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RESEARCH

Reclassification of clinical exome data leads to significant clinical assessment changes in almost half of the patients

Klinik ekzom verilerinin yeniden sınıflandırılması hastaların yaklaşık olarak yarısında klinik değerlendirmede anlamlı değişmelere neden olmaktadır

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Abstract

Purpose: With the global accumulation of genetic/clinical data, we are understanding the clinical significance of the reclassification of pathogenicity for gene variants. We hypothesized that this evolution in classification(s) may cause clinically-relevant discrepancies in the genetic risk assessment of subjects. In this study, we sought to reclassify the clinical exome sequence (CES) data of our patients to assess whether these changes would have clinical significance.

Materials and Methods: The study included CES data of 23 cases diagnosed with cancer or familial cancer predisposition. The variants were first classified in 2020 and then reclassified a year after based on the ACMG database. Chart reviews were performed to record clinical history and interventions.

Results: In the first classification of CES data, a total of 80 variants were identified as being not benign (26 likely pathogenic/pathogenic and 54 variants of undetermined significance (VUS)). The clinical significance of fifteen variants (19%) changed after reclassification in 10 patients (43%). The only upgraded variant was the c.9097 dup in exon 23 of *BRCA2* gene (likely pathogenic to pathogenic). Fourteen variants were downgraded at reanalysis in 9 patients: from pathogenic to likely pathogenic (2 variants), pathogenic to VUS (2), likely pathogenic to VUS (4), and VUS to benign (6).

Conclusion: Considering that the clinical significance of CES data changed due to reclassification in almost half of the studied patients, we believe genetic variant-related data should be assessed at regular intervals, regardless of follow-up status in the clinic.

Keywords: Cancer, clinical exome sequencing, likely pathogenic/pathogenic variants

Öz

Amaç: Gen varyantlarının patojenitelerindeki değişmelerin (yeniden sınıflandırma) klinik önemleri genetik/klinik veriler arttıkça giderek daha çok anlaşılmaktadır. Bize göre bu durum hastaların varyantları yeniden sınıflandırıldığında genetik risk değerlendirmesinde kliniksel açıdan önemli tutarsızlıklara yol açabilmektedir. Bu çalışmada, hastalarımızın klinik ekzom sekanslama (CES) verilerini yeniden sınıflandırarak bu verilerin klinik açıdan anlamlılığını değerlendirdik.

Gereç ve Yöntem: Kendisinde kanser teşhis edilmiş veya ailesel kanser yatkınlığı olan 23 hastanın CES verileri incelendi. İncelenen varyantlar ACMG veri tabanı kullanılarak 2020 yılında sınıflandırıldıktan sonra ertesi yıl tekrardan sınıflandırıldı. Hastaların klinik hikayelerini ve yapılan müdahaleleri kaydedebilmek için hasta dosyaları incelendi.

Bulgular: CES verilerinin ilk incelenmesinde, benign olmayan 80 varyant tespit edilmiştir (26 olası patojenik/patojenik ve 54 önemi bilinmeyen varyant (VUS)). Yeniden sınıflandırma sonrası 10 hastada (%43) 15 varyantın (%19) klinik önemi değişmiştir. Klinik önemi artan tek varyant *BRCA2* geninin ekzon 23'ündeki c.9097 dup varyantı olmuştur (olası patojenikten patojeniğe). Yeniden analiz sonrası dokuz hastada 14 varyantın klinik önemi azalmıştır: patojenikten olası patojeniğe (2 varyant), patojenikten VUS'a (2 varyant), olası patojenikten VUS'a (4 varyant) ve VUS'dan benigne (6 varyant).

Sonuç: Çalışılan hastaların yaklaşık yarısında CES verilerinin klinik öneminin yeniden analizler sonrası değiştiği düşünüldüğünde, genetik varyantlar ile ilişkili verilerin klinikteki takip durumlarından bağımsız olarak düzenli aralıklarla değerlendirilmesi gerektiğine inanmaktayız.

Anahtar kelimeler: Kanser, klinik ekzom sekanslama, olası patojenik/patojenik varyantlar

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INTRODUCTION

Cancer is a genetic disorder that is characterized by the loss of the control over cell division. Following the completion of the Human Genome Project in 2003 important advances occurred in the characterization of cancer-related genes and their sequencing[1].

