ORIGINAL ARTICLE

Rare Protein-Truncating Genetic Variants in a Tribal Family of Baluchistan with Familial Cancers

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ABSTRACT

Background: Inherited cancers are heterogeneous, involving the role of multiple genes. Identification of the underlying genes assists in early diagnosis, prognosis, and screening of the affected families.

Objective: This study aimed to perform genetic testing in a high cancer-risk tribal family of Baluchistan, Pakistan, having three patients with different cancers, including medulloblastoma, osteosarcoma, and colorectal carcinoma.

Methods: This was a familial genetic testing study in which a family from the Mandokhel tribe of Quetta, Pakistan (South Asia), with three cancer patients, was recruited at the Patel Hospital, Karachi, Pakistan, during March to August 2018. The medulloblastoma and osteosarcoma probands, along with both the parents, three healthy siblings, and the osteosarcoma proband's son, were recruited. Next-generation sequencing of the TruSight cancer panel (94 genes and hotspots of 284 SNPs) was performed using Illumina MiSeq. The bioinformatics analysis of sequencing data was performed to determine the potential pathogenic genetic variants.

Results: The bioinformatics analysis identified two ultra-rare protein-truncating heterozygous single-nucleotide variants (SNVs). These included a pathogenic variant in TP53 (p.R342X), associated with hereditary cancer-predisposing syndrome, detected in the father, two probands, and two unaffected sisters, as well as a *de novo* variant (p.S35X) in *SDHC* in the medulloblastoma proband. Additionally, a new *de novo* deleterious missense variant (p.L167P) in *STK11* was identified in the medulloblastoma proband. In the osteosarcoma patient, two missense variants (p.T1777I and p.H1927R) in *PLCE1*, previously associated with an elevated cancer risk, were also detected.

Conclusion: The findings are suggestive of a likely pathogenic role of protein-truncating SNV rs730882029 of *TP53* synergistically with other *de novo*/missense SNVs in a compound heterozygous manner, in this familial cancer.

Keywords: *Inherited cancer syndrome, pathogenic variant, compound heterozygous, deleterious, TP53.*

INTRODUCTION

The majority of cancers occur by chance, as a result of lifestyle choices or environmental conditions. Nevertheless, in some families, the incidence of cancer is more than might be expected to occur by chance [1]. The occurrence of multiple primary malignant tumors may be owing to a familial cancer syndrome. So, it is very crucial to determine which of the families carries an inherited gene mutation, as the cancer risk is much higher in hereditary cancer families than in the general population. Red flags indicate hereditary cancers include early age at onset, rare presentations of cancer, and a combination of cancers on the same side of a family [2].

World-wide research efforts on different ethnicities have enhanced our understanding of genetic predisposition to inherited cancers, but despite these discoveries, a large percentage of familial cancers remains unexplained, highlighting the fact that the majority of susceptibility genes remain unidentified [3]. Inherited mutations in *BRCA1* and *BRCA2* genes are associated with hereditary breast and ovarian cancer syndrome, with *BRCA1* having a higher mutation rate. *PALB2* is the second most commonly mutated gene for hereditary pancreatic cancer. Lynch syndrome (hereditary non-polyposis colorectal cancer) can be caused by a mutation in any of several mismatch repair (MMR) genes, including *MLH1, MSH2, MSH6, PMS1,* and *PMS2*. The most commonly somatically mutated gene in all cancers is TP53, which produces a protein that suppresses the growth of tumors. In addition, germline mutations in this gene can cause Li-Fraumeni syndrome, an inherited disorder that leads to a higher risk of developing certain cancers. Mutations in *the PTEN* gene are associated with Cowden syndrome, an inherited disorder that increases the risk of breast, thyroid, endometrial, and other types of cancer [4-7].

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Received: May 02, 2025; Revised: July 17, 2025; Accepted: August 07, 2025
DOI: https://doi.org/10.37184/lnjcc.2789-0112.6.9

  ISSN: 2789-0120 (Online) Liaquat National Journal of Cancer Care 2024; 6(2): 41-47

