RESEARCH ARTICLE

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A Case Study on PPM1D and 9 Other Shared Germline Alterations in a Family

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Abstract

Background: The use of high-throughput genotyping techniques has enabled us to identify the rare germline genetic variants with different pathogenicity and penetrance, and understand their role in cancer predisposition. We report here a familial cancer case, a study from Western Indian. **Methods:** NGS-WES was carried out in a lung cancer patient who has a family history of multiple cancers across generations, including tongue, lung, brain, cervical, urothelial, and esophageal cancer. The results were validated by data mining from available data bases. I-TASSER, RasMol and PyMol were used for protein structure modelling. **Results:** The sequencing by NGS-WES revealed PPM1D c.1654C>T (p.Arg552Ter) mutation in hotspot region exon 6 leading to sudden protein truncation and loss of the C-terminal, due to the substitution of C>T. This mutation was classified as a variant of uncertain significance (VUS), due to limited data on lung cancer, The three unaffected siblings of proband did not show any pathogenic variants and comparative analysis of the four siblings indicate 9 shared genetic variants, classified as benign as per ClinVar. **Conclusion:** PPM1D constitutional genetic alterations are rare and uncommon in different ethnic populations. This gene encodes a phosphatase playing role in regulating the P53 tumor suppressor pathway and DNA damage response. Genetic alterations in the PPM1D gene maybe linked to history of gliomas, breast cancer, and ovarian cancer onset in the proband's family.

Keywords: ClinVar- whole-exome sequencing- glioma- lung cancer- I-TASSER

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Introduction

Familial cancers are an outcome of interaction between environmental factors and genetic makeup, of an individual. The constitutional genetic variants which play role in rare diseases have high penetrance and low allelic frequency for e.g., RAD50 and PPM1D mutations have 0.0003% and 0.003% of allelic frequency which play role in breast, ovarian, brain and lung cancers. The detection of such important rare genetic variants can be missed when the targeted gene panel is only used.

This constraint has been overcome with the use of NGS-WES, enabling the study of rare variants broadly across human exomes, for e.g., reports from Southern India indicate role of CHEK2 in addition to BRCA1, BRCA2 in early onset of HBOC (Hereditary Breast and Ovarian cancer) and lung cancer in families with a history of breast and ovarian cancer (Rajkumar, 2003), while genetic alterations in the MRE11A (p.Arg364Ter) are reported in breast cancer without the BRCA1 and BRCA2 mutations (Bhai, 2017). NGS-WES in breast cancer patient revealed three recurrent mutations L719fs, K994fs, H1269fs in RAD50 gene, reduce the chances of recurrence free survival (Fan, 2018).

Our earlier study involved NGS-WES in a patient with breast and ovarian cancer and her asymptomatic son, with a family history of pancreatic, prostate, and intestinal cancer across the generations. Reports showed BRCA2, NOS3 and NTHL1 genetic mutations. In this study we report a PPM1D heterozygous mutation in the exon 6 of a lung cancer patient. We also report 9 other shared genetic variants in the patient and his three asymptomatic siblings.

Materials and Methods

Informed consent of the participants was taken and 6 ml of venous blood was collected in an EDTA vial aseptically, stored at -20ºC. DNA isolation was performed using Qiagen Blood MidikitTM. NGS-WES was done with Exome capture kit. The libraries were sequenced 80-100x coverage on the Illumina sequencing platform to generate paired-end output.

Clinical details and pedigree

We report a WES study in a lung cancer patient 66Y/M (DM_01). The pedigree in Figure I shows family history tongue, lung, brain, cervical, urothelial, and esophageal cancers across the generations. Genotyping has been done in patient and his three unaffected siblings. None, of them have a history of tobacco chewing, smoking, or alcohol

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consumption or other significant cancer-causing exposure.

