ARTICLE

Consistency of variant interpretations among bioinformaticians and clinical geneticists in hereditary cancer panels

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Next-generation sequencing (NGS) is used increasingly in hereditary cancer patients' (HCP) management. While enabling evaluation of multiple genes simultaneously, the technology brings to light the dilemma of variant interpretation. Here, we aimed to reveal the underlying reasons for the discrepancy in the evidence titles used during variant classification according to ACMG guidelines by two different bioinformatic specialists (BIs) and two different clinical geneticists (CGs). We evaluated final reports of 1920 cancer patients and 189 different variants from 285 HCP were enrolled to the study. A total of 173 of these variants were classified as pathogenic ($n = 132$) and likely pathogenic ($n = 41$) by the BI and an additional 16 variants, that were classified as VUS by at least one interpreter and their classification would change the clinical management, were compared for their evidence titles between different specialists. The attributed evidence titles and the final classification of the variants among BIs and CGs were compared. The discrepancy between P/LP final reports was 22.5%. The discordance between CGs was 30% whereas the discordance between two BIs was almost 75%. The use of PVS1, PS3, PP3, PP5, PM1, PM2, BP1, BP4 criteria markedly varied from one expert to another. This difference was particularly noticeable in PP3, PP5, and PM1 evidence and mostly in the variants affecting splice sites like *BRCA1* (NM_007294.4) c.4096 + 1 G > A and *CHEK2*(NM_007194.4) c.592 + 3 A > T. With recent advancements in precision medicine, the importance of variant interpretations is emerging. Our study shows that variant interpretation is subjective process that is in need of concrete definitions for accurate and standard interpretation.

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INTRODUCTION

Next-generation sequencing is widely used in the assessment of hereditary cancers. Targeted cancer panels provide a fast, accurate, and cost-effective approach in the evaluation of affected cases and their unaffected relatives who are at higher risk of developing cancer [1]. Different screening panels, targeting a wide range of hereditary cancers are developed constantly and the number of covered genes is increasing. Advancements in sequencing technology have helped clinicians to provide the most appropriate clinical management for a small yet crucial proportion of cancer patients by detecting germline pathogenic variants in cancer susceptibility genes. Defining variants in the genes that predispose to cancer is important for diagnosis, surveillance, risk reduction strategies, and prophylactic procedures for the patient and family members. Besides, preventive surgery alternatives or individualized therapies might be based on evaluating the affected gene(s) [2, 3]. Ultimately, this advanced sequencing method brings thousands of variants into consideration. Although the treatment options are mainly based on variant interpretations, the functional data on how these variants are related to the phenotype can not be obtained as fast as the variant itself. With the increasing number of patients undergoing NGS panel testing, the benefits of hereditary cancer syndrome management depend on the accuracy and preciseness of how these variants are interpreted.

The validity and utility of the variant interpretations rely on the clinical geneticist (CG) approach, which should take reasonable hypotheses into account and be objective and evidence-based. The guidelines issued by the American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP) in 2015 provide a framework for standardizing variant interpretations [4]. According to ACMG/AMP guidelines, a variant is evaluated by using the criteria for pathogenicity, including very strong (PVS1), strong (PS1-4), moderate (PM1-6), or supporting (PP1-5), and for benignity, including stand-alone (BA1), strong (BS1-4), or supporting (BP1-7). These criteria arise from population-based, computational, and predictive data, functional studies, familial segregation analysis, allelic data, and parental origin of the variant. Evaluation according to these criteria puts the variant into one of the following groups; pathogenic (P), likely pathogenic (LP),

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variant of uncertain significance (VUS), likely benign (LB), and benign (B) [4]. The accurate classification of variants is important for rapid and effective clinical management [2]. However, despite the utilization of the same criteria, the outcome may vary from one expert to another, and identifying the source of inconsistencies may help to improve the present guidelines or create new ones.

Here we analyzed the genetic testing reports from the two cancer genetic diagnostic centers, each reporting around 400 patients annually, based in the Marmara Region of Turkey. The medical reports are prepared in two steps, a pre-report by bioinformaticians (BIs) and a final report prepared by CGs. Evaluation of the reports for this number of patients revealed wide discrepancies between the BIs' and the CGs' variant interpretations. This study aimed to determine the underlying reasons for the discordant variant interpretations by BIs and CGs and expose the need for more objective and standardized methods for interpreting variants.