Pakistan is the sixth most populous country in the world, facing a lot of healthcare issues due to poor socio-economic conditions [8, 9]. Consanguinity has imparted a 2.5 times higher risk of breast cancer in Pakistan than in neighboring countries [10]. Despite this, little research has been carried out on cancers in Pakistan. Most of the studies on the genetics of inherited cancers in this population focus on mutation screening in the well-associated genes in different cancers, mostly the breast/ovarian cancers. Earlier, three families with hereditary ovarian cancer syndromes were identified, but a genetic determinant could not be found due to a lack of facilities [11]. A study identified 30 different deleterious mutations in the *BRCA1* and *BRCA2* genes of breast and ovarian cancer patients in Pakistan [12]. Another report indicated 9% involvement of BRCA1 alterations and 3% BRCA2 alterations in non-selected breast and ovarian cancers in Pakistan, while the remaining 88% of breast and ovarian cancers can be attributed to the involvement of other genes or develop somatically without prior germline predisposition [13]. A recent study identified *PALB2* germline mutations in early onset of breast/ovarian cancers with negative *BRCA1, BRCA2, TP53, CHEK2*, and *RAD51C* mutations in Pakistan [14]. These studies highlight the importance of discovering inherited cancer-related genes using a broader screening approach. In this study, a family with three male siblings suffering from three different cancers, *i.e.*, colorectal carcinoma (deceased), medulloblastoma, and osteosarcoma, was recruited and analyzed genomically to determine the mutations responsible for frequent representations of cancer. Both of the cancer types (medulloblastoma and osteosarcoma) are rare presentations of cancer, which is an important red flag for tumor predisposition. Moreover, the age at onset was 30, 12, and 22 years for osteosarcoma, medulloblastoma, and colorectal carcinoma patients, respectively. The early age at onset of cancers is also a typical characteristic of hereditary cancer.

MATERIALS AND METHODS

Study Design

This was a familial genetic testing study in which a family from the Mandokhel tribe of Quetta, Pakistan (South Asia), with three cancer patients, was recruited at the Patel Hospital, Karachi, Pakistan, during March to August 2018. The living family members with cancer were included, while the third one with colorectal carcinoma was excluded due to his prior death. The index individuals were two brothers, one with osteosarcoma (D13) and the other with medulloblastoma (D11). Six healthy members of the family, including both parents (C3 & C4), one healthy brother (D12), two healthy sisters (D7 & D9) and Proband-1's son (E1), were also recruited (**Fig. 1**). The study was approved by the Independent Ethics Committee (IEC) of the institute (ICCBS/IEC-071-HT-2021/Protocol/1.0). Written and informed consent was obtained from all the individuals.

Sample Collection and DNA Extraction

The peripheral blood samples were collected from all the subjects in EDTA blood collection tubes after consent. Samples were stored at 4°C until further processing. The genomic DNA was extracted from the whole blood using the QIAamp DNA mini kit (QIAGEN, Hilden, Germany). Quality of the genomic DNA was assessed using 1% agarose gel electrophoresis. The concentration of the extracted DNA was determined with Qubit™ 2.0 Fluorometry using HS DNA kit (Life Technologies, Oregon, USA).

Sequencing of TruSight Cancer Panel Genes

TruSight cancer panel targets a 255 kb genomic region, which comprises 94 genes and hotspots of 284 SNPs, which have been described previously to exhibit predisposition for various cancers. The DNA sequencing library was prepared using the Illumina TruSight Rapid Capture kit (Illumina, San Diego, CA, USA) according to the manufacturer's protocol. For this, 50 ng of genomic DNA was tagmented, followed by adaptor ligation, and amplification of the fragments using sample-specific indexes. The enrichment of fragments corresponding to TruSight cancer panel genes/regions was carried out through hybridization of biotinylated region of interest (ROI) probes. The quality of the library was assessed with Bioanalyzer 2100 DNA 1000 kit (Agilent, Santa Clara, CA, USA), and quantified by using QIAseq Library Quant Assay kit (Qiagen, Hilden, Germany). The 2 x 150 bp paired-end sequencing was carried out using MiSeq reagent kit V2 (Illumina, San Diego, CA, USA) for ~500x depth of coverage.

