



Retrospective Re-evaluation of Variants of Uncertain Significance Identified by Clinical Exome Sequencing in Breast Cancer Patients

Meme Kanseri Hastalarında Klinik Ekzom Dizileme ile Belirlenen Klinik Önemi Belirsiz Varyantların Retrospektif Olarak Yeniden Değerlendirilmesi

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ABSTRACT

Objective: Variants of uncertain significance (VUSs) remain a major challenge in clinical molecular genetics because their interpretation may change as population, computational, and disease-specific evidence accumulates. This retrospective descriptive study aimed to re-evaluate VUSs identified by clinical exome sequencing in breast cancer patients and to characterize recurrent observations and genes of potential biological interest.

Methods: Clinical exome sequencing data from 25 individuals referred for breast cancer mutation analysis were retrospectively re-evaluated. The study population comprised individuals with breast cancer or a first-degree family history of breast cancer. Sequencing was performed on the Illumina NextSeq 550 platform. Thirteen individuals were analyzed using Sophia Clinical Exome Solution v2 with Sophia DDM, and 12 were analyzed using the Berry Genomics Clinical Exome workflow with Verita Trekker Variants Detection System and/or the Enliven Variants Annotation Interpretation System. An exploratory filtering strategy was used to focus on VUS observations outside genes already prioritized in the literature for breast cancer or cancer. Candidate variants were subsequently reviewed using public variant resources and *in silico* evidence.

Results: After sequential filtering and manual re-evaluation, four candidate observations in the once-observed group and seven candidate observations in the twice-observed group were examined in detail. Variants were identified in *ZDHC15*, *COL17A1*, *MFN1*,

Öz

Amaç: Klinik önemi belirsiz varyantlar (VUS), popülasyon, hesaplamalı analiz ve hastalığa özgü kanıtların zaman içerisinde birikmesiyle yorumlarının değişebilmesi nedeniyle klinik moleküler genetikte önemli bir sorun olmaya devam etmektedir. Bu retrospektif tanımlayıcı çalışmada, meme kanseri hastalarında klinik ekzom dizileme ile belirlenen VUS'ların yeniden değerlendirilmesi ve tekrarlayan gözlemlerin yanı sıra biyolojik açıdan potansiyel ilgi taşıyan genlerin karakterize edilmesi amaçlandı.

Yöntemler: Meme kanseri mutasyon analizi amacıyla yönlendirilen 25 bireye ait klinik ekzom dizileme verileri retrospektif olarak yeniden değerlendirildi. Çalışma popülasyonu, meme kanseri tanısı bulunan veya birinci derece akrabasında meme kanseri öyküsü olan bireylerden oluşturuldu. Dizileme, Illumina NextSeq 550 platformunda gerçekleştirildi. On üç birey Sophia Clinical Exome Solution v2 ve Sophia DDM kullanılarak, 12 birey ise Berry Genomics Clinical Exome iş akışı ile Verita Trekker Variants Detection System ve/veya Enliven Variants Annotation Interpretation System kullanılarak analiz edildi. Meme kanseri veya kanser açısından literatürde daha önce önceliklendirilmiş genlerin dışındaki VUS gözlemlerine odaklanmak amacıyla keşifsel bir filtreleme stratejisi kullanıldı. Aday varyantlar daha sonra kamuya açık varyant kaynakları ve *in silico* kanıtlar kullanılarak değerlendirildi.

Bulgular: Ardışık filtreleme ve manuel yeniden değerlendirme sonrasında, bir kez gözlenen gruptaki dört aday gözlem ile iki kez gözlenen gruptaki yedi aday gözlem ayrıntılı olarak incelendi.

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ABSTRACT

ZNF141, *MUC6*, *VEZT*, *MYBPC1*, *ZSWIM1*, *TDRD3*, *PRDM6*, and *PIGG*. *MUC6* and *ZNF141* were considered of exploratory interest. Two different *MUC6* variants were observed among four individuals; however, two carriers of one *MUC6* variant were related and were not considered independent observations. Two *ZNF141* missense VUSs were identified in a single breast cancer patient. No control cohort, statistical enrichment analysis, segregation analysis, or functional validation was available.

Conclusion: The findings represent descriptive and hypothesis-generating observations rather than evidence of a causal or statistical association with breast cancer. *MUC6* and *ZNF141* may warrant further investigation in larger, population-matched cohorts with segregation, gene-level enrichment, and functional studies. The study also emphasizes the importance of transparent and periodic reinterpretation of VUSs.