MATERIALS AND METHODS

Patients

We retrospectively analyzed the NGS based hereditary cancer panel results of 1920 index cases or the cases that were tested due to positive family history, who were referred to the Hereditary Cancer Clinic in the Genomic Laboratory (GLAB) of Umraniye Training and Research Hospital and Medical Genetics Department of Bursa Uludag University, Medical Faculty between November 2017 and June 2021. Prior to genetic testing, patients' medical history and family history were gathered and evaluated according to the current National Comprehensive Cancer Network (NCCN) criteria by CGs [5, 6]. The most frequent indication for genetic testing was breast cancer and 13 individuals were tested due to positive family history (Table 1).

In our clinical practice, the variants that are classified as VUS by the BI are not presented in the final report by the CG, unless they are detected in a gene that is related to the disease phenotype. For instance, a VUS variant detected in *BRCA1/2* is added to the final report of an HBOC case but not to Hereditary Colorectal Cancer (HCC) patient.

Sample preparation and NGS sequencing

Genomic DNA was automatically purified from peripheral blood using a QIAamp DNA Mini QIAcube Kit (Qiagen). The Sophia Hereditary Cancer Solution kit (Sophia Genetics), consisting of coding regions of 27 genes (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, FAM175A, MRE11A, NBN, PALB2, PIK3CA, RAD50, RAD51C, RAD51D, TP53, XRCC2, MLH1, MSH2, MSH6, EPCAM, PMS2, PMS2CL, MUTYH, APC, PTEN, STK11*) was used in the library preparation. The NextSeq 500/550 Mid Output v2 kit was applied to sequence the prepared libraries on the NextSeq500 (Illumina) instrument with paired end-reads (2x150bp).

Table 1. Distribution of age, gender and cancer types of the cohort whose reports were evaluated.

	Female ($n = 251$)	Male ($n = 34$)	Mean age years (Min–Max)
Breast cancer	177	2	44.5 (27–83)
Ovarian cancer	30	-	51.9 (28–83)
Colorectal cancer	14	18	47.4 (32–74)
Multiple primary cancers	14	-	53.7 (44–71)
Other cancers	8	9	46.7 (24–75)
Screened due to high risk family history	8	5	46.9 (25–61)
Total	251	34	

The variants that were detected in 285 patients were evaluated for their final reports and evidence titles.

Variant interpretation

In this study, the variant interpretations were performed by two different BIs and two different CGs independently. The BIs are molecular biologists with a PhD in bioinformatics. The CGs are medical doctors with a PhD in medical genetics and/or who had four years residency in medical genetics.

Both centers used the same analysis platform and workflow for the variant interpretation. The raw data were analyzed via Sophia DDM (Sophia Genetics v4.2) and related software. The BIs provided a pre-report for all the variants detected in the NGS panel analysis. The CGs examined the patients, evaluated the laboratory findings and family history, performed variant interpretation, prepared the final report according to ACMG/AMP guidelines, and presented the final report to the patients during post-test genetic counseling (Fig. 1).

BIs and CGs systematically analyzed the germline variants located at the coding regions and ±10 flanking regions. The effect of the variant on the amino acid sequence (missense, frameshift, etc.); variant frequency in the population databases (1000 G, ESP, gnomAD, and in house database); and in-silico prediction tools, such as SIFT [7], Polyphen [8], CADD [9], Human Splicing Finder (HSF) [10], MutationTaster [11], were used for variant pathogenicity predictions. Besides online databases, tools such as PubMed, OMIM, VarSome, and BRCA Exchange were used in variant evaluations (Fig. 1). All the variants that are presented in the study are submitted to the ClinVar database.

RESULTS

Within the scope of the study, 189 different variants in 285 patients were evaluated for their final reports and the evidence titles used by different specialists were compared. A total of 173 different variants from 246 patients reported by the BIs as *P* ($n = 132$) or LP ($n = 41$) were retrospectively evaluated, and the interpretation of 39 showed discordance between at least one BI and/or one CG (22.5%, Supplementary Table 1). The interpretations of two CGs were compared and 27 (10 P, 17 LP) out of 39 final reports found compatible. The 12 (30%) discordant variants among CGs were LP to P or vice versa and did not affect the clinical approach. Among the two BIs, 10 variants were reported concordantly (5 P and 5 LP), whereas 29 (74.3%) variant reports were discordant (Supplement Table 1).