Analysis of Sequencing Data

The primary base calling from the raw sequencing data, alignment of the short reads with the reference human genome (hg19), and variant calling were performed on MiSeq as its default built-in standardized pipeline of Genome Analysis Tool Kit (GATK) best practices. The VCF files generated by the MiSeq system were retrieved. The low-quality variants with quality score QUAL<50, genotype quality GQ<20, and depth DP<20 were filtered out, as described earlier [15]. To find the pathogenic variants, guidelines of the American College of Medical Genetics and Genomics [16] were followed. For this, multiple tools were employed to highlight potentially deleterious genetic variants. First, variants having a minor allele frequency $\geq$ 0.01 in publicly available databases, including 1000 Genomes Project phase 3, Exome Aggregation Consortium (ExAC), gnomAD_genome, and gnomAD_exome, were filtered out to remove population-specific polymorphisms. Then the variants were annotated using ANNOVAR [17] and Combined

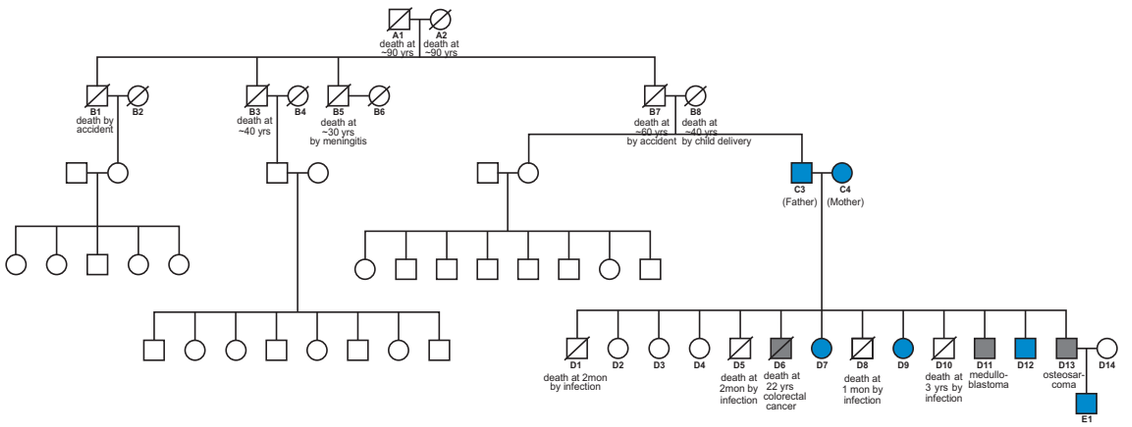

Fig. (1): Pedigree of the family. Squares: males, circles: females. The shaded squares/circles indicate the individuals recruited in this study. Grey: affected individuals, blue: healthy individuals. Crosses: deceased.

Annotation Dependent Depletion (CADD) [18] tools. The ANNOVAR annotates the variants with gene-based, region-based, and filter-based annotations. The CADD tool is integrated with multiple annotations, based on which, it predicts a Phred-scaled C-score of variants, where the higher CADD C-score represents higher pathogenicity. A variant was considered aeleterious for which the CADD C-score $\geq$ 15, SIFT score <0.05, and PolyPhen2_HDIV score was >0.957, as described by their authors. Then the variants were filtered with ClinVar [19] and COSMIC [20] datasets to determine the pathogenic variants previously associated with various types of cancers. The allele frequencies of prioritized variants in various populations were determined from the online Ensembl portal (https://www.ensembl.org/). The variants were also filtered with the ClinPred database, which is a machine learning prediction tool trained to determine pathogenic variants specifically in the perspective of rare genetic and cancer conditions. The variants having a score >= 0.5 were considered as pathogenic, as described by the authors [21]. The variants were further subjected to filtration with the dbDSM database to determine the deleteriousness of synonymous variants [22].

The family data was also analyzed for autosomal recessive variants to assess the genetic risk imparted from the parents. For this, homozygous variants in either of the parents were excluded, and subsequently heterozygous variants in parents but homozygous in the offspring were determined. A 2 x 2 contingency table was constructed (**Table 1**), and the Odds ratio was calculated by using Fisher's exact test.

Table 1: Number of homozygous and heterozygous genetic variants in the affected and healthy siblings of the family.

Variants	Affected n(%)	Healthy n(%)
Homo variants	29 (44)	16 (22)
Hetero variants	37 (56)	56 (78)

RESULTS

The individual with osteogenic sarcoma was a male of 30 years (D13). He underwent excision of this lesion, which showed a 5.7 x 5.5 x 3.0 cm osteogenic sarcoma (chondroblastic variant). He subsequently received systemic chemotherapy and stayed in remission for ~ 15 months, when he developed a recurrent swelling in his right mandible. This was treated with radiation therapy and is currently being managed with systemic chemotherapy.

The individual with a midline cerebellar tumor was a male of 12 years (D11). The tumor was excised, and the histopathology confirmed medulloblastoma grade IV. He subsequently received chemoradiation followed by chemotherapy and is in remission. He developed a transient hearing deficit that was considered secondary to cisplatin-induced ototoxicity.