After the first identification of somatic mutations in human cancer genes, researchers began to define and describe various specific cancer genes and their biological functions[2]. These genes are often those which are associated with the control of cell proliferation, differentiation, and death. Additionally, many of the mechanisms that cause cancer stem from mutations in the genes that participate in DNA repair[3].

While some cancer types are associated with a single gene or a single mutation, many are associated with multiple genes. As such, ideally, sequence analyses should cover the whole exome. However, multi-gene panels and clinical exome sequencing (CES) are being increasingly used in the assessment of cancer risk. Different laboratories have varying interpretations about whether the variants detected in sequence analysis are pathogenic and the potential relationships of these variants with disease phenotypes[4]. There may also be differences between laboratories in terms of the classifications of variants. To overcome these variations in interpretation, a new classification by ACMG and ClinGen was proposed in 2015[5,6]. With this approach, a systemic methodology with semi-quantitative, point-based data was developed.

Cancer genetics is a field that is continuously improving and changing. Therefore, the reevaluation of data, especially concerning variants of undetermined significance (VUS), is of particular importance to detect changes in cancer genetics[7]. Improvements in bioinformatics are also critical to define previously unknown variants and to associate them with clinical characteristics. Repetitive analyses increase the probability of diagnosis in many diseases. One of the best examples of this was shown in patients with intellectual disorders. When exome sequence analyses without pathogenic variants in previous analyses were reevaluated using ANOVAR, new gene associations and various variants were identified, including those that were pathogenic and likely pathogenic[8].

Advances in sequencing technology and bioinformatics will likely lead to the definition of new variants associated with cancer and the detection of previously unrecognized cancer genes. We aimed to reclassify likely pathogenic/pathogenic and VUS variants to determine whether reclassification can lead to important changes in clinical interpretation in the cancer-related genes of cases who had undergone CES analysis. By doing this, we assessed the temporal changes in the reclassification of variants, which is an important matter for the purpose of genetic counseling and also changes in variant classification can have significant implications on patient management and prognosis. Our hypothesis in this study is the reclassification of the variants will change in time because of the improvements in the bioinformatic techniques and the improvements in the genetic knowledge.

As a result, in this study we aimed to show how the interpretations of the genetic data can evolve in time. As new information about the issue comes, we acquire invaluable insights into complex diseases such as cancer. The ability to detect these changes through clinical exome sequencing has a huge impact in the diagnosis, prognosis, and treatment of cancer.

MATERIALS AND METHODS

This study was ethically approved by Baskent University Health Sciences Research Board and Ethics Committee (project No KA21/303) with the approval number 21/113 on 16/06/2021.

Sample

This study is a retrospective, cross-sectional, descriptive and observational study. All twenty-three cases who were diagnosed with cancer or had a predisposition to cancer and underwent CES analyses that were performed between November 2019 and November 2020 at the Baskent University Faculty of Medicine Department of Medical Genetics, Genetic Diseases Evaluation Center, were included in this study. The patients who were diagnosed with cancer at the old ages and did not have any familial cancer history were excluded in this study. Enrollment in the study, evaluation and CES analysis of the patients were done by clinical geneticists and molecular geneticists at the Baskent University Faculty of Medicine Department of Medical Genetics.

Data collection

The demographic data of the research groups, their clinical findings and pathology results were recorded from patients' follow-up files and hospital data system. The CES analyses data were obtained from the database of the Baskent University Faculty of Medicine Medical Genetics Department. Cancer-associated genes in CES data were determined based on Pan Cancer Panel, SOPHIA Cancer Gene Panel, Berry Genomics Cancer Gene Panel and other relevant literature.

Bioinformatic tools and databases

SOPHIA Clinical Exome Solution V2 and Berry Genomics Clinical Exome Test were used in the analysis of CES data. Raw data were analyzed using bioinformatic tools. In silico prediction tools (SIFT, Provean, Polyphen and Mutation Taster and Revel) were utilized for variant annotation and classification of pathogenicity. The databases like 1000Genome, ExAc, and gnomAD were used to indicate the frequency of the variants. If the frequency of variants was above 1%, they were not included from the study. Different databases were used to interpret variants, including Human Genome hg19/GRCh37, Refseq (release 61), GeneCards, dbSNP (v151), OMIM, VarSome, ClinVar, and Franklin.