Keywords: Breast cancer, variant of uncertain significance, VUS, clinical exome sequencing, variant reinterpretation

Öz

ZDHC15, *COL17A1*, *MFN1*, *ZNF141*, *MUC6*, *VEZT*, *MYBPC1*, *ZSWIM1*, *TDRD3*, *PRDM6* ve *PIGG* genlerinde varyantlar saptandı. *MUC6* ve *ZNF141* keşifsel açıdan ilgi çekici bulundu. Dört bireyde iki farklı *MUC6* varyantı gözlemlendi; ancak *MUC6* varyantlarından birini taşıyan iki bireyin akraba olduğu ve bu nedenle bağımsız gözlemler olarak değerlendirilmediği belirlendi. Tek bir meme kanseri hastasında iki farklı *ZNF141* missense VUS saptandı. Kontrol kohortu, istatistiksel zenginleşme analizi, segregasyon analizi veya fonksiyonel doğrulama verisi bulunmamaktaydı.

Sonuç: Bulgular, meme kanseri ile nedensel veya istatistiksel bir ilişkiyi ortaya koyan kanıtlardan ziyade tanımlayıcı ve hipotez oluşturu gözlemler niteliğindedir. *MUC6* ve *ZNF141* genleri; segregasyon, gen düzeyinde zenginleşme ve fonksiyonel çalışmaların gerçekleştirileceği, daha geniş ve popülasyon açısından eşleştirilmiş kohortlarda ileri araştırmaları hak edebilir. Çalışma ayrıca VUS'ların şeffaf ve düzenli aralıklarla yeniden yorumlanmasının önemini vurgulamaktadır.

Anahtar Sözcükler: Meme kanseri, klinik önemi belirsiz varyant, VUS, klinik ekzom dizileme, varyantın yeniden yorumlanması

INTRODUCTION

Cancer is a multifactorial disease characterized by disruption of cellular homeostasis and uncontrolled cell proliferation resulting from the interaction of genetic and environmental factors (1). Breast cancer is a heterogeneous malignancy arising predominantly from epithelial cells of the mammary ducts or lobules (2). Its development is influenced by age, hormonal and reproductive factors, lifestyle, breast density, environmental exposures, and family history (3).

Although breast cancer is a genetic disease at the cellular level, it is not always hereditary. *BRCA1* and *BRCA2* are the best-established high-penetrance hereditary breast and ovarian cancer genes. Additional genes, including *ATM*, *CHEK2*, *PALB2*, *TP53*, *PTEN*, *CDH1*, and *STK11*, contribute to breast cancer susceptibility with variable penetrance (4-6). The increasing use of next-generation sequencing and clinical exome sequencing has expanded the number of variants detected during genetic evaluation and has consequently increased the number of variants of uncertain significance (VUSs) requiring interpretation.

A VUS is a sequence variant for which the available evidence is insufficient or conflicting for classification as pathogenic/likely pathogenic or benign/likely benign. A VUS should not be considered evidence of causality or used alone to establish a clinical association. Because population databases, computational predictions, clinical submissions, and functional evidence evolve over time, periodic reinterpretation of previously identified VUSs may provide additional information.

The aim of the present study was to retrospectively re-evaluate VUSs identified by clinical exome sequencing in breast cancer patients and to characterize recurrent observations and genes of potential biological interest. The study was designed as a descriptive and exploratory reanalysis and was not intended to establish statistical gene-disease associations.

MATERIALS AND METHODS

Study Design and Breast Cancer Patient Cohort

This retrospective descriptive study included 25 individuals referred to the Department of Medical Genetics, Başkent University Hospitals, for breast cancer mutation analysis between December 2019 and October 2021. The study population comprised individuals with a diagnosis of breast cancer or a first-degree family history of breast cancer. Throughout the manuscript, the cohort is referred to in the context of breast cancer patients and breast cancer genetic evaluation. All included individuals had previously undergone clinical exome sequencing as part of their genetic assessment.

The study was approved by the Başkent University Medical and Health Sciences Research Board on April 12, 2022 (decision no. 119004; project no. KA22/45) and was supported by the Başkent University Research Fund. Written informed consent was obtained from all participants.

Clinical Exome Sequencing and Primary Analysis

Clinical exome sequencing was performed using the Illumina NextSeq 550 platform. Thirteen individuals were analyzed using the Sophia Clinical Exome Solution v2 assay, and primary analysis was performed using Sophia DDM software. The remaining 12 individuals were analyzed using the Berry Genomics Clinical Exome workflow. Variant detection and annotation for the Berry Genomics data were performed using the Verita Trekker Variants Detection System and the Enliven Variants Annotation Interpretation System. The gene contents of the Sophia Clinical Exome Solution v2 and Berry Genomics Clinical Exome assays are provided in Supplementary S1 and S2, respectively.