The third individual was a 22-year-old male (D6) who developed tenesmus and mucus discharge from the rectum. A biopsy was performed, and he was found to have rectal adenocarcinoma. He underwent systemic chemotherapy followed by chemoradiation. His disease progressed on this treatment, and he eventually expired because of his advanced disease.

The ages of the healthy siblings were D7 9 years, D9 11 years, and D12 15 years at the time of recruitment. In the genetic testing, 375 different genetic variants were found after filtering out the low-quality sites (QUAL<50, GQ<20, and DP<20). The summary of the variants pertaining to different genomic locations in all the individuals is shown in Table **2**.

To determine the variants possibly involved in the onset of cancers in the probands, all the individuals were screened for the 284 TruSight SNPs list of the panel. This filtration showed a high number of SNPs (161-175 SNPs) found in all members of the family, with the medulloblastoma patient having 175 SNPs, and the osteosarcoma patient having 168 SNPs. The father had 168, the mother 174,

Table 2: The number of total genetic variants in different genomic regions of the 94 genes in each individual.

Individuals	C3	C4	D13	D11	D7	D9	D12	E1
No. of variants	273	297	283	290	285	284	282	279
Exonic	111(40.66)	130(43.77)	115(40.64)	125(43.1)	120(42.11)	124(43.66)	126(44.68)	121(43.37)
Upstream	1(0.37)	1(0.34)	1(0.35)	1(0.34)	1(0.35)	1(0.35)	0(0)	1(0.36)
Downstream	1(0.37)	2(0.67)	2(0.71)	1(0.34)	1(0.35)	2(0.7)	1(0.35)	2(0.72)
Intergenic	68(24.91)	68(22.9)	71(25.09)	71(24.48)	70(24.56)	67(23.59)	62(21.99)	62(22.22)
Intronic	59(21.61)	63(21.21)	62(21.91)	58(20)	63(22.11)	56(19.72)	63(22.34)	60(21.51)
ncRNA_exonic	4(1.47)	5(1.68)	5(1.77)	5(1.72)	4(1.4)	4(1.41)	4(1.42)	5(1.79)
ncRNA_intronic	23(8.42)	21(7.07)	19(6.71)	20(6.9)	19(6.67)	23(8.1)	19(6.74)	20(7.17)
Splicing	1(0.37)	1(0.34)	1(0.35)	3(1.03)	1(0.35)	1(0.35)	1(0.35)	1(0.36)
UTR3	3(1.1)	5(1.68)	5(1.77)	4(1.38)	4(1.4)	4(1.41)	4(1.42)	5(1.79)
UTR5	2(0.73)	1(0.34)	2(0.71)	2(0.69)	2(0.7)	2(0.7)	2(0.71)	2(0.72)

The numbers in () indicate % of the total variants in an individual. C3 = Father, C4 = Mother, D13 = Proband1, D11 = Proband2, D7 = Healthy sister 1, D9 = Healthy sister 2, D12 = Healthy brother, E1 = Son of Proband 1 (D13)

the three healthy siblings contained 161, 164, 170, while the son of proband 1 contained 162 TruSight SNPs. For comparison, this 284-SNP list was also filtered with in-house whole-exome sequencing data of five dilated cardiomyopathy patients [15], which showed that there were 18-29 TruSight SNPs (average=24, SD±4) in each individual.

Filtration of the genetic data with the ClinVar database showed a pathogenic SNV rs730882029 (heterozygous and stopgain) in *TP53* (NM_000546.6; p.R342X) in the father, both of the probands, and two healthy sisters. Search through publicly available databases showed this variant as ultra-rare and somatic, as it was not present in 1000 Genomes Project, ExAC, gnomAD_genome, and gnomAD_exome datasets, rather catalogued in the COSMIC database with COSM11073 and COSM99721 IDs. Analysis of the variants with VEP showed another stopgain SNV rs778212096 in *SDHC* (NM_001035513: p.S35X) in the medulloblastoma proband. Further evaluation revealed that 4 protein-coding transcripts were affected by this premature-truncating variant. This SNV was also ultra-rare and its allele frequency has been reported in Non-Finnish Europeans of the gnomAD_exome database as 3.598×10^{-5}. Further, a heterozygous frameshift deletion rs369823368 in *FANCD2* (NM_001018115.3: c.1278+3_1278+6del) with conflicting classifications of pathogenicity was found in all the members of the family studied here. The population's allele frequency of this deletion is 8.89×10^{-6} in Non-Finnish Europeans of the gnomeAD_exome database. The assessment of deleteriousness of missense variants by using SIFT, Polyphen2, and CADD tools prioritized a novel missense SNV chr19:1220407, T>C (*STK11*) in the D11. The evaluation of variants using ClinPred divulged three missense SNVs as pathogenic. These included rs760678574 in *SDHC* (in D11), rs760056622 in TSC2 (in C4, D13, D12, D7, and E1), and the novel SNV chr19:1220407,T>C in *STK11* (in D11).

