IJMS
Vol 48, No 4, July 2023

Brief Report

A Novel Germline Pathogenic Variant of *RECQL4* Gene in an Iranian Pedigree with Familial Squamous Cell Carcinoma: A Brief Report

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Received: 13 February 2022
Revised: 19 May 2022
Accepted: 18 July 2022

Abstract

Squamous cell carcinoma (SCC) is the most common human solid tumor and the leading cause of cancer death. SCC of the breast is a very rare type of cancer that has not been well researched. Early identification of the genetic factors involved can lead to early diagnosis and targeted treatment. The present study was conducted in 2018 at Isfahan University of Medical Sciences (Isfahan, Iran). The proband was a 66-year-old woman with SCC of the breast and a positive family history of cancer. Blood DNA samples were used for whole-exome sequencing to identify germline pathogenic variants. Variant annotation and prioritization were done on variant call format files using bioinformatics software tools. The screened variants were confirmed using the Sanger sequencing method. Co-segregation analysis was performed on the blood DNA samples of the first- and second-degree relatives of the proband to assess the presence of the mutation. A novel germline pathogenic variant was identified in the *RECQL4* gene of the family. *RECQL4* is a known protein in DNA repair and replication. Considering its effect on other types of SCC, it may play an important role in SCC initiation and progression in the breast.

Please cite this article as: Amin M, Mahmoodi-Khaledi E, Narrei S, Zeinalian M. A Novel Germline Pathogenic Variant of *RECQL4* Gene in an Iranian Pedigree with Familial Squamous Cell Carcinoma: A Brief Report. Iran J Med Sci. 2023;48(4):420-424. doi: 10.30476/IJMS.2022.94539.2587.

Keywords ● Cancer ● Next generation sequencing ● Whole exome sequencing ● Carcinoma ● Squamous cell

What's Known

- Squamous cell carcinoma (SCC) is the most common human solid tumor and the leading cause of cancer death.
- Identification of genetic factors involved in SCC can lead to early diagnosis and targeted treatment.

What's New

- For the first time, a new clinical feature caused by a germline novel mutation in the *RECQL4* gene is presented.
- An applicable method for genetic testing of other types of SSC is presented.

Introduction

Squamous cell carcinoma (SCC) of the breast is a very rare type of cancer that has not been well-researched. Differentiation should be made between the primary skin keratinizing squamous carcinoma and squamous metaplastic cancer.[1] SCC of the skin is generally the most common type of cancer in humans. It can, however, be observed in unusual organs, such as the thyroid, prostate, and breast of unknown origin.[2] The most common cause of SCC is prolonged exposure to ultraviolet light that damages DNA in skin cells and tumorigenesis.[2, 3]

Deleterious mutations in genes such as *TP63*, *TP53*, *RB1*, *CDKN2A*, *CCND1*, *MYC*, *FBXW7*, *HRAS*, *SOX2*, *NRF2*, *EP300*, *NOTCH-1*, and *RECQL4* may lead to the development of a variety of SCCs.[4, 5] Mutations in cell cycle-regulated genes, such as *CDKN2A*, *RB1*, and *TP53*, were commonly reported in SCC. Moreover, mutations in some signaling pathway-related genes, including *RAS*, *MAPK*, and *PI3K*, could lead to different types of

SCCs.[6] In this study, a family with familial SCC was examined to identify pathogenic variants.

Patients and Methods

This study was conducted in 2018 at Isfahan University of Medical Sciences in collaboration with Ala Cancer Prevention and Control of Charity Center (Isfahan, Iran). The study was approved by the Ethics Committee of Kashan University, Kashan, Iran (code: 925090). Written informed consent was obtained from the patient and her relatives for the publication of this brief report.

The proband was a 66-year-old woman (III-6) with SCC of the breast and a positive family history of cancer (figure 1). Six cases of such malignancies were present in 27 family members across three generations. In addition to the patient, her mother (II-3) was diagnosed with SCC of the lip at the age of 72, and her 75-year-old sister (III-4) with SCC of the esophagus.

Immunohistochemical (IHC) staining for ER, PR, Cerb-B2, and P53 was negative but was positive for CK 5/6. Core needle biopsy of the right breast showed neoplastic proliferation of squamous cells composed of cellular sheets with abundant keratin pearl formation, which indicated SCC. In addition, fine-needle aspiration (FNA) smears were moderately cellular and showed some epithelial cells with extensive eosinophilic cytoplasm and enlarged nuclei, indicative of positive malignant cells.