The present study used retrospectively available variant outputs generated by these clinical workflows. Because two clinical exome workflows and different output structures were used during the

study period, the datasets were standardized before exploratory filtering. The Sophia-derived dataset initially contained 101,225 records. The Berry Genomics outputs contained 195,241 records from the Verita Trekker Variants Detection System and 49,751 records from the Enliven Variants Annotation Interpretation System; because the latter two datasets had similar output characteristics, they were combined for subsequent evaluation.

Exploratory VUS Reanalysis and Filtering Strategy

The objective of the exploratory reanalysis was to examine VUS observations outside genes already prioritized in breast cancer and general cancer literature. At the first stage, a literature-derived list of 2,156 cancer-related genes was used as an exclusion list. This strategy was not intended to imply that known cancer genes are biologically unimportant; rather, it was used to focus the exploratory analysis on observations that would not routinely be prioritized as established cancer-gene findings. The complete list of excluded genes is provided as Supplementary S3.

After application of the 2,156-gene exclusion list, 64,677 records remained in the Sophia dataset and 165,881 records remained in the combined Berry Genomics dataset. Known benign and pathogenic variants were then removed to enrich the dataset for variants reported as VUSs or variants requiring further review. This sequential process yielded 38,173 records for additional evaluation.

The original filtering description did not adequately distinguish automated consequence filtering from the subsequent manual and *in silico* review. In the revised workflow, non-coding and synonymous observations were deprioritized during the initial consequence-based screen; however, recurrent records that remained unresolved or inconsistently classified by the source analysis systems were manually reviewed. Therefore, selected intronic observations were retained for descriptive evaluation. Their retention did not constitute evidence of pathogenicity, and intronic or synonymous observations were not interpreted as disease-associated in the absence of splicing, RNA, or other functional evidence.

Using record counts across the standardized datasets, 14 variants were initially identified in the once-observed group and 868 in the twice-observed group. These variants were individually re-evaluated using *in silico* and public variant interpretation resources. Variants that could be resolved as benign during this review were deprioritized. Four candidate observations from the once-observed group and seven candidate observations from the twice-observed group were retained for detailed descriptive assessment. In this manuscript, “once-observed” and “twice-observed” refer to occurrence counts within the standardized retrospective dataset and should not be interpreted as population frequencies or statistical recurrence.

Variant Interpretation and Database Review

Candidate variants were reviewed using Franklin, VarSome, and gnomAD, together with available literature and gene-level biological information. ACMG/AMP evidence codes were treated as evidence criteria rather than numerical scores. In accordance with current ClinGen Sequence Variant Interpretation guidance, rarity/absence evidence and computational or splicing evidence were interpreted cautiously and in the context of the relevant gene–disease relationship. PM2, BP4, and BP7 codes reported by

the source databases are presented as database-reported evidence criteria; they were not independently counted as proof of breast cancer association. BP4 and BP7 were explicitly recognized as benign-supporting evidence. Because several candidate genes do not have an established breast cancer gene–disease relationship and transcript-specific raw evidence was not uniformly available for retrospective reevaluation, we did not claim a *de novo* definitive ACMG/AMP classification for every variant. Instead, the revised tables distinguish database-reported ACMG/AMP evidence, public database classification, and the interpretation adopted in the present reanalysis.

Clinical Variables and Analytical Approach

The cohort comprised 25 individuals aged 25–64 years. The retrospective clinical records identified 18 individuals with a breast cancer diagnosis, including one individual with fibroadenomatous hyperplasia and breast cancer, and 7 individuals referred primarily because of family history.

Because the total cohort size was small and no healthy, population-matched, or internal control cohort was available, no inferential gene-level burden or enrichment analysis was performed. Related individuals were not treated as independent observations in frequency-based interpretation. Available clinical characteristics were summarized descriptively. Molecular subtype, receptor status, tumor stage, and tumor grade were not consistently available in the retrospective dataset and therefore could not be used for adequately powered subgroup analyses.