Then the family data was also analyzed for autosomal recessive variants to find the risk imparted from the parents. For this, homozygous variants in either of the parents were excluded, and subsequently heterozygous variants in either of the parents but homozygous in the offspring were determined. This analysis revealed a higher number of homozygous variants in both the probands (D13=34, D11=25) as compared to healthy siblings of the family (D12=18, D7=12, D7=17). The odds ratio was found as 2.6943 (95% CI: 1.2866-5.6418, p=0.008). To determine the risk in Proband1's son (E1), the homozygous variants were also calculated in him and found to be 17. The odds ratio of both the probands and E1 was calculated as 1.8903 (95% CI: 0.8968-3.9843, p=0.009). Further, the analysis of the *de novo* mutations in the probands and healthy siblings showed that six *de novo* mutations were present in the medulloblastoma proband, including the heterozygous stopgain SNV rs778212096 of SDHC, and three nonsynonymous SNVs, including the novel SNV (chr19:1220407, T>C in *STK11*). The osteosarcoma proband contained only one heterozygous synonymous *de novo* SNV rs61742551 in *RHBDF2*.

In addition to the pathogenic/likely pathogenic variants, a synonymous SNV rs200435277 in PTCH1, catalogued as likely benign in ClinVar but associated with hereditary cancer-predisposing syndrome, was observed in mother (C4) and Proband 1 (D13) and Proband 2 (D11), but absent in father (C3), healthy siblings, and E1 individual. Further, two homozygous missense SNVs (rs3765524,and rs2274223) in PLCE1

(NM_016341.4: p.T1777I, and p.H1927R) found in Proband1 only.

DISCUSSION

The study deals with the genomic analysis of a family exhibiting multiple occurrences of different cancers to determine potential pathogenic variants. Three of the siblings in the family were suffering from three different cancer types, *i.e.*, osteosarcoma, medulloblastoma, and colorectal carcinoma. The sibling suffering from colorectal carcinoma had died. The genomes of two cancer patients, as well as healthy members of the family, including parents, one brother, two sisters, and the son of the osteosarcoma patient, were subjected to enrichment with TruSight cancer panel, and sequencing, followed by germline variant calling. The analysis of variants was carried out for the autosomal recessive model, as well as for independent risk variations.

The presence of a relatively higher number of TruSight SNPs in the family than in non-cancerous individuals represents the predisposition of the family to cancers. The ClinVar pathogenic stopgain rare SNV rs730882029 of *TP53*, in the father, two probands, and two healthy sisters, suggests further risk of cancers. To determine whether the effect of this protein-truncating SNV is protected through the nonsense-mediated decay (NMD) pathway, exploration in the Ensembl database (https://www.ensembl.org/index.html) revealed 8 protein-coding transcripts being affected by this variant, while one transcript (ENST00000635293.1) was found to undergo NMD. Through exploration of the COSMIC database, this SNV was found to affect seventeen pathways, including the androgen receptor signaling pathway, DNA damage response, MAPK signaling pathway, and TGF-beta receptor signaling pathway *etc.* The disruption of signaling pathways of cell survival and proliferation due to *TP53* genetic alterations is associated with multiple types of cancers, termed as Li-Fraumeni syndrome (LFS) [OMIM#151623], breast cancer, somatic [OMIM#114480], hepatocellular carcinoma, somatic [OMIM#114550], and nasopharyngeal carcinoma, somatic [OMIM#607107]. This protein-truncating variation was also found previously in two unrelated patients with Li-Fraumeni syndrome at age <14 years [23]. The clinical manifestation of the family is also suggestive of the typical picture of LFS, because it is characterized by early onset of tumors, or multiple tumors within an individual, or multiple family members affected. LFS presents a variety of tumor types rather than site-specific cancers, the most common types being soft tissue sarcomas, osteosarcomas, brain tumors, breast cancer, leukemia, and adrenocortical carcinoma [24, 25].