Classification of the variant pathogenicity

The pathogenicity of the variants was classified as benign, likely benign, VUS, likely pathogenic and pathogenic according to the ACMG criteria[5].

Likely pathogenic/pathogenic and VUS variants in cancer-associated genes were the only targets included in the study. The associations of these variants with clinical data were assessed in the research groups.

Reclassification of the variant pathogenicity

Because of the pathogenicity of the variants can change in a timely manner all variants were reanalyzed using ClinVar, VarSome and Franklin databases after approximately one year and the differences were recorded and compared.

Statistical analysis

The parametric values were given as mean ± standard error of mean. All frequencies were shown as percentages.

RESULTS

The mean age of 23 individuals in the research group was 44.74 ± 10.8 (22-64 years). Female-to-male distribution was 20-to-3. Menarche age for female cases was 11.5±1.6 (11-16 years). The demographic and clinical characteristics of the cases are shown in Tables 1 and 2.

At baseline, 80 variants were found to be associated with cancer (pathogenic, likely pathogenic and VUS) which had been identified in 23 cases. Twenty-six of these variants (32.5%) were likely pathogenic/pathogenic variants. The remaining 54 variants (67.5%) were classified as VUS. The distribution of variants in cases and their properties together with clinical features are shown in Supplementary Table 1.

As a result of variant reclassification performed for the 80 variants, the defined pathogenicity of 15 of these variants changed (18.75%). All properties of reclassified variants and clinical data were shown in Supplementary Table 1.

Table 1. Demographic profiles of cases

Variable	Frequency (%)
Sex	
Female	20 (86.6)
Male	3 (13.4)
Marital status	
Single	3 (13.4)
Married	17 (73.2)
Divorced/widowed Unknown	3 (13.4)
Menapausal status	
Premenopausal	8 (40)
Menopausal	12 (60)
Parity (For female cases)	
0	4 (20)
1	4 (20)
2-4	9 (45)
>4	3 (15)
Smoking	7 (30.4)
Hormone replacement therapy	2 (10)
Oral contraceptive use	4 (20)

The pathogenicity of one variant was reclassified from likely pathogenic to pathogenic. This variant was in a case diagnosed with breast cancer at an early age (29 years old). She had a second-degree relative with breast cancer, a first-degree relative with testicular cancer, and a third-degree relative with pancreas cancer. The likely pathogenic variant in the

BRCA2 gene (23 exon c.9097dup) gained the PP5 criterion and was upgraded to pathogenic.

Two variants in the pathogenic category were reclassified as likely pathogenic (Table 3). The SNP variant in the exon 73 of the *LRP1B* gene of a patient with small cell lung cancer was downgraded to likely pathogenic by losing PP3 evidence for variant pathogenicity. Similarly, in a case with endometrial and ovarian cancer history and a family history of lung and colon cancer, the variant c.1187G>A in the exon 13 of the *MUTYH* gene was downgraded from pathogenic to likely pathogenic with the loss of PM1, PS3 and BS1 evidence.

While the variants c.1337A>G in the exon 10 of the *MBTPS2* gene and c.1248-2A>G in the exon 11 of the *TYRO3* gene were pathogenic at the first evaluation (baseline), they were reclassified as VUS at the second classification due to losing PVS1 and PVS1, PP3 respectively (Table 3).

Four variants originally classified as likely pathogenic were reclassified as VUS (Table 3). The pathogenicity of the *MUTYH* gene in case 2 (exon 10 c.925C>T) and the variant in *FGFR1* gene (exon 4 c. 386A>C) were changed due to losing PS1 and gaining BP6 and losing PS1/PM2 and gaining BP6 respectively. The only likely pathogenic variant of case 14 who had familial breast cancer (*PTCH1* gene exon 8 c.1128C>G) was reclassified as VUS due to loss of the PM1 and PM5 criteria. The likely pathogenic variant in the *DDR2* gene (exon 6 c.476T>C) in case 20 who had endometrial and ovarian cancer was identified to be VUS due to loss of PM1, PM2, PP2 criteria and the addition of the BS1 criterion.