Bioinformatic analysis with GATK Sentieon and GATK4

The GATK Sentieon was used for raw data analysis and detection of constitutional genetic variants. The GRCh38.p13 was used as the reference genome which was aligned by Sentieon aligner, followed by removal of duplicates, base quality score recalibration, and re-alignment of indels. Sentieon haplotype caller was implemented for the detection of clinically relevant variants. Gene annotation was aided by the VEP (Variant Effect Predictor) program (McLaren, 2016), against the Ensembl release 99 human genome model. The results obtained using GATK Sentieon (Freed, 2017) was revalidated using GATK4 (McKenna, 2010).

Open source database and in-silico tools used for validation of results and protein modelling

The genetic variants were annotated from existing literature and databases like ClinVar, OMIM (Online Mendelian Inheritance in Man) (Hamosh, A., 2005), GWAS (Genome Wide Association Studies)(MacArthur, 2017), HGMD (Human Gene Mutation Database) (Stenson, 2017), and Swiss Var (Mottaz, Anaïs, 2010), while the common variants were filtered based on allele frequency from 1000 Genome Project (Purcell, 2007), GenomAD (Chen, 2022), dbSNP (Sherry, 1999). The effect of the non-synonymous variants in the coding regions and the splice sites were determined using algorithms like PolyPhen-2 (Adzhubei, 2010), SIFT (Sorting Intolerant from Tolerant) (Ng, 2003), MutationTaster2 (Schwarz, 2014), and LRT (Likelihood Ratio Test). Protein structure model was generated with the help of I-TASSER (Yang, 2015). Stability of the protein structure was determined by C-score, Tm-score and RMSD (Root Mean Square Deviation) values of 2.04, 0.47 and 12.7 respectively and a cluster density of 0.0465 with 600 decoys. Mutagenesis in the native protein structure was developed using RasMol (Mantis, 2023) and PyMol.

Results

In DM_01 a PPM1D c.1654C>T (p.Arg552Ter) heterozygous variant was detected in exon 6. It has been

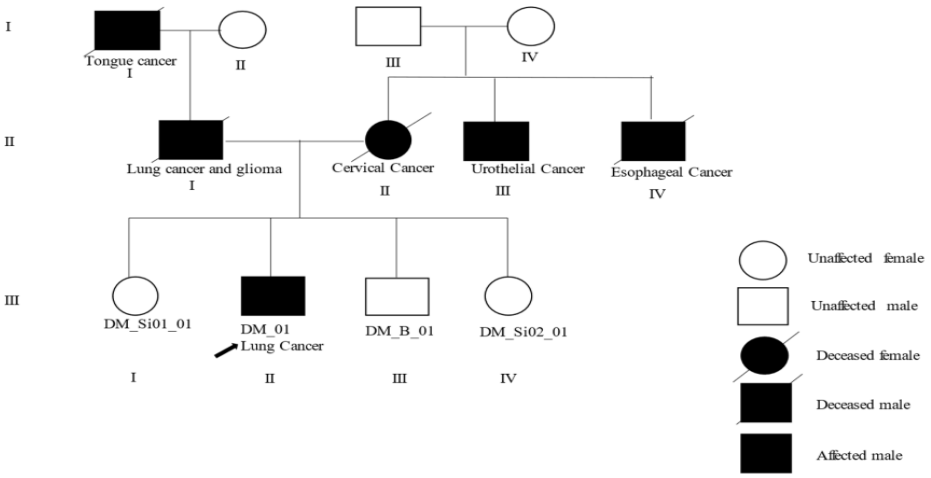

Figure 1. Pedigree of Lung Cancer Patient with Family History of Cancer

Table 1. The Shared Variants in the Lung Cancer Patient and Three Unaffected Siblings