Statistical Analysis

Statistical analysis was descriptive. Categorical clinical variables (diagnostic category and referral indication) were summarized as counts, age as a range, and variant-level results as record counts at each filtering step; “once-observed” and “twice-observed” denote occurrence counts in the standardized retrospective dataset, not allele frequencies. Related individuals were not counted as independent recurrence events. No inferential analysis (hypothesis testing, p-values, confidence intervals, odds ratios, gene-level burden or enrichment tests, or multiple-testing correction) was performed, because no control cohort was available, the sample size was small, and the study was not powered for association testing; accordingly, no finding should be interpreted as statistically significant. Clinical variables that were not consistently available (molecular subtype, receptor status, tumor stage, and tumor grade) were neither analyzed nor imputed.

RESULTS

A total of 25 individuals referred for breast cancer mutation analysis were included in the retrospective reanalysis. The cohort was evaluated as an exploratory breast cancer series rather than as a case-control association cohort. At the beginning of the bioinformatic review, variant records were evaluated independently of the individual clinical characteristics; clinical context was considered only after the filtering and candidate-review stages.

After application of the literature-derived 2,156-gene exclusion list and removal of known benign and pathogenic variants, 38,173 records remained for further evaluation. The initial occurrence-

based review identified 14 variants in the once-observed group and 868 variants in the twice-observed group. Individual *in silico* and database review further reduced these groups to four and seven candidate observations, respectively.

The detailed once-observed candidate group involved *ZDHC15*, *COL17A1*, *MFN1*, and *ZNF141*. The *ZDHC15* c.137-51_137-50delGA, *COL17A1* c.1688-45_1688-44dupCA, and *MFN1* c.1663-8_1663-6dupTTT variants were intronic observations. Public interpretation resources included benign-supporting evidence for these variants, and *COL17A1* and *MFN1* were classified as benign or likely benign in at least one reviewed resource. Accordingly, these observations were retained only for descriptive completeness and were not interpreted as evidence of breast cancer susceptibility (Table 1).

Two *ZNF141* missense variants, c.136G>A (p.Ala46Thr) and c.193G>T (p.Val65Leu), were identified in the same 46-year-old female breast cancer patient. Both variants were reported as VUSs in the reviewed resources and had BP4 benign-supporting computational evidence. The patient also had previously reported VUSs in *ERBB2*, *PMS1*, and *TSC1*. The co-occurrence of two *ZNF141* VUSs in one patient was considered an exploratory observation and not evidence of a *ZNF141*–breast cancer association.

The detailed twice-observed candidate group involved *MUC6*, *VEZT*, *MYBPC1*, *ZSWIM1*, *TDRD3*, *PRDM6*, and *PIGG*. Several of these observations were synonymous or intronic and were therefore interpreted cautiously. Gene-level literature suggesting roles in cancer-related biological processes was not considered variant-level evidence of pathogenicity.

Two different *MUC6* variants were identified among four individuals. One *MUC6* variant was observed in two unrelated individuals aged 25 and 49 years; the 25-year-old individual had fibroadenomatous hyperplasia and breast cancer, whereas the 49-year-old individual was referred in the context of a family history of breast cancer. The other *MUC6* variant was observed in two sisters aged 43 and 45 years from a family with breast cancer history. Because the sisters were related, they were not considered independent recurrence events.

No formal segregation analysis involving affected and unaffected relatives was performed (Table 2).

No control-based comparison, population-matched enrichment test, gene-level burden analysis, formal segregation study, or functional validation was performed. Therefore, the present data do not demonstrate a statistical or causal association between the identified variants or genes and breast cancer.

DISCUSSION

In this retrospective descriptive study, clinical exome sequencing data from breast cancer patients were re-evaluated with a specific focus on VUS observations outside genes already prioritized in cancer literature. The study identified a limited set of recurrent or potentially noteworthy observations after sequential filtering and manual review. The findings should be interpreted as hypothesis-generating and do not establish gene–disease associations.

The original exploratory strategy excluded a literature-derived list of 2,156 genes associated with breast cancer or cancer in general. We acknowledge that excluding established cancer genes would be inappropriate in a comprehensive clinical diagnostic analysis. In the present study, however, the strategy was used only after the original clinical exome evaluation and was intended to focus a secondary research reanalysis on less routinely prioritized genes. The known cancer-related findings from the original clinical reports were not considered absent or clinically irrelevant; rather, they were outside the specific exploratory question of this secondary analysis.

MUC6 was considered of exploratory interest because two different variants were observed among four individuals. However, the interpretation is limited by family relatedness, the absence of segregation analysis, and the absence of a control population. *MUC6* encodes a secreted mucin involved in epithelial protection and has been studied as a biomarker in several epithelial malignancies (7-9). In breast cancer, mucin expression studies have reported variation in *MUC6* expression according to tumor characteristics, including estrogen receptor status and mucinous histology (10). These gene-

Table 1. List of recurring variants.