Seventeen variants remained unchanged in the likely pathogenic/pathogenic group according to the initial evaluation. However, four variants in this group were downgraded from likely pathogenic to VUS, and two variants from pathogenic to VUS. One variant was upgraded from likely pathogenic to pathogenic. In all cases, there were 26 likely pathogenic/pathogenic variants in the first evaluation, whereas there were 20 in the second evaluation.

Six of the 54 variants that were classified as VUS in the first evaluation were reclassified as likely benign/benign (Table 3). Therefore, overall, the pathogenicity changed in six of the 54 variants (11.1%) identified at baseline.

Table 2. Clinical characteristics of the research group

	Diagnosis	**Age**	**Age of Diagnosis**	**Family History**
Case 1	Breast Cancer	63	39	Sister and uncle's daughter's breast, mother colon cancer, father lymphoma, uncles' breast and prostate cancer
Case 2	Complex fibroadenoma LIN3	22	22	Aunt early diagnosed with breast cancer, grandmother breast cancer
Case 3	Undiagnosed mass in the breast	42	42	Aunt, mother's still daughter and her daughter early diagnosed with breast cancer
Case 4	Fibro glandular hyperplasia of the breast	40	40	Mother and one of her aunt's breasts, the other aunt's breast and ovarian, aunt's breast, father rectum, uncle liver cancer
Case 5	Invasive ductal carcinoma in the breast	46	46	Sister's and aunt's gastric, brother's bladder and father's prostate cancer
Case 6	Ductal carcinoma in situ in the breast (triple +)	29	29	Aunt's breast diagnosed at 40 years, brother's testis, second-degree cousin's pancreas cancer
Case 7	İnvasive ductal carcinoma in the breast	45	44	Father's, uncle 's and aunt's lung cancer, other aunt's lymphoma, uncle's prostate, grandfather's kidney cancer
Case 8	Ductal carcinoma in situ in the breast	27	27	Grandmother's breast, grandfather's thyroid cancer
Case 9	Breast cancer (triple +)	34	34	Grandmother's breast, father's lung cancer
Case 10	İnvasive breast cancer (ER+, PR+, HER-2 -) + gastric cancer	48	47 48	Uncle's pancreas, cousin's colon cancer
Case 11	Papillary neoplasia in the breast	46	46	Daughter's B cell ALL, sister's breast (35 age), aunt's ovarian cancer

Case 12	İnvasive breast cancer (triple -)	32	29	
Case 13	Breast cancer family history	47		Mother's bilateral, aunt's daughter (32 age), and mother's aunt's daughter's breast cancer
Case 14	Breast cancer family history	44		Mother's breast cancer
Case 15	Breast cancer family history	47		Mother's breast cancer (bilateral), elder sister's ovarian, two uncle's lung, aunt's endometrial cancer
Case 16	Breast cancer family history	45		Mother and an aunt's breast, an aunt's breast+ ovarian, aunt's breast, father's rectum, uncle's lung cancer, sister's fibro glandular hyperplasia in the breast
Case 17	Ovarian cancer	51	50	
Case 18	Ovarian cancer	64	63	Uncle's prostate, uncle's daughter's breast + ovarian cancer, aunt's son's lung cancer
Case 19	Ovarian + endometrial cancer	56	56	
Case 20	Ovarian + endometrial cancer	51	51	Father's colon, uncle's lung cancer
Case 21	Small cell lung cancer	54	54	Uncle's cancer (organ?)
Case 22	Prostate adenocarcinoma	55	55	Two aunts' advanced age breast cancer, father's thyroid, son's Burkitt lymphoma + malign melanoma, grandmother's brothers' lung cancer
Case 23	Family history of cancer	41		Father's larynx, brothers' brain stem and skin cancer, uncle's prostate cancer, uncle's son's lymphoma

