

Genotype-Phenotype Analysis in an Indian Family Affected with Li-Fraumeni Syndrome-Role of Genetic Counselling

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ABSTRACT Li-Fraumeni syndrome (LFS) is associated with the high risk of a diverse spectrum of childhood and adult-onset malignancies with a predominance of the soft-tissue sarcomas, osteosarcoma, breast cancer, brain tumor, adrenocortical carcinomas, Wilms tumor, leukaemia and several other LFS-associated cancer types. This paper reports a case of a 43 years male diagnosed with an undifferentiated, high grade sarcoma. Genetic testing by Next Generation Sequencing revealed a heterozygous likely novel pathogenic germline mutation in the TP53 gene (c.323delG; p.Gly108ValfsTer15) in the proband. Post-test genetic counselling referred the family screening and the other eight family members were found to be carrier for the same variant. Thus the researchers have tried to describe the genotype-phenotype correlation for the LFS with the TP53 mutation which may have contributed to the variable phenotypes in the reported family with reduced/ incomplete penetrance. In this paper the researchers have also tried to highlight the cancer genetic counselling to detect an inherited cancer syndrome

INTRODUCTION

Li-Fraumeni is reported as a familial cancer syndrome with a rare incidence in the global stage. It is an autosomal dominant inherited cancer syndrome with a rare incidence that increases the risk of developing multiple primary neoplasms. Germline mutations in the TP53 gene are the underlying cause of LFS (Vogel et al. 2004; Shannon and Patel 2010). The syndrome is associated with high risks of both the paediatric and the adult-onset malignancies (Bennett et al. 1995; Bennett et al. 2008; Joerger and Fersht 2010) with a predominance of the soft-tissue sarcomas, osteosarcoma, breast cancer, brain tumor, adrenocortical carcinomas, Wilms tumor, leukaemia (Birch 1994; Olivier 2010). The most

commonly occurring cancer in LFS is the early onset (<30 years) breast cancer (25%), followed by the soft tissue sarcomas (20%), bone sarcoma (15%), brain tumor (13%) and other LFS-associated cancers. The LFS-associated cancer prevalence is higher and more pronounced in women than in men primarily because of the high incidence of female breast cancer (McBride 2014). Approximately 400 individuals from 64 families have been reported to have the disorder worldwide (Nichols 2001). There are very few cases that have been reported in India (Olivier 2003).

This familial cancer syndrome is primarily diagnosed based on the adaptable revised Chompret criteria (Table: 1) (Nichols 2001). Genetic study is an additional diagnostic tool to confirm the syndrome prevalence. The genetic evidence endorses germline mutations in two important genes causing the disease development, TP53 (17p13.1) and CHEK2 (22q12.1) (Olivier 2003). Tumor protein p53 is a 53KDa protein encoded by TP53 gene and acts as a tumour suppressor in the oncogenic meshwork. At the molecular level of investigation, germline mutation in TP53 gene has been reported to result in LFS. In case of the wild type or functional p53,

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the *CHEK2* (22q12.1) gene mutation has come up with some significant genetic alterations, notably 1100delC. According to the International agency for Research on cancer (IARC) which is the largest TP53 mutation database (version R18, April 2016, <http://p53.iarc.fr/>), to date, about 700 germline mutations have been described. Although most mutations are scattered throughout the gene, only few hotspots mutations in codons 125, 158, 175, 196, 213, 220, 245, 248, 273, 282, and 337 have been reported (Bougeard et al. 2015).

The majority of *TP53* germline mutations that cause LFS are missense substitutions and occur in the highly conserved core DNA binding domain of the protein, rendering the p53 unable either to bind the DNA or to activate the transcription (Li et al. 1988; Garber 1991; Lindor 2008) however, the truncation mutations have also been reported in patients affected with LFS (Varley 2003; Nagy et al. 2004; Mukesh 2011). A previous research re-ported that earlier onset of cancer is associated more with the missense mutations rather than the nonsense or frameshift mutations (Bougeard et al. 2015). LFS shows the autosomal dominant mode of inheritance in the families, thus the children of the affected parents have a fifty percent chance of inheriting the mutated allele either from their paternal or from their maternal side. Every generation has a tendency of expressing the disease phenotype at variable ages of the onset depending on the heterogeneous cancer types.

Recently two novel frameshift mutations at exons 3 and 4, have been reported in the Korean patients along with the previously re-ported missense mutations within exons 5-8 encoding DNA-binding region providing further insights into the TP53 mutation spectrum (Park et al. 2016).