The presence of ClinPred pathogenic, deleterious synonymous, and frame-shift variants seems to further the predisposition of the family to cancers. The variants prioritized as deleterious by more than one method/tool used are suggestive of their strong role in cancer pathophysiology in the family. This included the novel *de novo*, heterozygous missense SNV chr19:1220407(T>C) of *STK11*, found in a medulloblastoma proband, and predicted as deleterious by SIFT, Polyphen 2, having a CADD_phred score of 26.9, and predicted as pathogenic by the ClinPred tool. The gene encodes serine/threonine kinase 11, mutations in which are associated with Peutz-Jeghers Syndrome [OMIM#175200]. This T>C transition causes NM_000455: p.L167P substitution, and likely introduces a premature bend in the protein chain. Physiologically, this kinase possesses multiple cellular functions in the regulation of cell bioenergetics metabolism, embryo development, cell cycle arrest, cell polarity, apoptosis, and maintenance of function and dynamics of hematopoietic stem cells [26]. Also, the stk11 physically associates with p53 in the nucleus and phosphorylates p53 Ser15 and Ser392 residues. These two residues of p53 are required for stk11-dependent cell cycle arrest at the G1 stage.

The findings showed multiple cancer-predisposing deleterious/pathogenic genetic variants in the family members, nevertheless, to highlight the variants possibly causing neoplasms in the probands only, a mutation spectrum was constructed to determine deleterious mutational load in all the study subjects using the prioritized variants including the stopgain, splicing, frameshift indel, and predicted deleterious variants (**Table 3**). This showed that D11 possibly acquired medulloblastoma due to multiple heterozygous mutations in *TP53* (rs730882029), *SDHC* (rs778212096, rs760678574), and/or *STK11* (chr19:1220407, T>C). The protein-protein network analysis (https://string-db.org/) indicated strong interactions of co-expression,

Table 3: The spectrum of deleterious mutations in the family. The red colour shows high risk, while green low. The ClinPred pathogenic variant in D11 (underlined) was same as predicted deleterious, so it was counted once. The numbers in bold represent the proposed compound heterozygous variants.

Effect	Gene	C3	C4	D13	D11	D12	D7	D9	E1
ClinVar Pathogenic	***TP53***	**1**		**1**	**1**		1	1	
ClinPred Pathogenic	***SDHC***				1				
	STK11				1				
	TSC2		1	1		1	1		1
Predicted deleterious by in silico tools	***FANCI***		1	1		1	1		
	ERCC4								1
	STK11				1				
Stopgain	***SDHC***				1				
Frameshift	***FANCD2***	1	1	1	1	1	1	1	1
Synonymous	***PTCH1***		**1**	**1**	**1**				
TOTAL		**2**	**4**	**5**	**6**	**3**	**4**	**2**	**3**

experimental, and the curated databases between the *TP53* and *STK11*. The cellular tumor antigen p53 acts as a tumor suppressor in many tumor types, and induces growth arrest or apoptosis depending on the physiological circumstances and cell type [27] . The serine/threonine-protein kinase (STK11) is also a tumor suppressor that controls the activity of AMP-activated protein kinase (AMPK) family members, thereby playing a role in various processes such as cell metabolism, cell polarity, apoptosis, and DNA damage response [28]. The additive effect of heterozygous mutations is in line with previous reports [29]. The mutations in TP53 and STK11 are previously associated with lower overall survival in various cancers [30]. The mutation spectrum (**Table 3**) also showed the highest risk in the medulloblastoma proband, followed by osteosarcoma and healthy sister 1 (D7). The prioritized variants in the osteosarcoma proband were the same as in healthy sister 1 (D7). Further, the *TP53* mutation was also detected in D7 in addition to D9 and both the probands. The onset of cancer in proband 1 seems to be the effect of multiple heterozygous variations, which were absent in healthy siblings, for example, the likely benign synonymous SNV rs200435277 (*PTCH1*), inherited from the mother and present in both the probands D11 and D13, but absent in the healthy siblings. This rare SNV is associated with hereditary cancer-predisposing syndrome, and absent in 1000 Genomes Project phase 3, ExAC datasets, but present in ESP6500 European_Americans with an alternate allele frequency of 0.000116. These findings suggest the role of the synergistic contribution of rs730882029 (*TP53*) and rs200435277 (*PTCH1*). *PTCH1* encodes patched 1 protein, involved in the hedgehog signaling pathway, and associated with Gorlin syndrome [OMIM#109400]. Further, two homozygous missense SNVs (rs3765524 and rs2274223) in *PLCE1* (NM_016341.4: p.T1777I and p.H1927R) were found in Proband1 only, suggesting their additional role in cancer pathophysiology in the family. This gene encodes a phospholipase C epsilon-1 enzyme involved in the hydrolysis of phosphatidylinositol-4,5-bisphosphate to generate two secondary messengers: inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). These two SNVs have been shown in a meta-analysis to induce a higher risk of cancers [31].