Table 3. Variants that were downgraded in the reclassification

Case No	Gene/ Chromosome	Exon	Variant /Protein	Reference Genome No	First Analysis	ACMG criteria 1	Second Analysis	ACMG criteria 2
Case 20	*MUTYH* (1)	13	c.1187G>A p. G396D	NM_001128425.1	P	ps3 pm1 pm5 pp3 pp5 bs1	LP	pp3 pm2 pm5 pp5
Case 21	*LRP1B* (2)	73	c.11169C>A p.C3723*	NM_018557.2	P	pvs1 pm2 pp3	LP	pvs1 pm2
Case 22	*MBTPS2* (X)	10	c.1337A>G p. Lys446Arg	NM_015884.4	P	pvs1 pm2 pp3	VUS	pm2 bs2 pp3
Case 22	*TYRO3* (15)	11	c.1248-2A>G	NM_001330264	P	pvs1 pm2 pp3	VUS++	pm2
Case 2	*MUTYH* (1)	10	c.925C>T p. R309C	NM_001128425.1	LP	pm1 pm2 ps1	VUS	pm2 bp6
Case 2	*FGFR1* (8)	4	c.386A>C p. D129A	NM_023110.2	LP	ps1 pm2 pp2 pp3	VUS	pp3 pp2 bp6
Case 14	*PTCH1* (9)	8	c.1128C>G p. F376L	NM_000264.5	LP	pm1 pm2 pm5	VUS	pm2
Case 20	*DDR2* (1)	6	c.476T>C p. I159T	NM_006182.2	LP	pm1 pm2 pp2 pp3	VUS	pp3 bs1
Case 10	*ESR1* (6)	4	c.286C>T p. R96C	NM_001328100.2	VUS++	pm1 pm2 pp3	LB	pm2 bs2 pp3 bp6
Case 10	*NOD2* (16)	4	c.2051G>A p. R684Q	NM_001370466.1	VUS	Pm2 bp4	LB	bs1 bp4 bp6
Case 11	*ERCC2* (19)	11	c.974C>T p. T325M	NM_000400.4	VUS	pm2	LB	pp2 bs1 bp6
Case 19	*MET* (7)	2	c.850A>G p. Ile284Val	NM_000245.4	VUS	pm2 bp4	LB	pm2 bp4 bp6
Case 23	*ATM* (11)	6	c.544G>C p. V182L	NM_000051.4	VUS	pm1	B	ba1 bs2 bp4 bp6
Case 23	*CEBPA* (19)	1	c.564_566del p. P189del	NM_004364.5	VUS	pm1 bp3	LB	pm2 bp3 bp6

There were five variants among the VUS variants whose classification did not change but whose classification power increased (VUS++). The VUS variant in the *ERBB2* gene (exon 25 c.3044G>A) in the case with invasive ductal carcinoma of the breast and familial cancer history was upgraded to VUS++ grade by gaining the PP2 criterion. Similarly, the VUS variants in the *NOTCH1* (exon 23 c.3788G>A) and in the *ERBB2* (exon 26 c.3182T>C) genes of a case diagnosed with triple negative invasive ductal carcinoma of the breast at 29 years of age (with no family history) lost their BP4 criterion, resulting in increased VUS grades. In a case who had a family history of breast cancer and was followed up for an undefined breast mass, the VUS variant in the *KRT6A* gene (exon 2 c.721G>A) gained PM1 & PP2 and the VUS variant in the *PRF1* gene (exon 3 c.1390C>T) lost the BP4 criterion, and both were evaluated as VUS++.

As a result, six of the 80 variants were ultimately defined as likely benign or benign, and a total of 74 variants remained. Twenty of these 74 variants (27.03%) were classified as likely pathogenic/pathogenic. While the frequency of likely pathogenic/pathogenic variants decreased significantly at the second classification, there was no significant change in VUS variants.

DISCUSSION

According to the results of CES, likely pathogenic/pathogenic and VUS variants were detected in 23 cases with a cancer diagnosis or familial cancer predisposition, especially breast and ovarian cancers. There were twenty-six (32.5%) likely pathogenic/pathogenic variants.

Like our study, likely pathogenic/pathogenic variants were detected in 30.8% of the cases in a study of 52 patients with high risk for breast/ovarian cancer without mutations in the *BRCA1/BRCA2* genes[9]. The frequency of likely pathogenic or pathogenic variants was reported to be 9% in one of the largest studies in the literature which included 10.000 cases with breast, ovarian, or colorectal/gastric cancer[10]. The most important reason for the low percentage in this study was that only 29 genes with high and moderate risks were sequenced. While the variant frequency in the genes accepted as high-risk genes such as *BRCA1/2*, *TP53* and *RET* was 51.8%, this frequency decreased to 41.8% in moderate-risk genes.

