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– ORIGINAL PAPER –

ACMG-Based Reclassification of TP53 Variants in Oncogenetic Practice

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Abstract

Introduction*: Variants of uncertain significance (VUS) in cancer predisposition genes, such as TP53, represent a major challenge in clinical interpretation. The p53 protein functions as a tumor suppressor involved in cell cycle regulation, apoptosis, and DNA repair mechanisms. Loss-of-function variants may contribute to the development of various cancer types or to the Li-Fraumeni syndrome. The DNA-binding domain (DBD) is essential for p53 function, and variants occurring within this region may have significant functional impact.*

Materials and Methods: *TP53 variants located within the DNA-binding domain and classified as VUS were extracted from the ClinVar database. A total of 93 variants met the inclusion criteria and were re-evaluated according to ACMG/AMP guidelines and available literature data. Bioinformatic tools, including gnomAD and MutationTaster, were used to support the reclassification process.*

Results: *Among the 93 VUS variants analysed, 6 variants (6.45%) retained their VUS status, whereas 87 variants (93.55%) were reclassified as Likely Pathogenic, supporting the clinical relevance of their localization within a key functional domain. Although six variants initially reached a cumulative score of 5 points, partly due to their absence from large population databases such as gnomAD, the available evidence was insufficient to justify a change toward a benign classification.*

Conclusions: *Reclassification of TP53 variants within the DNA-binding domain highlights the importance of periodic re-evaluation of VUS. The integration of functional, bioinformatic, and clinical data, together with expert genetic assessment, is essential for accurate diagnostic interpretation and appropriate clinical management.*

Keywords*: TP53 variants, Variants of uncertain significance (VUS), ACMG/AMP guidelines*

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Introduction

TP53 gene, also referred to as the "*guardian of the genome*", plays an essential role in maintaining genomic stability and regulating the cell cycle. Variants occurring within the coding regions of the TP53 gene lead to the formation of a functionally inactive or absent p53 protein, an event directly involved in the initiation and progression of malignant pathologies. For this reason, the classification of TP53 gene variants represents a major challenge, requiring periodic evaluation and reclassification based on newly available functional and clinical data. The most frequent types of cancer associated with alteration of the TP53 gene are high-grade serous ovarian cancer and small-cell lung cancer; however, the frequency of these alterations is heterogeneous across

other types of neoplasms (Baliakas & Soussi, 2025). In addition, in malignant hematological disorders, TP53 gene alterations are frequently encountered, particularly in advanced stages of the disease (Olivier & Hainaut, 2008; Soussi & Wiman, 2015).

The main functions of the TP53 gene include regulation of the cell cycle and activation of DNA repair mechanisms, but the gene is also involved in other essential biological processes, such as cellular metabolism and the immune response. When a cell undergoes irreversible alterations of its genetic material, functional p53 protein induces apoptosis, thereby preventing propagation of cells with an unstable genome. TP53 is considered a tumour suppressor due to its role in initiating programmed cell death in affected cells (Bouaoun et al., 2016). Given the essential roles of the TP53 gene, any alteration affecting it, followed by the expression of an aberrant p53 protein may lead to the development of neoplastic processes.

TP53 gene is located on the short arm of chromosome 17 (17p31.1), consists of 11 exons, and encodes the cellular tumour antigen p53 (Doffe et al., 2020a). The p53 protein is structured into five functional domains: the transactivation domain (TAD), the proline-rich domain (PRD), the DNA-binding domain (DBD), the tetramerization domain (TET), and the negative regulatory domain (NEG-REG). The TAD is divided into two regions, both responsible for the transcriptional activation of downstream target genes. The PRD is involved in regulation of apoptosis, the TET domain mediates oligomerization of p53 monomers, and the DBD enables specific DNA binding (Baliakas & Soussi, 2025; Giacomelli et al., 2018a; *TP53 Variations in Human Cancers: New Lessons from the IARC TP53 Database and Genomics Data – IARC*, n.d.).

In healthy cells, p53 protein expression levels are low, with the protein being predominantly present in the cytoplasm in the form of dimers, exhibiting reduced DNA-binding capacity. In response to cellular stress signals (hypoxia, oncogene activation, DNA damage, ribosomal dysfunction), p53 accumulates intracellularly, and adopts an active tetrameric structure associated with increased DNA affinity. Activation of p53 initiates transcriptional processes that regulate the cellular response to stress (Baliakas & Soussi, 2025).

The DNA-binding domain constitutes the functional core of the p53 protein and represents the most extensive domain of the TP53 gene, comprising approximately 600 nucleotides. At the same time, it is a fragile domain, as nucleotide substitutions located within the DBD have major consequences on p53 protein function. Genetic variants lead to partial or complete loss of DNA-binding capacity, resulting in dysregulation of cell cycle control mechanisms. The most frequently encountered alterations are missense nucleotide substitutions, some of which exhibit preferential distribution depending on the histopathological type of the neoplasm.