CONCLUSION

Given the aforementioned, the family was at risk of multiple types of cancers due to the presence of a higher number of cancer predisposing SNPs, rare deletions, rare stopgain, missense, and synonymous SNVs in a complex heterozygous manner. The findings of the study will not only help clinicians in taking prompt measures in cancer predisposed healthy members of the family, but will also contribute to cancer data repositories from the South Asian region.

ETHICS APPROVAL

The study was approved by the Independent Ethics Committee (IEC) of the institute (ICCBS/IEC-071-HT-2021/Protocol/1.0). All procedures performed in studies involving human participants were following the ethical standards of the institutional and/ or national research committee and the Helsinki Declaration.

CONSENT FOR PUBLICATION

Written and informed consent was obtained from all the patients for publishing the genetic results anonymously.

AVAILABILITY OF DATA

All the results and associated data have been presented in the manuscript.

FUNDING

The study was funded by Searle Pharmaceuticals Ltd., Pakistan. The funding entity has no role in study design, results interpretation, manuscript preparation, or submission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

AUTHORS' CONTRIBUTION

MS, AA performed experimentation, data analysis, and manuscript write-up; RHS, SKN, and AHA identified, consulted, and treated family, and provided clinicopathological information; IAK supervised the study.

REFERENCES

1. Corso G, Feroce I, Intra M, Veronesi P, Sacchini V, Bonanni B, *et al*. Prognostic impact of germline mutations in inherited cancer syndromes. Future Med 2017; 13(24): 2125-7. DOI: https://doi.org/10.2217/fon-2017-0296

2. Sijmons RH. Identifying Patients with Familial Cancer Syndromes. Bethesda (MD), USA: National Center for Biotechnology Information; 2010. Available from: https://www.ncbi.nlm.nih.gov/books/NBK45295/

3. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW. ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. Am J Gastroenterol 2015; 110(2): 223. DOI: https://doi.org/10.1038/AJG.2014.435

4. National Cancer Institute. The Genetics of Cancer 2018. Available from: https://www.cancer.gov/about-cancer/causes-prevention/genetics.

5. Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev 2009; 23(5): 537-48. Available from: DOI: http://www.genesdev.org/cgi/doi/10.1101/gad.1756509

6. Pritchard CC, Mateo J, Walsh MF, De Sarkar N, Abida W, Beltran H, *et al*. Inherited DNA-repair gene mutations in men with metastatic prostate cancer. N Engl J Med 2016; 375(5): 443-53. DOI: https://doi.org/10.1056/NEJMOA1603144

7. Hu C, Hart SN, Polley EC, Gnanaolivu R, Shimelis H, Lee KY, *et al*. Association between inherited germline mutations in cancer predisposition genes and risk of pancreatic cancer. JAMA 2018; 319(23): 2401-9.
DOI: https://doi.org/10.1001/JAMA.2018.6228

8. The World Bank. The World Bank data for world populations 2017. Available from:
http://databank.worldbank.org/data/download/POP.pdf.

9. Zaidi S, Saligram P, Ahmed S, Sonderp E, Sheikh K. Expanding access to healthcare in South Asia. BMJ 2017; 357: j1645.
DOI: https://doi.org/10.1136/BMJ.J1645

10. Shaukat U, Ismail M, Mehmood N. Epidemiology, major risk factors and genetic predisposition for breast cancer in the Pakistani population. Asian Pac J Cancer Prev 2013; 14(10): 5625-9.
DOI: https://doi.org/10.7314/APJCP.2013.14.10.5625

11. Aziz AB, Sabih M, Malik IA. Hereditary Ovarian Cancer Syndromes in Three Pakistani Families. J Pak Med Assoc 1996; 46: 201-3.

