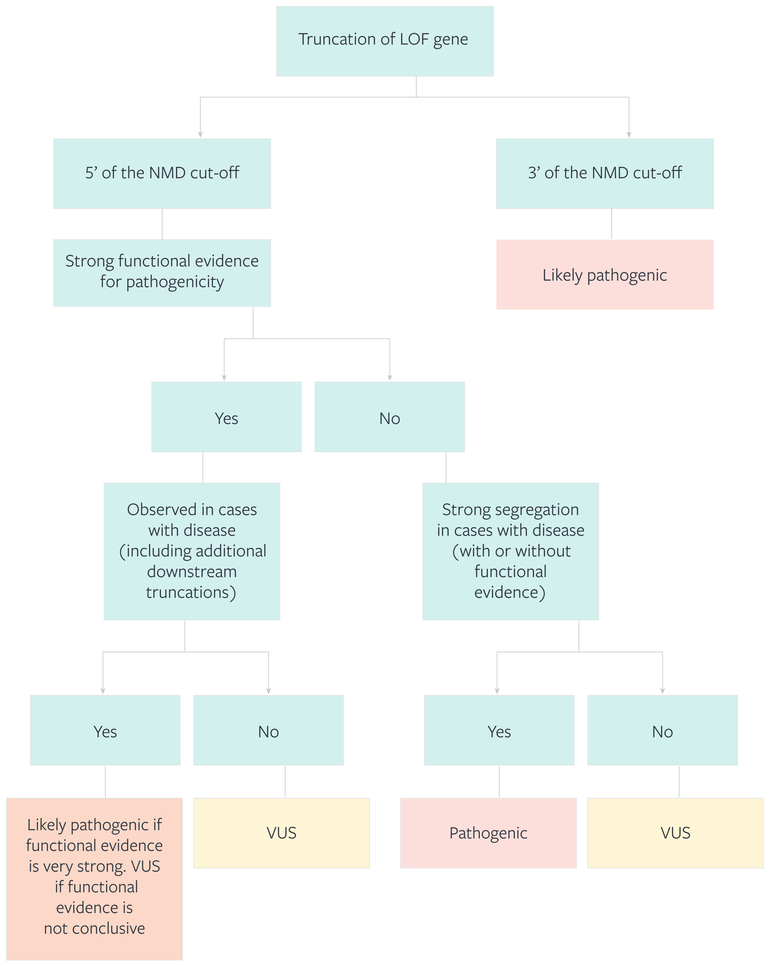
Introduction

Most genetic variants that result in premature protein truncation subject to nonsense-mediated decay (NMD) are pathogenic by their nature. Because of this, classification of truncating variants in LOF genes is relatively straightforward based on ACMG guidelines. However, truncations after the theoretical NMD boundary, generally considered to be 50 bp upstream of the penultimate exon, may not be subject to NMD. While many non-NMD truncating variants are pathogenic, case, functional, and structural evidence must be carefully evaluated in order to assign an appropriate classification. Other mechanisms, such as disruption of the C-terminus or another critical functional domain, may be causal. Evaluating the evidence for pathogenicity of non-NMD truncations can be especially challenging in genes associated with hereditary cancer given the high rate of disease occurrence in the population. Here, we sought to evaluate the evidence for pathogenicity of non-NMD truncations on a per-gene basis by aggregating the evidence available for classification of variants observed at Counsyl beyond the NMD boundary.

Methods

We performed a systematic review of truncations past the NMD boundary observed on Counsyl's ReliantTM Cancer Screen panel for hereditary cancers. We then reviewed each of the variants observed at Counsyl along with the most 3' pathogenic truncation annotated in ClinVar to establish where the last known pathogenic mutation for each gene was located. Each variant observed at Counsyl was classified based upon Counsyl's criteria (Figure 1). While these criteria allow for classification of the majority of variants, there are rare exceptions.