Altogether, clinical manifestations, mammograms, and laboratory results confirmed the presence of SCC of the breast in the proband. Subsequently, genetic counseling was done to determine the pedigree and the inheritance pattern (figure 1). Genomic DNA was extracted from the peripheral blood sample of the proband (III-6) using the genomic DNA purification kit (GeNet Bio, South Korea). Qualified DNA samples were sent to CENTOGENE GmbH (Germany) for whole-exome sequencing using the NovaSeq system and Illumina software suite (www.illumina.com). Variant annotation and prioritization were performed by CENTOGENE using bioinformatics platforms such as Toolkit (https://toolkit.tuebingen.mpg.de/), BWA (https://bio.tools/bwa), Pcard (https://broadinstitute.github.io/picard/), and GATK (https://gatk.broadinstitute.org/hc/en-us) to determine probable disease-causing variants. The results of variant calling analysis, presented in variant call format (VCF), were filtered as proposed in a previous study.[7] Briefly, variants with homogeneous changes were excluded, since they are not usually seen in the carrier state. Novel variants within 3´UTR, 3´intronic, 5´UTR, 5´intronic, introns, and upstream genes were also excluded. Variants with a frequency greater than 1% (according to different databases) were excluded, given the assumption of their safety and normal function. Prediction software tools such as wANNOVAR (https://wannovar.wglab.org/), CADD (https://cadd.gs.washington.edu/), PolyPhen (http://genetics.bwh.harvard.edu/), Clin-Var (https://www.ncbi.nlm.nih.gov/clinvar/), Mutation Taster (https://www.mutationtaster.org/), and GeneCards (https://www.genecards.org/) were used to select the related genes.

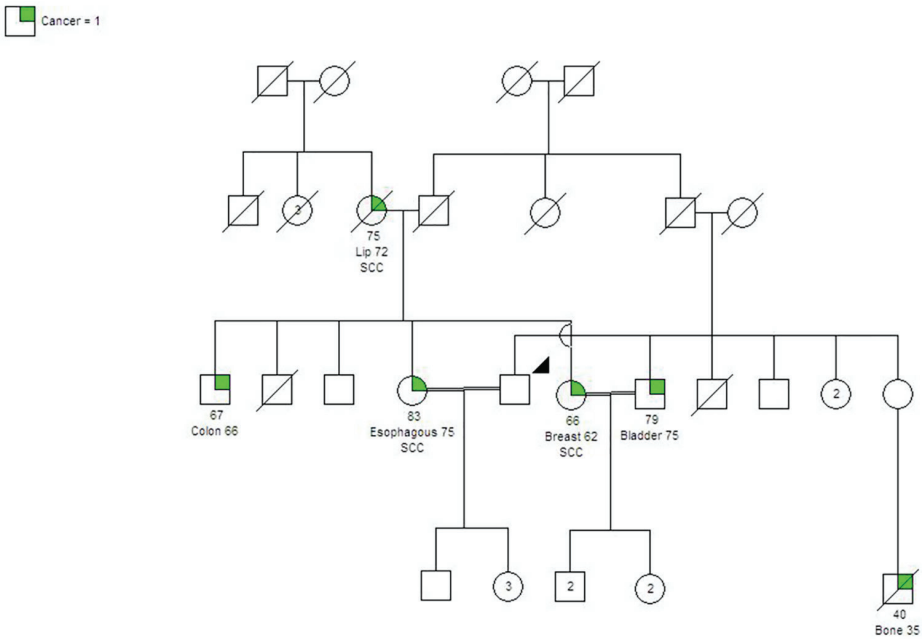

Figure 1: The family pedigree of a 66-years-old woman with SCC of the breast, as the proband and a positive family history of cancer in three successive generations is shown.

Finally, variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[8] The screened variants were confirmed using the Sanger sequencing method to rule out possible false-positive results.

Ideally, after confirming the variants in the genomic DNA of the proband, her parents and siblings should have been investigated. However, since her parents had already died, her sister (III-4) and three offspring (IV-5, IV-6, IV-7) were evaluated. The genomic DNA extracted from the four family members was amplified using 1.5 µL forward and reverse primers for the *RECQL4* gene (5'-GCCTGCCTGCATCTGACAT-3' / 5'-GCCTGCCTGCATCTGACAT-3'). The PCR program was set to 95 °C for two minutes (1 cycle), 95 °C for 20 sec, 62 °C and 59 °C for 30 sec, and 72 °C for 30 sec (35 cycles), with a final extension of five minutes at 72 °C. The PCR products were analyzed in 2% agarose gel electrophoresis.

Results

Out of the 7,176 variants reported in the VCF file, 1,114 homozygous variants were removed. Of the remaining 6,062 variants, 3,690 were removed, as they were within the non-coding regions of the genes. In addition, of the remaining 2,372 variants, those with a frequency greater than 1% of the population were removed, and eventually, 265 variants were analyzed. Following genotype-phenotype association analysis, only one disease-causing variant (*RECQL4*: c.3104_3105insA with NM_004260.4 and consequence frameshift) was identified because of its compatibility with the disease phenotype (table 1).