While the likely pathogenic/pathogenic variants detected in this study were found in 15 cases, *in 8 cases (34.75%), we detected only VUS variants*. Only one of the cases with the VUS variants had no family history of cancer. This case was diagnosed with invasive breast cancer at an early age and had VUS variants in the *ERBB2* and *NOTCH1* genes which are two critical protooncogenes. In patients who had relatives with breast cancer history but were without any likely pathogenic/pathogenic variants, we identified VUS variants in genes such as *ZFHX3*, *MNX1*, *KMT2C*, *PAX8*, *ERBB2,* and *ATM*.

The frequency of pathogenic variants in the *BRCA1/BRCA2* genes is very high in cases with familial breast and ovarian cancer predisposition. Likely pathogenic or pathogenic variants in the *BRCA2* genes were detected in two of the 20 cases and were associated with breast/ovarian cancer predisposition. The frequency of mutations in the *BRCA1/BRCA2* genes has been reported to be 20-25% in the other studies[11,12].

In our cases, likely pathogenic or pathogenic variants were found in genes that were reported as having high to moderate frequencies associated with cancer in the literature such as *PARK2*, *MUTYH*, *CHEK2*, *PAX3*, *PTCH1*, *ALK*, *TP53*, *RET*, *ERCC2*, *DDR2*, *WAS* other than *BRCA2*[13].

In the reclassification of our 23-case series, approximately one year later, alterations of pathogenicity were detected in 15 of the 80 variants (18.75%). There was one variant whose pathogenicity was upgraded (*BRCA2* c.9097dup) from likely pathogenic to pathogenic. The pathology of this patient was triple (+) ductal carcinoma of the breast. This variant was interpreted as pathogenic after gaining PP5 evidence at follow-up assessment.

There were 14 downgraded variants. In the patient with prostate adenocarcinoma who had family cancer history, the variants in the metalloproteinase-encoding *MBTPS2* gene (c.1337A>G) and in the tyrosine kinase-encoding *TYRO3* gene, were all downgraded to VUS in the follow-up evaluation. *MBTPS2* lost PVS1 criterion which is the strongest evidence of pathogenicity. *TYRO3* also lost PVS1 evidence in addition to PP3 evidence. It is difficult to

explain these pathogenicity changes which occurred in such a short period of time.

Pathogenicity alteration of two grades has been rarely reported. Garber et al. reclassified 1017 variants to test the differences in variant interpretations between laboratories. They found 998 changes according to the ACMG classification. While 307 of them showed a single-grade alteration in terms of severity (from benign to likely benign, from likely benign to benign, from likely benign to VUS, etc.), 668 variants showed two-grade alteration (from benign to VUS, from pathogenic to VUS, from VUS to benign, etc.). There were three variants that demonstrated a three-grade change in classification. These had downgraded from pathogenic to likely benign. Twenty variants that changed four grades downgraded from pathogenic to benign[14].

One of the longest studies about variant reclassification in the literature is a follow-up study including data from 1.9 million people –obtained at a single laboratory. The variants detected in this study were reclassified more than once. During follow-up, 276 variants were reclassified. While 82.1% of these variants were downgraded, 17.9% were upgraded. Initially 82.8% of these variants were classified as VUS. Researchers have argued that reclassified variants are often sparsely detected, which leads to the necessity of reclassification, and that it would be appropriate to develop new annotation tools[15]. Because VUS rates differ according to ancestry, variant reclassification rates also demonstrate variations associated with ancestry. In one study, when Asian individuals were compared with European individuals, VUS rates were found to be 13-42% in Asians and 6-27% in Europeans. Similar trends were shown between Spanish Americans and African-descent Americans[16].