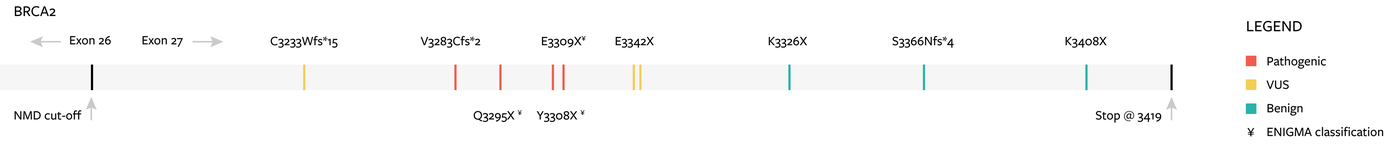
Figure 1



Results

- A total of 55 non-NMD truncating variants were observed. The data used for classification and a summary of available evidence for each gene is described in Table 1.
- The truncations were observed in 15 of 28 genes associated with hereditary cancer tested at Counsyl. The other 13 genes had no non-NMD truncations observed at Counsyl.
- The genes with conclusive evidence for pathogenicity for non-NMD truncations include ATM, BRCA1, CDKN2A, MLH1, MSH6, PALB2 and PTEN. These genes had a combination of strong functional evidence and observation in multiple cases with associated cancers OR strong segregation in families with associated hereditary cancer syndromes.
- APC, BRIP1, CHEK2, MSH2, NBN, and RAD51D had less compelling evidence for pathogenicity. While some of these genes had evidence for pathogenicity, the evidence was not conclusive in all cases.
- BRCA2 was unique in that there is compelling evidence that truncations past residue 3326 are benign (Figure 2).

Figure 2



Conclusions

- Evaluating a single non-NMD truncation in isolation may not provide an accurate picture of the mechanism for pathogenicity in the C-terminal region for a given gene. Comprehensive review of all truncations past the NMD boundary on a per-gene basis can provide more complete data on which to base classification decisions.
- APC, BRIP1, CHEK2, MSH2, NBN, and RAD51D may require further definition of the mechanism and precise boundary for pathogenicity. Segregation studies in hereditary cancer families with non-NMD truncations in these genes may provide valuable data regarding pathogenicity.
- By compiling the information available for classification of non-NMD truncations, variant classification scientists can more accurately determine which mutations may benefit from additional functional analysis and/or segregation studies in families with disease.