Given the significant functional impact of variants occurring within the DBD, periodic classification and reclassification of these variants are essential for the accurate interpretation of their biological and clinical relevance.

In the context of the essential role of the DNA-binding domain and the high frequency of variants identified at this level, the accurate interpretation of variants of uncertain significance (VUS) remains a major challenge in oncological clinical practice. The lack of conclusive functional data frequently leads to the classification of these variants as VUS, thereby limiting their clinical and therapeutic utility. The primary objective of this study is the reclassification of VUS identified within the DBD of the TP53 gene by integrating updated classification criteria, with the aim of improving the interpretation of their clinical relevance (Doffe et al., 2020b; Giacomelli et al., 2018a).

Materials and Methods

In this study, 93 genetic variants initially classified as variants of uncertain significance (VUS) were analysed. These variants were located within the DNA-binding domain (DBD) of the TP53 gene and were retrieved from the public ClinVar database, which provides information on the clinical significance of reported genetic variants. The inclusion criteria were as follows: variants identified in the TP53 gene; localization of the variants within the DBD; initial classification as VUS; missense-type variants. Variants that did not simultaneously meet all inclusion criteria were excluded from the analysis.

Reclassification was performed in accordance with the updated criteria of the ACMG/AMP guidelines for the interpretation of genetic variants. Each variant was individually re-evaluated through the systematic application of classification criteria, considering available data on allelic frequency and the potential impact on p53 protein function.

The ACMG/AMP guideline (Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical

Genetics and Genomics and the Association for Molecular Pathology) represents the internationally accepted reference document for the classification of genetic variants. This guideline employs standardized terminology (pathogenic, likely pathogenic, variant of uncertain significance, likely benign, benign) to describe variants identified in genes with Mendelian inheritance. The assignment of variants to one of these five categories is based on evidence criteria, including population data, computational or predictive data, and functional evidence, which support the pathogenic or benign nature of a given variant (Giacomelli et al., 2018b).

For the assessment of allelic frequency, providing population-level data on each variant, the gnomAD (Genome Aggregation Database) was utilized. The functional impact of missense variants on the p53 protein was estimated using the MutationTaster in-silico tool, which integrates information regarding structural alterations and effects on splicing. Data obtained from these sources were correlated and integrated into the variant reclassification process, in accordance with the recommendations of the ACMG/AMP guidelines, ensuring a comprehensive and standardized interpretation framework.

Results

A total of 93 germline missense variants located within the DNA-binding domain (DBD) of the TP53 were analyzed. All variants were initially classified as variants of uncertain significance (VUS) in the ClinVar database (accessed in August 2025). Applying the 2024 ACGS Best Practice Guidelines for Variant Classification in Rare Disease led to the use of the following criteria: PS4, PM1, PM2, PM5, and PP3. The scoring system assigned 4 points for the strong criterion PS4, 2 points for the moderate criteria PM1, PM2, and PM5, and 1 point for the supporting criterion PP3, as shown in Table 1. Thresholds for final classification were defined as ≥10 points (Pathogenic), 6-9 points (Likely Pathogenic), and 0-5 points (VUS).

Following re-evaluation, 87 out of 93 variants were reclassified as Likely Pathogenic, whereas 6 variants retained their VUS status (Figure 1). This reclassification highlights the substantial contribution of the scoring system in assigning pathogenicity based on accumulated evidence.

Table 1. *Reclassification from VUS to Likely Pathogenic: Applied ACMG Criteria*

Variant	*Protein*	*PS4*	*PM1*	*PM5*	*PP3*	*SCORE*	*Initial Status*	*Reclassification*
c.845G>T	*Arg282Leu*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.800G>A	*Arg267Gln*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.776A>G	*Asp259Gly*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.764T>C	*Ile255Thr*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.753C>G	*Ile251Met*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.730G>C	*Gly244Arg*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.720T>A	*Ser240Arg*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.710T>C	*Met237Thr*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.595G>A	*Gly199Arg*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.587G>A	*Arg196Gln*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.568C>G	*Pro190Ala*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.540G>T	*Glu180Asp*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.530C>A	*Pro177His*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.523C>T	*Arg175Cys*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.488A>C	*Tyr163Ser*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.467G>A	*Arg156His*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*
c.393C>A	*Asn131Lys*	*PS4*	*PM1*	*PM5*	*PP3*	*9*	*VUS*	*Likely pathogenic*