Sr no.	The segregated constitutional variants	HGVS nomenclature	Last evaluation and ACMG classification
1	rs1042522	TP53(NM_000546.6) c.215C>G p.Pro72ARG	(Jun 18, 2022) Benign
2	rs12628	HRAS (NM_005343.4) c.81T>C p.His27=	(Jan 18, 2022) Benign
3	rs1566734	PTPRJ(NM_002843.4) c.827A>C p.Gln276Pro	(May 04, 2022) Benign
4	rs4792311	ELAC2(NM_018127.7) c.650C>Tp.Ser217Leu	(Oct 04, 2021) Benign
5	rs1670283	ALK(NM_004304.5) c.4381A>G p.Ile1461Val	(Jan 12, 2022) Benign
6	rs1801131	MTHFR(NM_005957.5) c.1286A>C p.Glu429Ala	(Oct 21, 2021) Benign
7	rs1881421	ALK (NM_004304.5) c.4587C>G p. Asp1529Glu	(Jan 06, 2022) Benign
8	rs1881420	ALK (NM_004304.5) c..4472A>G p.Lys1491Arg	(Dec 18, 2021) Benign
9	rs201315884	MET(NM_000245.4) c.110T>C p.Val37Ala	(Dec 15, 2021) Benign

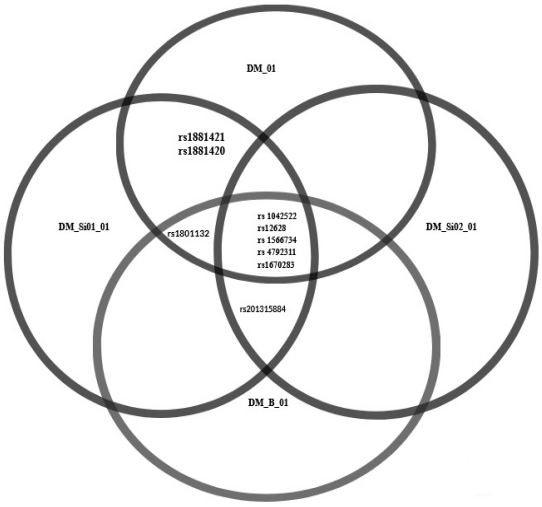

Figure 2. Segregation of Genetic Variants in the Lung Cancer Patient and Three Unaffected Siblings

classified as VUS according to the ACMG guidelines. WES of his three siblings did not show any pathogenic variant, while VEP analysis for the four siblings showed 9 shared genetic variants which are classified as benign as per the evaluation in ClinVar as shown in Table 1 and Figure 2.

Discussion

We studied this family for inheritance of germline mutations and performed NGS-WES on the proband having PPM1D gene mutation which was also checked in three of his siblings. However, since samples are not available from parents and progeny of the proband, it is difficult to establish significance of this finding in terms of cancer onset.

The PPM1D c.1654C>T (p.Arg552Ter) heterozygous variant in exon 6 on chromosome 17 has been classified as pathogenic in May 04, 2022 with respect to breast and ovarian cancer, but due to lack of report in lung cancer the genetic variant is classified as VUS in our NGS-WES report. PPM1D gene encodes proteins of PP2C (Protein Phosphatase 2C) family Ser/Thr protein phosphatases, that negatively regulate the cell stress response pathways by disrupting DNA damage response pathways, in a P53 dependent manner. This is necessary to recover the cells from pre-stress state after DNA damage is repaired as shown in Fig III (Husby, 2021).

Truncation of PPM1D protein as shown in Fig IV (a, b, c) has a considerable effect on the phosphatase activity of PPM1D leading to its upregulation and deregulation of P53 in the same cell, leading to inhibition of double stranded break repair, nucleotide excision repair and base excision repair followed by tumorigenesis (Li, 2019).

PPM1D genetic alterations although rare are reported in various types of cancers like breast cancer, ovarian cancer, thyroid cancer, sarcoma, lung cancer, pancreatic cancer, glioma etc. (Deng, 2020).