Table 1: Review of evidence for non-NMD truncating variants

Gene	# of non-NMD truncating variants observed at Counsyl I	Most 3' **Pathogenic** at **VUS** at Counsyl	Most 3' Pathogenic at ClinVar*	Length of protein (amino acids)	Summary of Evidence
APC	14	c.6371T>A (p.L2124X)	c.8047delA (p.I2683Lfs*40; stop at 2722)	2843	The last exon of APC comprises 77% of the gene, therefore the majority of truncations are past the NMD cut-off. There are numerous pathogenic truncations observed in both ClinVar and at Counsyl in this region. Functional data for pathogenicity for the most 3' truncations is limited. Literature suggests that truncation of the PDZ-binding motif in APC abolishes linkage of microtubules to the plasma membrane in Xenopus A6 epithelial cells (PMID 17295841). The most 3' truncation at Counsyl is c.8441-8444delAGAA (p.K2814Sfs*26) which is classified as VUS.
ATM	4	c.9139C>T (p.R3047X)	c.9139C>T (p.R3047X)	3056	There is functional evidence in patient cell lines supporting pathogenicity (PMID 19431188); There are patients reported with Ataxia Telangiectasia (AT) phenotype as well as breast cancer. The AT phenotype provides additional support for pathogenicity in ATM.
BRCA1	5	c.5558dupA (p.Y1853*fs; stop at 1853)	c.5534delA (p.Y1845Sfs*10, stop at 1854)	1864	There is functional evidence that the BRCA1 C-terminal hydrophobic cluster is necessary for transcription activity (PMID 10811118); Additionally, there are multiple patients with classic HBOC phenotype with truncations in this region.
BRCA2	6	c.9845dupC (p.V3283Cfs*2; stop at 3284)	c.9946G>T (p.G3316X)	3419	There is functional evidence that truncations past amino acid 3326 are not pathogenic (PMID 18607349, 24123850, 15695382). There is also some phenotypic evidence that non-NMD truncations may lead to a reduced penetrance phenotype.
BRIP1	4	c.3390_3393delCTAT (p.Y1131LfS*18; stop at 1148)**	c.3232A>T (p.K1078X)	1250	There is functional evidence that truncations may disrupt a key phosphorylated threonine at 1133 which is important for TOPBP1 interaction (PMID 21127055, 21059562). Additionally, truncations may remove two modified residues at 1227 and 1249 which are important for DNA damage response (PMID 22792074). Because of the reduced penetrance nature of this gene phenotypic evidence has limited utility. There are multiple truncations in this region withtruncation at conflicting classifications in ClinVar which highlights the complexity of interpreting the evidence for pathogenicity of this region in BRIP1.
CDKN2A	1	c.225-243del19 (p.A76Cfs*64; stop at 139)	c.358delG (p.G120Sfs*26, stop at 145)	157	There is functional evidence for pathogenicity including greatly reduced affinity for CDK4 and CDK6, mislocalization, reduced ki67 expression, and altered p16 distribution (PMID 20340136). Additionally, c.225-243del19 is a well described Dutch founder mutation. There are numerous cases with hereditary melanoma and pancreatic cancer that show segregation with truncations in this region.
CHEK2	1	c.1522dupC (p.L508Pfs*11; stop at 518)	c.1528C>T (p.Q510X)	544	There is functional evidence to suggest that the NLS-3 motif may be disrupted which is required for nuclear localization of Chk2 in cells (PMID 12909615). However, because of the reduced penetrance nature of this gene phenotypic evidence has limited utility.
MLH1	1	c.2252_2253delAA (p.K751Sfs*3; stop at 753)	c.2252_2253delAA (p.K751Sfs*3; stop at 753)	757	While there is limited functional evidence for pathogenicity, c.2252_2253delAA is a well described Italian founder mutation with segregation in multiple HNPCC families (PMID 24802709).
MSH2	1	c.2785C>T (p.R929X), VUS**	c.2680dupA (p.M894Nfs*5; stop at 898)	935	There are no pathogenic non-NMD truncations observed at Counsyl. While there are known functional domains in the last exon of MSH2, there is no functional evidence establishing a mechanism for pathogenicity in this region. While p.R929X has been observed in cases with the HNPCC phenotype, there is also co-observation with other pathogenic variants in patients that do not have CMMRD.
MSH6	5	c.3840_3846del7 (p.E1281Lfs*44; stop at 1324)	c.4004_4007dupAAGT (p.C1337Sfs*5, stop at 1341)	1361	There is functional evidence for pathogenicity in non-NMD truncations including reduced expression in patient cells (PMID 16418736). There is also phenotypic evidence with multiple alleles segregating with disease in HNPCC families. One variant, (c.4068_4071dupGATT) is observed at high frequency in control populations (ExAC EAS 3.15%; 5 hom) and is classified as benign.
NBN	4	c.2205delA (p.E736Kfs*15; stop at 750), VUS	NA (no pathogenic truncations in last exon)	755	All four non-NMD truncations observed at Counsyl are classified as VUS. While there is functional evidence that the C-terminus is necessary for MRE11 binding and cellular survival after radiation exposure, there is no clear role definition of this region (PMID 11238951). Given the reduced penetrance nature of this gene, phenotypic evidence has limited utility.
PALB2	5	c.3549C>A & c.3549C>G (p.Y1183X)	c.3549C>A & c.3549C>G (p.Y1183X)	1187	Functional studies include patient lymphoblastoid cells showing no protein expression and disruption of RAD51 and BRCA2 binding (PMID 17200671, 19584259). Additionally, non-NMD truncations in this region are shown to segregate in patients with disease.
PTEN	1	c.1003C>T (p.R335*)	c.1048dupA (p.T350Nfs; stop at 360)	404	Functional evidence for pathogenicity includes reduced protein expression in patient cells (PMID 23475934). Additionally, non-NMD truncations are observed in multiple patients with Cowden syndrome.
RAD51D	2	c.955C>T (p.Q319*), VUS**	c.803G>A (p.W268*)	329	Both non-NMD truncations in RAD51D observed at Counsyl are classified as VUS. Functional evidence includes yeast two-hybrid analysis and shows that the c-terminal region is needed for interaction with RAD51C, however the function of the last 23 amino acids was not evaluated (PMID 14704354, 19327148). Given the reduced penetrance nature of this gene, phenotypic evidence has limited utility.

* ClinVar variants selected had a consensus classification by all submitters
** ClinVar classification discrepancy

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