Additional 16 different variants from 39 patients, that were classified as VUS by at least one interpreter, of which their downgrading or upgrading (P to VUS, LP to VUS or vice versa) would change the clinical management (Table 2), are exemplified by the following variants

The NGS panel analysis detected a heterozygous missense *APC* (NM_000038.6) c.3920 T > A variation which affects a partially conserved functional domain. The BI_1 coded PS3, PM2, PP5, BP1 evidence and reported the variant as LP. BI_2, coded BP1, and BP4 evidence but reported the variant as LP with support of literature. On the other hand, the CGs attributed BS1, BS2 titles by referring to GenomAD frequencies and reported the variant as VUS with the support of ClinVar records.

The deepest discrepancy between the two CGs was detected in the *BRCA1*, c.4096 + 1 G > A variant that was classified as VUS by the CG_1 and P by CG_2. The BI_1 and BI_2 classified this variant as LP and P respectively. Since the variant is a splice donor +1 and causes loss of function by disrupting the protein structure, the two BIs and CG_2 coded the PVS1 evidence. Both the CGs and BIs coded the PM2 criteria for the variant. On the other hand, the CG_1 did not code the PVS1 criterion due to the finding that the variant was causing an increased expression of a shorter in-frame transcript and CG_1 classified the variation as VUS.

The *CHEK2* (NM_007194.4) c.592 + 3 A > T variant causes skipping of exons 4–5 and truncates the CHEK2 protein [12] and the BI_1 coded PS3 evidence. The variant, which by consensus of the CGs and BIs contained evidence of PM2, was reported as LP by the BI_1, but CGs and BI_2 did not attribute PS3 and reported the variant as VUS. The other *CHEK2* c.1427 C > T variation was classified by two BIs as VUS, controversially the CGs classified