Based on the results of Sanger sequencing and co-segregation analysis, variants similar to the *RECQL4* of the proband (figure 2A) were observed in two of the evaluated family members (IV-6 and IV-7) (figures 2B and 2C),

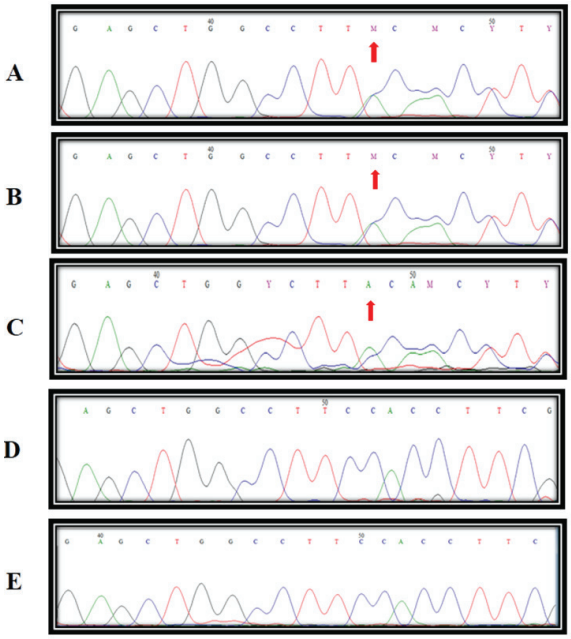

Figure 2: Electropherograms related to the co-segregation analysis of the *RECQL4* are illustrated. The c.3104-3105 insA variant was detected by Sanger-sequencing in the proband and her four first-degree relatives. The mutation is shown by the red arrow.

while no mutations were observed in the other two (III-4 and IV-5) (figures 2D and 2E, table 2).

Discussion

Primary SCC of the breast is a very rare cancer, and its definitive diagnosis is difficult, because this type of malignancy usually remains unclear with cytological and histopathological examinations. In the present study, a large pedigree with familial SCC and the proband with SCC of the breast has been genetically constructed.

Following variant prioritization and filtering of the extracted data using the whole-exome sequencing method, the *RECQL4* gene was identified as a pathogenic variant. The protein encoded by *RECQL4* is a DNA helicase belonging to a protein family called RecQ

Table 1: The identified gene variant as a possible cause of familial squamous cell carcinoma in the family pedigree

Name	Chr.	Ref.	Alt.	Transcript	Consequence	Base change
RECQL4	Chr8	G	GT	ENST00000617875.4	Frameshift	c.3104_3105insA

Chr: Chromosome; Ref: Reference; Alt: Alternative; G: Guanine; T: Thymine; ins: Insertion

Table 2: Gene sequences and the location of the identified novel mutation in the pedigree with familial squamous cell carcinoma

Proband and her four first-degree relatives	*RESQL4* variant
Proband III-6	AGCTGGCCTTM*C
Family member III-4	AGCTGGCCTTCC
Family member IV-5	AGCTGGCCTTCC
Family member IV-6	AGCTGGCCTTM*C
Family member IV-7	AGCTGGYCTTA*C

helicases. *RECQL4* helicase is a molecular motor that unwinds DNA, an essential process during DNA replication and DNA repair.[9] *RECQL4* is believed to be a tumor suppressor, but its role in human breast cancer is not fully determined.[10] The *RECQL4* gene has 21 exons and plays an important role in maintaining genome stability, aging, and cancer.[11] Its association with susceptibility to cancer and premature aging due to deficiency in DNA helicase and germline mutations of the RECQL4 gene were reported.[12]

In line with a previous study,[13] we identified a pathogenic variant in the *RECQL4* gene (exon 18, c.3104_3105insA) that could cause SCC. Given the family pedigree and the observed inheritance pattern, tumor suppressor genes and their germline mutations are considered candidates for pathogenesis. Although the identified variant has been frequently reported in the SCC of the skin, there is no report of this mutation in the SCC of the breast as a rare disease.[14] This gene variant was confirmed by the Sanger sequencing method in the samples from the proband, her living sibling, and their offspring. As mentioned earlier, the variant in the *RECQL4* gene was detected in two relatives of the proband, which could be used as a diagnostic biomarker for the early detection of at-risk family members. Based on the autosomal dominant pattern of the disease in the family pedigree, a carrier state is expected in half of the first-degree relatives of the proband. It appears that the carriers of the *RECQL4* pathogenic variant should undergo regular screening for early detection of cancer and be more aware of cancer risk factors and prevention methods.

The main limitation of the study was the low sample size due to the rarity of the disease. Further studies with larger sample sizes are required to substantiate our findings.

Conclusion

It is recommended to include similar cases to better assess the pathogenicity of the novel variant and identify other variants. Considering the time and costs involved with the Sanger sequencing method to identify deleterious and/or disease-causing variants related to this phenotype, the use of next-generation sequencing technology, particularly whole-exome sequencing, is recommended.

Acknowledgment

The authors would like to thank the patient and her family members for participating in the study and for permission to publish the manuscript. We also would like to thank the staff at Ala Cancer Prevention and Control Center for their support.

Authors' Contribution

M.A: Formal analysis, investigation, methodology, original draft preparation. E.M-Kh: Conceptualization, funding acquisition, resources, supervision, review, and editing. S.N: Formal analysis, investigation, original draft preparation. M.Z: Conceptualization, funding acquisition, resources, supervision, review, and editing. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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