12. Rashid MU, Zaidi A, Torres D, Sultan F, Benner A, Naqvi B, *et al*. Prevalence of BRCA1 and BRCA2 mutations in Pakistani breast and ovarian cancer patients. Int J Cancer 2006; 119(12): 2832-9.
DOI: https://doi.org/10.1002/ijc.22269

13. Farooq A, Naveed AK, Azeem Z, Ahmad T. Breast and ovarian cancer risk due to prevalence of BRCA1 and BRCA2 variants in Pakistani population: a Pakistani database report. Journal of Oncology. 2011; 2011(ID 632870): 1-8.
DOI: https://doi.org/10.1155/2011/632870

14. Rashid MU, Khan FA, Muhammad N, Loya A, Hamann U. Prevalence of PALB2 germline mutations in early-onset and familial breast/ovarian cancer patients from Pakistan. Cancer Res Treat 2019; 51(3): 992-1000.
DOI: https://doi.org/10.4143/crt.2018.356

15. Shakeel M, Irfan M, Khan IA. Rare genetic mutations in Pakistani patients with dilated cardiomyopathy. Gene. 2018; 673: 134-9.
DOI: https://doi.org/10.1016/j.gene.2018.06.019

16. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17(5): 405.
DOI: https://doi.org/10.1038/gim.2015.30

17. Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc 2015; 10(10): 1556-66.
DOI: https://doi.org/10.1038/nprot.2015.105

18. Kircher M, Witten DM, Jain P, O'roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 2014; 46(3): 310-5.
DOI: https://doi.org/10.1038/NG.2892

19. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, *et al*. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res 2013; 42(D1): D980-5.
DOI: https://doi.org/10.1093/nar/gkt1113

20. Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, *et al*. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res 2014; 43(D1): D805-11.
DOI: https://doi.org/10.1093/nar/gku1075

21. Alirezaie N, Kernohan KD, Hartley T, Majewski J, Hocking TD. ClinPred: prediction tool to identify disease-relevant nonsynonymous single-nucleotide variants. Am J Hum Genet 2018; 103(4): 474-83.
DOI: https://doi.org/10.1016/j.ajhg.2018.08.005

22. Wen P, Xiao P, Xia J. dbDSM: a manually curated database for deleterious synonymous mutations. Bioinformatics. 2016; 32(12): 1914-6.
DOI: https://doi.org/10.1093/bioinformatics/btw086

23. Gonzalez KD, Buzin CH, Noltner KA, Gu D, Li W, Malkin D, *et al*. High frequency of *de novo* mutations in Li–Li-Fraumeni syndrome. J Med Genet 2009; 46(10): 689-93.
DOI: https://doi.org/10.1136/jmg.2008.058958

24. OMIM. Li-Fraumeni Syndrome; LFS 2019.
Available from: https://www.omim.org/entry/151623.

25. Li FP, Fraumeni JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, *et al*. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988; 48(18): 5358-62.

26. Zhao R-X, Xu Z-X. Targeting the LKB1 tumor suppressor. Curr Drug Targets 2014; 15(1): 32-52.
DOI: https://doi.org/10.2174/1389450114666140106095811

27. Chen X, Zhang T, Su W, Dou Z, Zhao D, Jin X, *et al*. Mutant p53 in cancer: from molecular mechanism to therapeutic modulation. Cell Death Dis 2022; 13(11): 974.
DOI: https://doi.org/10.1038/s41419-022-05408-1

28. Rho SB, Byun HJ, Kim BR, Lee CH. LKB1/STK11 Tumor Suppressor Reduces Angiogenesis by Directly Interacting with VEGFR2 in Tumorigenesis. Biomol Ther 2023; 31(4): 456-65.
DOI: https://doi.org/10.4062/biomolther.2023.106

29. Kamphans T, Sabri P, Zhu N, Heinrich V, Mundlos S, Robinson PN, *et al*. Filtering for compound heterozygous sequence variants in non-consanguineous pedigrees. PLoS One 2013; 8(8): e70151.
DOI: https://doi.org/10.1371/journal.pone.0070151

30. Krishnamurthy N, Goodman AM, Barkauskas DA, Kurzrock R. STK11 alterations in the pan-cancer setting: prognostic and therapeutic implications. Eur J Cancer 2021; 148: 215-29.
DOI: https://doi.org/10.1016/j.ejca.2021.01.050

31. Li X, Li X, Jiang M, Tian W, Zhou B. Single nucleotide polymorphisms in PLCE1 for cancer risk of different types: a meta-analysis. Front Oncol 2018; 8: 613.
DOI: https://doi.org/10.3389/fonc.2018.00613