Whole genome analysis of 170 pediatric high-grade gliomas indicated PPM1D as driver mutation` in diffused

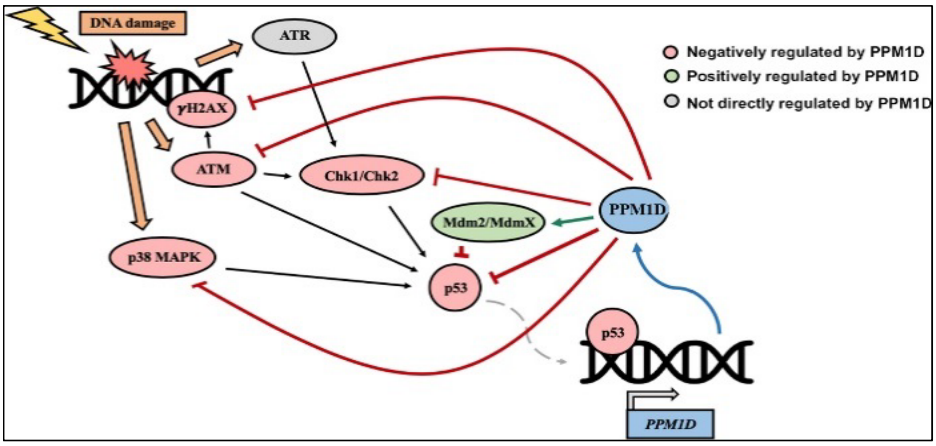

Figure 3. PPM1D Regulatory Pathway

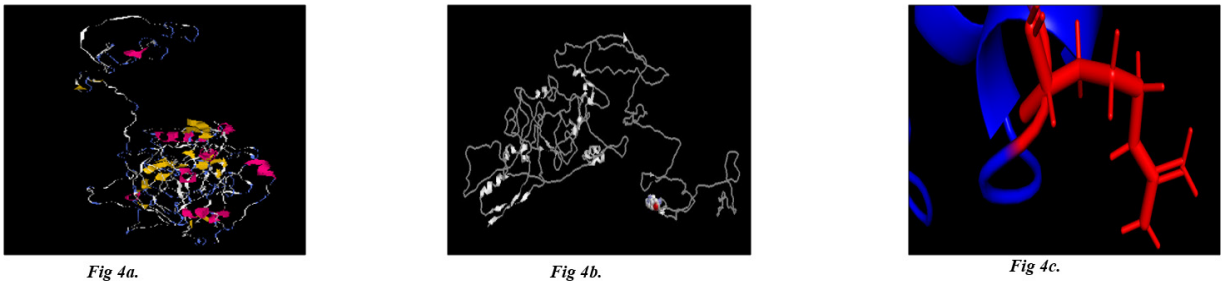

Figure 4. a, Shows the native protien structure generated by I -TASSER. b, The location of the mutation has been denoted with RasMol. c, The replacement of C>T and the predicted changes in the catalyitic site have been observed by inducing muatgenesis using RasMol andPyMol.

midline gliomas (DMGs). Reports revealed truncation in exon 6 causing PPM1D stability, indicating it to be clonal drivers in 11% DMGs and are enriched in primary pontine tumors (Khadka, 2022). The frequency of PPM1D in Non-Small Cell Lung cancer (NSCLC) patients is comparatively higher than the breast and colorectal cancer patients and similar to the ovarian cancer patients (Ruark et al. and Akbari et al.). Functional analysis showed two truncating (frame-shift) mutations Arg458X, Lys469G out of which Arg458X enhanced the dephosphorylation of PPM1D at serine 15 of p53, while Lys469G was equivalent to the wild type, yet none of them showed strong modulatory action on p53 (Zajkowicz, 2015). A study showed mutations on exon 6 in 20 out of 1295 BRCA1/BRCA2 negative ovarian cancer patients from Toronto. Reports concluded that PPM1D mutations is associated with an increased risk to ovarian cancer predisposition in first degree relative of non-carrier ovarian cancer patients (Akbari, 2014). Ruark et al., (2013) showed the predisposition to breast and ovarian cancers could be due to PPM1D mosaic mutations. Study in individuals of European descent showed, 18 protein truncating genetic variants identified in exon 6 in 6,912 breast cancer patients, and 12 mutations in 1,121 ovarian cancer patients. The effect of PPM1D c.1384C>T and PPM1D c.1420delC mutations were studied to suppress the p53 activity post Infrared exposure of human U2OS tumor cells transfected with the wildtype PPM1D construct, and indicated a gain of function of PPM1D hyperactive isoforms due to the suppression of P53.