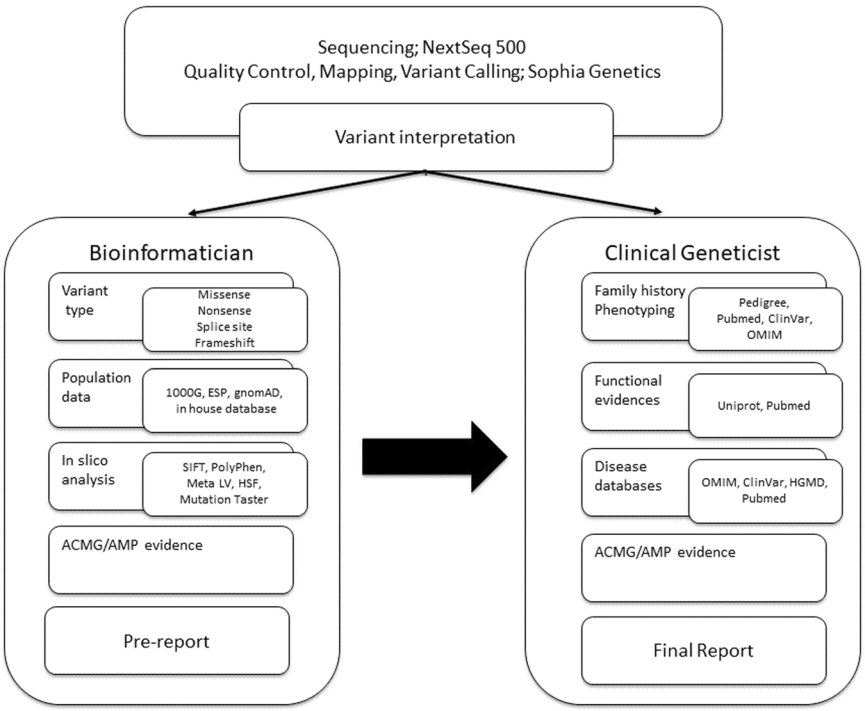

Fig. 1 The workflow of variant interpretation by bioinformation (BI) and clinical geneticist (CG). The BI follows a partially automated workflow during the variant interpretation. The reference alignment (hg19), variant filtering and annotations are done by an automated pipeline. The BI adds the population data and the impact on protein level by using different tools. Variant classification is done according to ACMG/AMP guideline and ClinVar database. On the other hand, the CGs follows a multistep approach consisting pre- and post- test genetic counseling. The patients are referred to cancer clinics mostly by medical oncologists. The CGs perform a pre-test evaluation which starts by taking a detailed family history, followed by clinical phenotyping in support of laboratory tests, images and the epicrisis of the oncologist. Following the NGS panel test, the CG evaluates pre-report of the BI with the information obtained during pre-test genetic counseling in six different steps; (1) Phenotype description (Pubmed, ClinVar, OMIM) (2) Population frequency (1000 G, GnomAD, Iranome, in house database) (3) in silico prediction tools (PolyPhen, SIFT, Meta LV, HSF, mutation tasting) (4) Functional evidence (Uniprot, Pubmed) (5) Disease database search (OMIM, ClinVar, HGMD, Pubmed) (6) Segregation analysis. The variant is classified by the relevant ACMG/AMP evidence that are selected in accordance with these findings and the final report is presented to the patient during post test genetic counseling by the CGs.

the variant as P and LP. Strikingly, none of the criteria were used with common consensus among the interpreters (Table 2).

We detected attribution discordance in PVS1, PS3, PP3, PP5, PM1, PM2, BP1, BP4 evidence. The usage of PP3, PP5, and PM1 titles showed higher variation than the others (Table 3). The PS2, PM3, PM6, BS1, BS2, BS3, BS4, BP2, BP3, BP5, BP7 criteria were not attributed to any of the variants.

DISCUSSION

Variant interpretation and genotype-phenotype correlation in cancer predisposition genes are not easy due to variable penetrance and expression phenomenon in cancer development. On the other hand, recent classifications and targeted therapies in cancer are mostly gene or variant specific. Therefore, the correct classification of the variants is even more important. ACMG/AMP criteria are currently the most valid universal guide for evaluating NGS data, and it is also widely used in interpreting germline variants in hereditary cancer cases [13–16]. The ACMG/AMP guideline for evaluating somatic variations was also published, and the major difference was that the somatic variants were assessed according to their role in treatment, diagnosis, and/or prognosis [17]. Here we enrolled 1920 hereditary cancer patients' reports that were evaluated according to the 2015 ACMG/AMP guideline and around 22.5% (39/173) of them showed discordance in the interpretations between BIs and CGs. The discrepancy rates among interpreters and/or laboratories for the same variants have been reported as >50% [1, 14, 15, 18, 19]. In line with our findings, the reported discordant ACMG/AMP evidence largely involved P/LP variants or VUS [14, 15, 19]. The final reporting of two CGs were mostly concordant and the difference was at the P and LP level, whereas the discrepancy was deeper among the BIs. Although the BIs, who share a common background, are using similar pipelines, their interpretations lack clinical data which might be one of the reasons for this discrepancy.

An important example of a discrepancy among the interpreters was detected in the *APC* variant I1307K (c.3920 T > A), which is considered as a risk factor in the NCCN guideline and suggested as pathogenic for colorectal cancer [5, 20]. The BI reported this variant as LP with attribution of PS3, PM2, PP5, BP1 titles. On the other hand, the ClinVar records are quite controversial, and the population frequency was found to be high in the databases. Additionally, in our cohort the case that presented this variation was diagnosed with breast cancer. In support of these findings, the CGs attributed BS1 and BS2 titles for this variant, and they reported as VUS. Neither the titles nor the last classification of the variant overlap between the BIs and CGs. Clearly functional studies would solve this dilemma, which is beyond the scope of this study.

The *CHEK2* c.1427 C > T variant is a clear example of discordance in variant interpretations. Although the interpreters attributed different evidence combinations, BIs reported the variant as VUS and CGs as P or LP. Reaching the same pathogenicity level by using different evidence titles can be considered as an indication of consistency for ACMG guideline but subjective approaches by different specialists is the biggest obstacle in variant interpretations.