Because our study includes only Turkish citizens, it is impossible to compare or discuss VUS frequencies according to ancestry. VUS frequency in our study was high, similar to subjects with Asian descent. Another difference of our study was that it included not only a certain cancer gene panel, but rather, most of the cancer susceptibility genes. While significant changes were not observed in classical cancer susceptibility genes like *BRCA2, TP53, CHEK2, RET, MUTYH* which were in the likely pathogenic/pathogenic group, a downgrade to a subcategory was observed in less-penetrating genes such as the *DDR2, PTCH1, FGFR, MBTPS2*, and *TYRO3* genes. A downgrade to a subcategory was observed in genes which were classified as VUS at baseline, such as those in the *NOD2, ERCC2, MET*, and *CEBPA* genes.

In another retrospective study, 8.4% of initially defined variants were reclassified and, in total, 23 reclassifications were performed in 194 individuals. While 10.3% of these reclassifications were upgraded, only 9.1% of them triggered a change in medical management. The median time to reclassification was 1.7 years[17].

Macklin et al. found 111 likely pathogenic/pathogenic variants and 266 VUS variants in 1103 genes associated with mostly breast and ovarian cancers. When forty of these variants were reclassified, most of the VUS variants were reclassified as likely benign, and one VUS variant was reclassified as likely pathogenic. One pathogenic variant and one likely pathogenic variant were reclassified as VUS. Additionally, three of the likely pathogenic variants were upgraded to pathogenic, while one pathogenic variant was downgraded to the likely pathogenic group. Researchers have argued that it is important to have an agreement among laboratories and clinicians with respect to variants upgraded to pathogenic[18].

In our study, the percentages of variants that were downgraded by one grade from the likely pathogenic/pathogenic groups (from pathogenic to likely pathogenic and from likely pathogenic to VUS) were 8.8% and 15.3% respectively. The percentage of VUS variants that were downgraded one grade was 11.1%. These frequencies were significantly lower than those reported by other studies in the literature. Two of the most important causes of this can be low patient count and sequencing of large gene panels. Besides these, the short period from baseline to follow-up assessment is another limiting factor. Performing longer follow-up with prospective study designs will enable better interpretation of variant evaluations and potential reclassifications.

As demonstrated by the literature and our study, definitive decisions regarding variant pathogenicity cannot be performed based on initial variant evaluations. Even when only bioinformatics-related data are concerned, genetic science is a rapidly changing discipline and analyses / interpretations may be subject to change. As the number of studies with evaluation of patients and their relatives (immediate, close, distant family members) increase, the outcomes of pathogenicity analyses are likely to

change. Even in cases where there is strong initial evidence for susceptibility, it is necessary to be careful in genetic counseling. The most important aim of cancer-related genetic studies is to determine genetic susceptibility in the patients' relatives and guide clinicians to take preventive measures. It seems that the high degree of success may depend on reclassification of variants in a timely manner and making decisions accordingly.

Although there is no recommendation regarding the frequency of reclassification, the results of various studies suggest a period between 1-2 years[17,18]. Reclassifications will make significant differences in genetic counseling. Therefore, clinical follow-up of these cases is necessary considering the possibility of repetitive reclassifications. Performing genetic analyses in not only the patients themselves, but also their relatives and siblings will increase the strength of genetic counseling.

Although there is no recommendation regarding the frequency of reclassification, the results of various studies suggest a period between 1-2 years[17,18]. Reclassifications will make significant differences in genetic counseling. So, the clinical follow up of these cases with repetitive reclassifications is necessary. Performing genetic analysis of not only the cases but also the relatives and siblings will increase the strength of the genetic counseling.

As a result, the reliability of pathogenicity classification in genetic evaluations for cancer susceptibility continues to be a matter of debate. This is an important problem since genetic tests have gained considerable use in patient care and follow-up, and in drug selection. Therefore, repetitive variant classifications are gaining more importance.

The most important limitation of this study is the low patient count. Significant differences can occur in reclassification frequencies when more subjects can be included. Another limitation is the shortness of the study period. Longer follow-up and time-bound assessment of alterations in variant pathogenicity with respect to clinical features can be more valuable for future studies.

In conclusion, it is important to acknowledge that the interpretation of CES data is ever-changing. As new information is discovered, our understanding of this data can shift, potentially leading to valuable insights into complex diseases such as cancer. The power to identify molecular perturbations through CES has the potential to greatly improve diagnosis, treatment, and prognosis in these cases. We believe that this approach offers a significant contribution to the interpretation and management of exome data in a dynamic and time-sensitive manner.

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