Kleiblova et al., (2013) sequenced the hotspot region in exon 6 of PPM1D gene, which detected two heterozygous mutations in c.1349delT and c.1372C>T, leading to truncation of p.L450X and p.R458X, validated in vitro in HCT116 and U2OS cells respectively. The mutations caused stabilization of the WIP1 protein leading to the inactivation of p53 and the DDR (DNA Damage Repair) pathway, thereby allowing the mutated cells to progress to S phase. Clinical analysis conducted in patients with colorectal cancer and high-risk patients with BRCA1/BRCA2 negative breast and ovarian cancer revealed the presence of four deleterious mutations located in exon 6, namely c.1372C>T and c.1602insT in colorectal cancer patients, and c.1601del15 and c.1451T>G in breast cancer patients. All the mutations were associated with the truncation of Wip1 proteins p.R458X, p.L484X, p.K535X, and p.F534X leading to P53 impairment (Kleiblova et al., 2013).

The rs1042522 (TP53), which is a tumor suppressor has a negative correlation in Iranian Breast cancer patients (Afzaljavan et al., 2020) and eastern Chinese children with neuroblastoma (Fang et al., 2020). Study on Polish post-menopausal females (Wujcicka et al.,, 2019) and Moroccan population showed increased predisposition to endometrial cancer (Ayoubi et al., 2018). The rs1801131 (MTHFR) can increase the predisposition to breast cancer in West Asian (Rezaee et al., 2021) and Egyptian population (Omran et al., 2021), along with ovarian (Xiong et al.,, 2020)and hepatocellular carcinoma (Su, 2019). The rs12628 (HRAS) increases risk to urinary bladder cancer and lung cancer in smokers (Laytragoon Lewin, 2021)and has been observed as one of the hot spot mutations in Uzbek lung cancer patients (Mirakbarova, 2021). Homozygous rs12628C (HRAS) mutations are found to increase melanoma risk in North American population (Tomei et al.,, 2012). The segregated variant rs1566734 (PTPRJ) with the highest frequency in Jewish population, is not associated with increased cancer risk (Laczmanska et al., 2019). Studies show a corelation between rs4792311 (ELAC2) and prostate cancer (Zahiri, 2020). The rs1881420 (ALK) and rs1881421 (ALK) is associated with the squamous cell carcinoma of lung (Mansour et al., 2020), rs1670283 (ALK) is shown to be associated with hereditary cancer predisposing syndrome and neuroblastoma 3 (Machlowska et al., 2020). There are lack of reports on rs201315884 (MET), but the variants of MET gene play a role in hereditary papillary renal cell carcinoma (Johnston et al., 2012)

In conclusion, Our findings of PPM1D gene mutation in a lung cancer patient corroborates with the history of lung cancer with brain metastasis in his deceased father as role of PPM1D is reported in lung and brain cancer (Deng et al., 2020), however, genetic analysis was not carried out in father. Hence, we would like to carry out study in both of his progeny to understand the likely contribution of PPM1D mutation to pathogenicity.

Author Contribution Statement

SRB: Conceptualization of the project and enrolment of cases based on their medical history and informed consent; SM: Analysis of the NGS-WES raw data and analysis using the GATK tools; SB: Blood sample collection, genetic analysis and interpretation using open databases and preparation of the manuscript.

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Ethical approval

The study design has been approved by the Nirma University, Institutional Ethical Committee (IEC), certificate number IEC/NU/20/IS/03

Availability of data and material

The datasets are available with the principal investigator. We believe in transparency and reproducibility, and making the dataset available to the public which will facilitate further research and collaboration in this area.

Conflict of interest

The authors declare no conflict of interests.

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