According to the ACMG/AMP criteria, the PVS1 title would be attributed to the *BRCA1* c.4096 + 1 G > A variation since it occurs at

Table 2. Variants classified as VUS by at least one interpreter. In total 27 variants' interpretations showed discordance between BIs and CGs.

Variant ID	Gene	Transcript	Genomic Location	HGVS	Nucleotide Exchange	Amino Acid Exchange	Variant Type	rs Code ClinVar ID	Evidence Coded by BI_1	Evidence Coded by BI_2	Evidence Coded by CG_1	Evidence Coded by CG_2	Final BI_1 Report	Final BI_2 Report	Final CG_1 Report	Final CG_2 Report
14	*APC*	NM_000038.6	chr5:112175211	NC_000005.9:g.112175211 T > A	c.3920 T > A	p.(Ile1307Lys)	Ms	rs1801155 822	PS3, PM2, PP5, BP1	BP1, BP4	BS1, BS2	BS1, BS2	LP	LP	VUS	VUS
15	*BRCA1*	NM_007294.4	chr17:41243451	NC_000017.10:g.41243451 C > T	c.4096 + 1 G > A	-	Spl	rs80358178 37565	PVS1, PM2	PVS1, PM2, PP3	PM2, PP3	PVS1, PM2, PP3	LP	P	VUS	P
16	*CHEK2*	NM_007194.4	chr22:29120962	NC_000022.10:g.29120962 T > A	c.592 + 3 A > T	-	SNV/ Spl**	rs587782849 142956	PS3, PM2	PVS1-M, PM2	PM2	PM2	LP	VUS	VUS	VUS
20	*MSH2*	NM_000251.2	chr2:47637348	NC_000002.11:g.47637348 T > C	c.482 T > C	p.(Val161Ala)	Ms	rs63750126 565544	PM1, PM2, PM5, PP3, BP1	PM2, PM5, PP3, BP1	PM2, PM5, PP3	PM2, PM5, PP3	LP	VUS	P	P
22	*CHEK2*	NM_007194.4	chr22:29121058	NC_000022.10:g.29121058 C > T	c.499 G > A	p.(Gly167Arg)	Ms	rs72552322 142524	PS3, PM1, PM2, PP3	PM2, BP1, PP3, PP5	PM2, PP5	PM2, PP3, PP5	LP	VUS	P	LP
25	*BRCA1*	NM_007294.4	chr17:41209088	NC_000017.10:g.41209088 C > G	c.5258 G > C	p.(Arg1774Thr)	Ms	rs397509246 55488	PS3, PM1, PM2, PP3, BP1	PM2, BP1, PP3, PP5	PM2, PP5	PS3, PM2, PP3, PP5	LP	VUS	P	P
37	*MUTYH*	NM_001128425.2	chr1:45798559	NC_000001.10:g.45798559_45798571del	c.504 + 19_504 + 31del	-	Intronic	rs781222233 406825	PM2, PP1, PP5	PM2, PP5	PM2, PP5	PM2, PP5	LP	VUS	LP	LP
45	*MLH1*	NM_000249.3	chr3: 37059093	NC_000003.11:g.37059093 A > C	c.884 + 3 A > C	-	Spl	rs267607803 90413	PM2, PP3, PP5	PVS1-M, PM2	PM2	PM2	LP	VUS	VUS	VUS
48	*BRCA2*	NM_000059.4	chr13:32954050	NC_000013.10:g.32954050 G > A	c.9117 G > A	p.(Pro3039 =)	Syn	rs28897756 38215	PVS1, PS3, PM2, PP3, PP5	PS3-M, PM2, PP5	PP5, PM2	PP5, PM2	P	VUS	P	P
49	*CHEK2*	NM_007194.4	chr22:29090054	NC_000022.10:g.29090054 G > A	c.1427 C > T	p.(Thr476Met)	Ms	rs142763740 128060	PM1, PP3, BS1	PP3, BP1	PM1, PM2, PM5, PP3, PP5, BP1	PM2, PM5, PP5	VUS	VUS	P	LP
50	*MUTYH*	NM_001128425.1	chr1:45796890	NC_000001.10:g.45796891_45796893del	c.1437_1439del	p.(Glu480del)	Fs	rs587778541 127838	PM2, PM4, PS3, PP5	PM2, PP5	PM2, PM4, PS3, PP5	PM2, PM4, PS3, PP5	VUS	P	P	P
51	*ATM*	NM_000051.3	chr11:108186590	NC_000011.9:g.108186590 A > G	c.6047 A > G	p.(Asp2016Gly)	Ms	rs587781302 140823	PM2, PP3, PP5, BP1	PM2, PP5, PP3	PM2, PP5, PP3	PM2, PP3, PP5	VUS	LP	LP	LP
52	*ATM*	NM_000051.3	chr11:108186638	NC_000011.9:g.108186638 G > A	c.6095 G > A	p.(Arg2032Lys)	Ms	rs139770721 181974	PM2, PP3, PP5, BP1	PM2, PP5	PM2, PP5	PM2, PP3, PP5	VUS	LP	LP	LP
53	*TP53*	NM_000546.6	chr17:7577114	NC_000017.10:g.7577114 C > T	c.824 G > A	p.(Cys275Tyr)	Ms	rs863224451 215997	PM2, PP3, PP5	PM1, PP2, PM2, PM5, PP3, PP5	PM1, PP2, PM2, PM5, PP3, PP5	PM1, PP2, PM2, PM5, PP3, PP5	VUS	P	P	P
54	*ATM*	NM_000051.3	chr11:108115727	NC_000011.9:g.108115727 C > T	c.875 C > T	p.(Pro292Leu)	Ms	rs747727055 229794	PM2, PP3, BP1	PM2, PP5, PP3, PP2	PM2, PP5, PP3, PP2	PM2, PP5, PP3	VUS	LP	LP	P
55	*BRIP1*	NM_032043.3	chr17:59885825	NC_000017.10:g.59885825_59885826delCA	c.918 + 2_918 + 3del	-	Spl	- SUB10324299	PM2	PVS1, PM2	PVS1, PM2	PVS1, PM2	VUS	LP	LP	LP