Gene	Nucleotide variation	Protein variation	dbSNP	Consequence	ACMG/AMP evidence reported in source databases	Public database classification	Interpretation in the present reanalysis
<i>ZDHC15</i>	c.137-51_137-50delGA	—	rs773803900	Intronic	BP7	VUS	Descriptive only; benign-supporting evidence
<i>COL17A1</i>	c.1688-45_1688-44dupCA	—	rs372506043	Intronic	PM2, BP7	Benign	Descriptive only; conflicting/benign evidence
<i>MFN1</i>	c.1663-8_1663-6dupTTT	—	rs75719342	Intronic	BP7	Likely benign	Deprioritized; benign-supporting evidence
<i>ZNF141</i>	c.136G>A	p.(Ala46Thr)	rs75245145	Missense	BP4; PM2 reported by VarSome	VUS	VUS; hypothesis-generating only
<i>ZNF141</i>	c.193G>T	p.(Val65Leu)	rs78725891	Missense	BP4; PM2 reported by VarSome	VUS	VUS; hypothesis-generating only

ACMG/AMP codes are evidence criteria, not numerical scores. PM2 is pathogenic-supporting rarity/absence evidence when appropriately applicable; BP4 and BP7 are benign-supporting evidence. Criteria shown here reflect evidence reported by the source databases used in the retrospective analysis and were not treated as independent proof of breast cancer association. VUS: Variant of uncertain significance, —: Not available/not applicable.

Table 2. Candidate variants in the twice-observed group.

Gene	Nucleotide variation	Protein variation	dbSNP	Consequence	ACMG/AMP evidence reported in source databases	Public database classification	Interpretation in the present reanalysis
<i>MUC6</i>	c.5T>G	p.(Val2Gly)	rs201440145	Missense	PM2 reported	VUS	VUS; hypothesis-generating only
<i>MUC6</i>	c.4967C>T	p.(Pro1656Leu)	—	Missense	PM2, BP4 reported	VUS	VUS; hypothesis-generating only
<i>VEZT</i>	c.634delC	p.(Arg212GlufsTer8)	—	Frameshift	PM2 reported	VUS	VUS; no PVS1 assigned without gene/disease-specific LoF assessment
<i>MYBPC1</i>	c.1793T>A	p.(Ile598Lys)	—	Missense	PM2 reported	VUS	VUS; no breast-cancer association inferred
<i>ZSWIM1</i>	c.627C>T	p.(Asn209=)	—	Synonymous	PM2, BP7 reported	Not available	Descriptive only; BP7 is benign-supporting
<i>TDRD3</i>	c.1019G>A	p.(Arg340Lys)	—	Missense	PM2 reported; BP4 reported by VarSome	VUS	VUS; hypothesis-generating only
<i>PRDM6</i>	c.561G>A	p.(Pro187=)	—	Synonymous	PM2, BP7 reported	Likely benign	Deprioritized; benign-supporting evidence
<i>PIGG</i>	c.2871C>T	p.(Tyr957=)	—	Synonymous	PM2, BP7 reported; BP4/BP7 by VarSome	Likely benign	Deprioritized; benign-supporting evidence

ACMG/AMP codes are evidence criteria, not numerical scores. PM2 is pathogenic-supporting rarity/absence evidence when appropriately applicable; BP4 and BP7 are benign-supporting evidence. Criteria shown here reflect evidence reported by the source databases used in the retrospective analysis and were not treated as independent proof of breast cancer association. VUS: Variant of uncertain significance, —: Not available/not applicable.

level observations provide biological context but do not demonstrate that the *MUC6* variants identified in our cohort confer breast cancer susceptibility.

ZNF141 was also of exploratory interest because two missense VUSs were observed in a single breast cancer patient. Zinc-finger proteins have diverse regulatory functions, and several members of the broader zinc-finger protein family have been implicated in cancer biology (11-16). Nevertheless, evidence concerning other zinc-finger genes cannot be extrapolated to *ZNF141*. Moreover, both *ZNF141* variants had BP4 benign-supporting computational evidence. The co-occurrence of two VUSs in one individual is insufficient to infer causality, and larger datasets and gene-specific functional studies are required.