This Indian research presents a unique case of sarcoma with multiple cancer phenotypes in a single family. The proband with the progressive disease and a strong family history of malignancies was recommended genetic testing for 94 unique genes associated with the hereditary cancers. The genetic analysis revealed a novel germline, likely pathogenic mutation in exon 4 of the TP53 gene associated with LFS and described the genotype-phenotype correlation for LFS patients with TP53 mutation. Referral of the patient to the cancer genetic clinics, timely and accurate identification of the hereditary mutation in the proband prompted the researchers to

screen the family members which showed the family to be on the terrain of LFS. The findings of this research will significantly benefit the 'at risk' family to mitigate the risk in a better way through timely monitoring and surveillance.

Case Presentation

A 43 years old male who was presented with the right gluteal pain for twenty days and a significant loss of weight and appetite for one and a half months, come for genetic counselling. A conventional MRI revealed a large heterogeneously enhancing mass in the ilium and the adjoining sacrum (Fig. 1a, 1b). PET CT demonstrated progression of the soft tissue mass, associated with the osteolytic lesion of right anterior iliac bone and the metabolically active osteolytic lesions in the right proximal sacrum, left scapula, and right 5th and 7th ribs indicating the stage IV disease. Histopathology analysis of CT guided the core biopsy from the right iliac bone was suggestive of metastatic poorly differentiated carcinoma. Microscopy revealed round to the oval tumor cells arranged in loose small clusters showed marked nuclear pleomorphism and unusual nuclei. Immunohistochemistry assay with the series of markers revealed the neoplastic cells to be positive for Vimentin (diffuse) and



Fig. 1a. PET CT Scan of the Sacrum

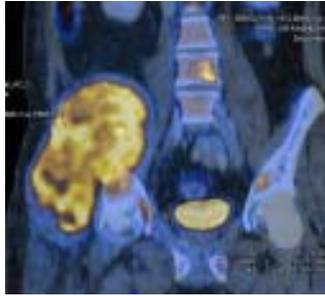


Fig. 1b. MRI Scan of the Sacrum

patchily for Bcl2, CD34, EMA and were negative for p53, CK, ALK-1 and Myf4 suggested of a high grade undifferentiated pleomorphic sarcoma of the bone (Fig. 2). The patient was commenced on appropriate chemotherapy and further molecular interventions were carried out.

Family History

Pre-genetic counselling disclosed that the proband had a strong family history of malignancies which was sketched in a pedigree using scientific symbols (Malkin et al. 1990; Chompret

et al. 2000; Birch et al. 2001; Senzer et al. 2007) (Fig. 3). The pedigree suggested that fifty percent of the family members descending from the father (I-3) of the proband were affected with cancer with the predominance of osteosarcoma at an early age in both paternal first (Fig. 3; I-3, II-1, II-2, II-7) and second degree (Fig. 3, III-3) relatives and an occurrence of the brain tumor in the second degree relatives (Fig. 3; III-13) of the proband (Fig. 3; II-4). The aggregation of such tumor types in a family at an early age supported the hereditary cancer syndrome hypothesis; hence germline mutation analysis by the Next Generation Sequencing (NGS) was suggested for the proband and his family members.

METHODOLOGY

With informed written consent, the researchers sequenced the patient's genomic DNA using the Trusight™ Cancer sequencing panel (Illumina, USA) that contained 94 genes suspected to play a role in cancer predisposition. Proband's saliva (0.5ml) was collected in "Oragene-DNA" saliva collection kit and genomic DNA was extracted using Prep IT-L2P kit (DNA

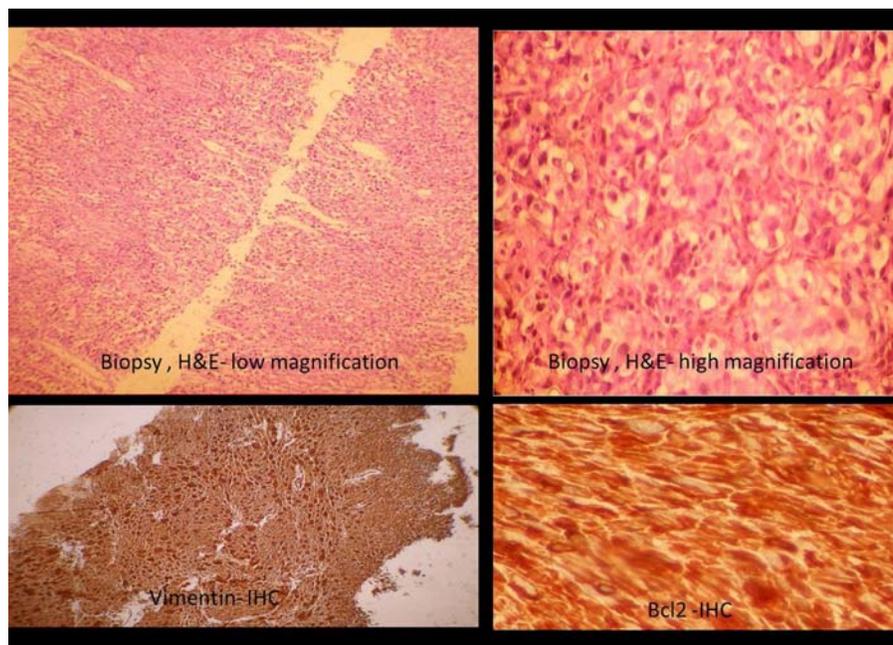


Fig. 2. Histopathology Analysis of CT guided core biopsy from the right iliac bone.