P pathogenic, *LP* likely pathogenic, *SNV* single nucleotide variation, *BI* bioinformatician, *CG* clinical geneticist, *Fs* frameshift, *Ins* insertion, *Ms* missense, *Spl* splice site, *Ns* nonsense, *Syn* synonymous, *SNV* single nucleotide variant.

**The variation is predicted to affect the splice site by human mutation finder tool [10].

Table 3. Criteria used non-concordantly among bioinformatician (BI_1) and clinical geneticist (CG_1) ($n = 173$).

Evidence	Coded by BI and CG *n* (%)	Not Coded by BI and CG *n* (%)	Coded Only by BI *n* (%)	Coded Only by CG *n* (%)	95% CI (LB-UB)
PVS1	149 (86.13)	19 (10.98)	2 (1.16)	3 (1.73)	3 (0.4–5)
PS3	6 (3.47)	151 (87.28)	13 (7.51)	3 (1.73)	9 (5–13)
PP3	42 (24.28)	91 (52.60)	18 (10.40)	22 (12.72)	23 (16.8–30)
PP5	55 (31.79)	93 (53.76)	5 (2.89)	20 (11.56)	14.4 (9–19)
PM1	6 (3.47)	137 (79.19)	25 (14.45)	5 (2.89)	17 (11.7–23)
PM2	160 (92.49)	1 (0.58)	9 (5.20)	3 (1.73)	7 (3–10)
BP1	2 (1.16)	162 (93.64)	9 (5.20)	-	5 (2–8)
BP4	-	166 (95.95)	5 (2.89)	2 (1.16)	4 (1–7)

The 95% CI was calculated only for the discordant data (Coded only by BI and Coded only by CG) between two interpreters.
LB lower bound, *UB* upper bound.

splice +1. On the other hand, it was previously shown that the variant was affecting the splicing of exon 10, and protein produced from this transcript may retain residual function [21–24]. With this supporting evidence, the CG_1 did not attribute PVS1 and classified the variant as VUS instead of LP. In line with the CGs' interpretation, it has been previously shown that different splicing variants in *BRCA2* remain as functional expressions of different transcripts [25–27], which might be relevant also for *BRCA1*. The BIs and CG_2 probably missed the warning in the ACMG guideline for splice site variations with residual functions and attributed the PVS1. Since PVS1 is the "very strong" pathogenicity evidence, definite findings and detailed information are needed for its attribution.