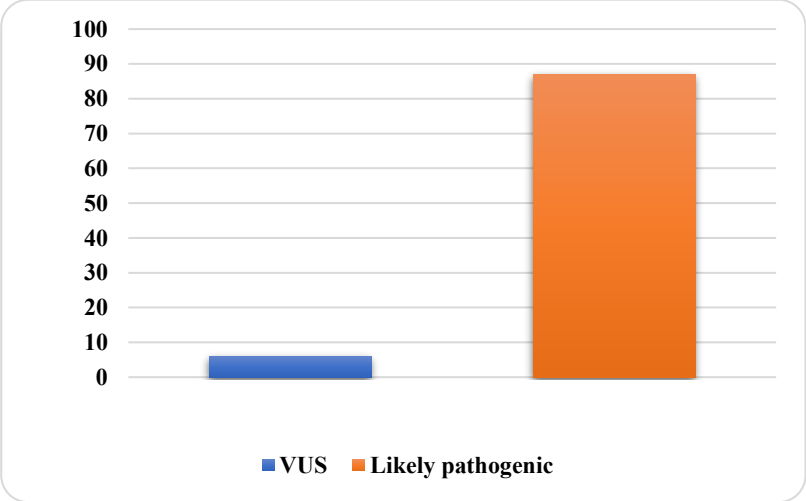

Figure 1. *Shift in variant classification after ACMG-based re-evaluation*

The distribution of applied ACMG criteria is summarized in Table 2. All 93 variants (100%) fulfilled the moderate criterion PM1, reflecting their localization within the DNA-binding domain of the TP53. The PM5 criterion was met by 87 variants (93.5%), corresponding to the presence of a previously reported pathogenic missense variant at the same amino acid residue, while 6 variants did not fulfill PM5. PM2 was met by 74 variants (79.6%). The PP3 criterion was fulfilled by 91 variants (97.8%), all supported by deleterious in silico predictions; only 2 variants (2.2%) did not meet PP3, being reported as benign by computational prediction tools. The PS4 criterion was applied to 19 variants (20.4%).

Table 2. *Distribution of applied ACMG criteria among reclassified TP53 variants*

Criterion	*Criterion Type*	*Variants, n (n=93)*	*Percentage(%)*
PS4	*Strong*	*19*	*20.4*
PM1	*Moderate*	*93*	*100*
PM2	*Moderate*	*74*	*79.6*
PM5	*Moderate*	*87*	*93.5*
PP3	*Supporting*	*91*	*97.8*

Cumulative scores of the variants are presented in Table 3. Six variants achieved a total score of 5 points, 68 variants scored 7 points, 2 variants scored 8 points, and 17 variants reached 9 points. Notably, variants with scores of 5 points retained their VUS classification, while those scoring 7–9 points were reclassified as Likely Pathogenic, demonstrating the direct impact of the cumulative scoring system on final variant classification.

Table 3. *Score-based distribution of reclassified variants*

Score	*Variants, n (n=93)*	*Percentage (%)*
5	*6*	*6.4*
7	*68*	*73.1*
8	*2*	*2.2*
9	*17*	*18.3*

Table 1 provides a detailed overview of all variants achieving the highest cumulative score (9 points), including their initial classification, applied criteria, individual scores, and final classification. Table 2 summarizes the number of variants fulfilling each ACMG criterion, and Table 3 presents the distribution of cumulative scores across the entire cohort. Figure 1 illustrates the proportion of variants classified as VUS versus Likely Pathogenic.

Conclusions and Discussion

The reclassification of missense variants located within the DNA-binding domain (DBD) of the TP53 highlights the critical importance of systematic and periodic reinterpretation of variants initially classified as variants of uncertain significance (VUS). As genomic databases expand and variant interpretation frameworks evolve, variant calls should not be regarded as static entities but rather as dynamic assessments subject to refinement over time. Our findings demonstrate that the application of updated classification criteria can substantially modify the interpretative landscape, leading to clinically meaningful reclassification in a significant proportion of cases.

A notable proportion of variants fulfilled the PM2 criterion due to their absence from large population databases such as gnomAD. However, absence from population datasets should not be interpreted as independent confirmation of pathogenicity. Instead, rarity represents only one component of the overall evidentiary framework and must be carefully contextualized alongside functional data, computational predictions, segregation evidence, and clinical correlations. These results underscore the need for cautious interpretation of population frequency data, particularly in genes with strong disease associations and domain-specific functional constraints.

Collectively, our study supports a dynamic and integrative approach to variant interpretation, in which functional, bioinformatic, and clinical evidence are continuously reassessed in light of emerging data. Regular re-evaluation of VUS is essential to prevent prolonged diagnostic uncertainty and to ensure appropriate genetic counselling and clinical management. Ultimately, expert clinical judgment remains indispensable for translating evolving genomic evidence into accurate diagnostic conclusions and informed therapeutic decision-making.

Ethics Statement and Conflict of Interest Disclosures

Financial support and sponsorship: All authors have declared that no financial support was received from any organization for the submitted work.

Conflict of interest:

No known conflict of interest correlated with this publication.

Availability of data and materials:

The data used and/or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interests:

The authors declared that they have no competing interests.

The use of generative AI and AI-assisted technologies:

AI technologies were used solely to refine English language and spelling

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