The intronic observations in *ZDHHC15*, *COL17A1*, and *MFN1* illustrate an important limitation of retrospective VUS review. Although such variants may remain unresolved in one resource or source workflow, benign-supporting evidence, location outside canonical splice regions, or benign/likely benign classifications in other resources substantially weaken disease-related interpretation. These variants were therefore discussed descriptively and were not used to support breast cancer association. The same caution applies to synonymous observations: gene-level cancer literature does not establish a functional effect for a specific synonymous variant.

The ACMG/AMP framework is an evidence-based classification system rather than a numerical scoring system (17). PM2, BP4, and BP7 are evidence criteria with different directions and strengths. Current ClinGen guidance emphasizes transparent, criterion-specific application of rarity, computational, and splicing evidence. In particular, BP4 and BP7 are benign-supporting criteria and cannot be used to support disease relevance. We therefore did not

convert automated Franklin or VarSome outputs into independent evidence of breast cancer association. The revised tables separately report database-reported ACMG/AMP evidence, public database classification, and our interpretation in this retrospective reanalysis. A definitive *de novo* ACMG/AMP classification was not assigned where the required gene–disease validity, transcript-specific, segregation, or functional evidence was unavailable.

Breast cancer is clinically and molecularly heterogeneous. The reviewer appropriately suggested analyses by age at diagnosis, family history, histopathological subtype, molecular subtype, stage, grade, and *BRCA1/BRCA2* status. Age and family history were available and are now presented descriptively. However, receptor status, molecular subtype, stage, and grade were not consistently available in the retrospective source dataset, and the cohort size of 25 individuals would result in very small subgroup counts. We therefore did not perform inferential subgroup analyses that could yield unstable or misleading estimates.

Periodic reinterpretation of VUSs remains important because population resources, clinical databases, computational evidence, and functional data evolve. Nevertheless, reinterpretation should not be conflated with association testing. Robust gene–disease association requires appropriate comparison cohorts, population matching, independent observations, statistical enrichment or burden analyses, and preferably segregation and functional evidence. The present study provides candidate observations that may inform future research under these more rigorous designs.

Study Limitations

This study has several limitations. The sample size was small and the retrospective cohort was not designed or powered for variant-level

or gene-level association testing. No healthy, population-matched, or internal control cohort was available, precluding enrichment and burden analyses. The cohort was clinically heterogeneous, and molecular subtype, receptor status, tumor stage, and tumor grade were not consistently available for subgroup analysis. Two clinical exome workflows with different output structures were used, requiring retrospective standardization of variant records. The 2,156-gene exclusion strategy was an exploratory research filter and was not a validated disease-association pipeline. Related individuals were present among the *MUC6* observations and no formal segregation analysis was performed. No RNA, splicing, or other functional validation was available. Finally, VUS classifications and database evidence evolve over time. These limitations preclude causal or statistical association claims.

CONCLUSION

In conclusion, this retrospective descriptive reanalysis of clinical exome data from breast cancer patients identified a limited number of exploratory VUS observations after sequential filtering and database review. *MUC6* and *ZNF141* were of potential biological interest; however, the current data do not demonstrate that variants in these genes are associated with breast cancer. The observations should be considered hypothesis-generating and require evaluation in larger, population-matched cohorts incorporating independent recurrence, segregation analysis, gene-level statistical testing, and functional validation. The study also emphasizes the importance of cautious, transparent, and periodic reinterpretation of VUSs in clinical molecular genetics.

Ethics

Ethics Committee Approval: The study was approved by the Başkent University Medical and Health Sciences Research Board on April 12, 2022 (decision no. 119004; project no. KA22/45) and was supported by the Başkent University Research Fund.

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: F.İ.Ş., Concept: S.G.E., F.İ.Ş., Design: S.G.E., F.İ.Ş., Data Collection or Processing: S.G.E., Analysis or Interpretation: S.G.E., Literature Search: S.G.E., Writing S.G.E., F.İ.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplement 1 Link: <https://d2v96fxpocvxx.cloudfront.net/cf9d60d6-523c-458a-a2e6-78728d3ffb0/content-images/4ddd9c81-7e43-453e-a8fa-1a48305e1313.pdf>

Supplement 2 Link: <https://d2v96fxpocvxx.cloudfront.net/cf9d60d6-523c-458a-a2e6-78728d3ffb0/content-images/11b3ce4a-8b7b-4def-b8a5-d5ba070331a2.pdf>

Supplement 3 Link: <https://d2v96fxpocvxx.cloudfront.net/cf9d60d6-523c-458a-a2e6-78728d3ffb0/content-images/e6702b2d-792d-4a16-a02f-d45a656a92cd.pdf>

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