PS3 evidence can only be used if reliable functional studies indicate that the variant is pathogenic. Brnich et al. published a long list of recommendations for assessing the reliability of PS3/BS3 evidence, which considers the disease mechanism, especially the reliability and validity of the assay [28]. ACMG/AMP guideline suggests using PVS1 evidence for variations at ±1 or 2 splices sites. Hence, the BI_1 attributed PS3 instead of PVS1 for *CHEK2* c.592 + 3 A > T variant that causes a truncated protein [12]. The CGs attributed neither PVS1 nor PS3 by referring to the same article which shows it to decrease, but not to eliminate the functionality of the protein [12]. Even by citing the same findings, the evidence titles can change, and it is important to define standards for functional studies and how to interpret the data provided by these analyses. Additionally, all the variants above have multiple entries with different classes in the ClinVar database, which shows the extent of discrepancy among different centers and/or evaluators.

Another discordant attribution of PVS1 and PS3 was for the synonymous variation *BRCA2* c.9117 G > A, which creates a truncating protein [25, 29] and the BI_1 encoded PVS1 evidence. However, the CGs did not attribute PVS1 considering both the ACMG/AMP guideline and the new ClinGens' recommendations for synonymous variations [4, 30]. The variant was reported as pathogenic by all the interpreters, except BI_2, with different attributions. As discussed previously, these examples show that evidence for PVS1 and PS3 needs quantitative data, gene-specific classification, clear experimental findings and definitive explanation for accurate usage [18, 28].

PM1 and PP5 evidence showed the high discordance between BIs and CGs. PM1 is coded for variants located in well-established critical regions with no benign variation in the same domain, and PP5 is about the entries of variant classification in reputable databases. For both titles the explanations are controversial. In PM1, the term "well-established" is not an objective definition, and there is no exact explanation on how the critical region is defined and which regions are considered as hotspots [18]. On the other hand, in PP5, regarding ClinVar, UniProt, and VarSome are dependent on individual submitters, and if there is no consensus on a variant in these databases, unfortunately, there is no information on how to use the evidence.

Another evidence that we found disagreement was related to PM2, which stands for variant frequency in population databases. Contra evidence of PM2 is BA1, and there is a threshold value for BA1(>5%). On the other hand, the lack of similar threshold and disease-specific population frequencies makes it hard to use the PM2 title. Evidence can be used confidently if a variant is absent from all general population databases. However, it is unclear how to attribute evidence when the variant frequency is low. The Sequence Variant Interpretation Working Group also offers to decrease the effect of the PM2 evidence due to the finding that rarity is quite common among individuals in the same population [31, 32].

The attribution of PP3 and BP4, which refers to the evaluation of the results from in-silico prediction tools, was also inconsistent in our study. When all in-silico tools disagree, it may be advisable not to use the evidence. For evaluating missense variants, some studies suggest using multiple prediction tools and taking into account the majority. On the other hand, for intronic variants, deletions, and silent variations, a consensus is needed. Additionally, splice variants should be evaluated with specific tools for this evidence to be attributed [33].

Although this study was conducted with a relatively limited number of patients, these findings show a fraction of discordance in variant interpretations. The possible reasons for this discrepancy can be summarized as; (i) the BIs are using the guideline with a limited initiative, (ii) the disease and population-specific frequencies are still lacking, (iii) in vitro and in vivo functional studies can not be performed in every laboratory and (iv) these tests are not always performed by experienced CGs.

Here, we evaluated the evidence titles just for P and LP variants and the VUS variants that are either upgraded or downgraded by the CGs. However, similar problems may be encountered in ACMG/AMP evidence titles that were not used in our study. Additional comparative analysis between BIs and CGs are needed for better and standard interpretations.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

NBA: formal analysis investigation methodology supervision writing — original draft writing — review & editing. BU: formal analysis investigation writing — original draft writing — review & editing. OAD: formal analysis methodology writing — review & editing. MOK: formal analysis writing — review & editing. PZ and SOS: formal analysis. SGT: formal analysis writing — review & editing. LD: project administration resources writing — review & editing.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving humans were in accordance with the ethical standards (Umraniye Teaching and Research Hospital No:49/24.03.2016).

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