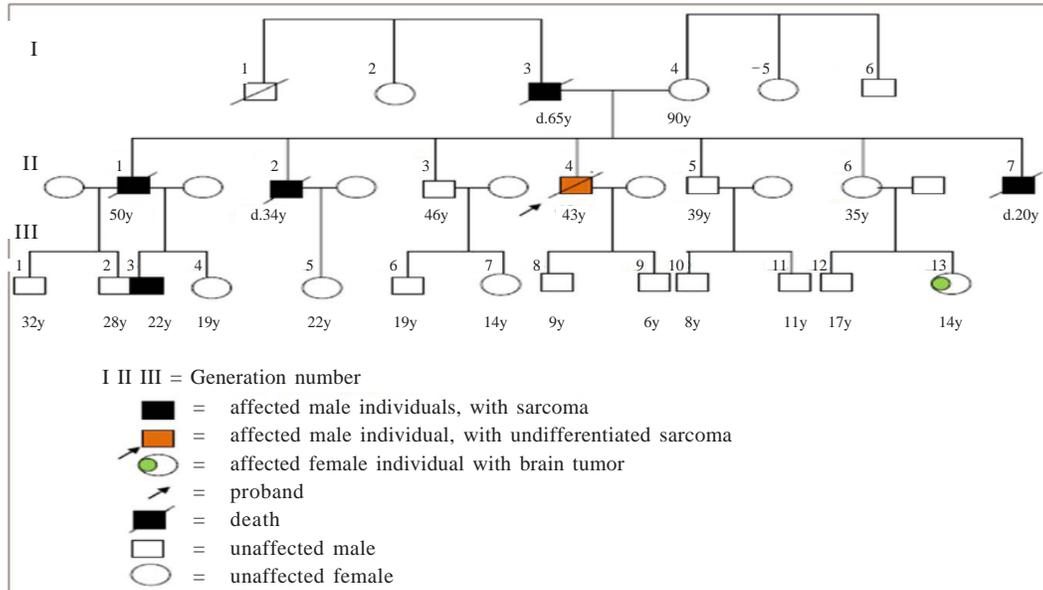


Fig. 3. Pre-genetic test pedigree chart

Genotek, Canada). The researchers used the Nextera DNA library preparation protocol (Illumina, USA) to convert the input genomic DNA (gDNA) into adapter-tagged indexed libraries. The pooled library was loaded and sequenced on the MiSeq platform (Illumina, USA), as per the manufacturer’s instructions. The variant detection algorithm in Strand NGS v2.1.6 was then used to detect variants in the target regions. The data were uploaded into StrandOmics v3.0 (<https://alpha.strandomics.com/>) which is a proprietary analysis and reporting platform from the Strand Life Sciences for all the clinical genomics analysis and variant interpretation. The identified variants in this research were classified according to the recommendations for interpretation and reporting of variants by the ACMG (American Society of Medical Genetics and Genomics). The variants were classified into five categories: a. pathogenic, b. likely pathogenic, c. variant of uncertain significance (VUS), d. likely benign, e. benign. To confirm the presence and absence of pathogenic/likely pathogenic variant in the other family members of the proband, who was reported to be positive, Sanger sequencing was performed using primers flanking the variant (Primer sequences and PCR conditions are available on request) using BigDye® Terminator v3.1 kit (Life Technol-

ogies, USA), on 3500DX Genetic Analyzer (Life Technologies, USA).

RESULTS AND DISCUSSION

This paper illustrated the importance of identifying hereditary cancer syndromes and the importance of cancer screening and early detection which can help the patients to be under constant surveillance for future cancers. The proband’s genomic DNA was screened for germline mutations in the 94 genes associated with the hereditary cancer by the NGS. A heterozygous germline likely pathogenic mutation (c.323delG) was identified in the exon 4 at codon 108 of the *TP53* gene (Transcript ID: NM_000546). The identified deletion was predicted to cause the frameshift and consequent premature termination of the protein (p.Gly108ValfsTer 15). The truncated protein was predicted to have a length of 121 amino acids (aa) as compared to the original length of 393 aa. In the resultant protein, the functionally important core DNA binding domain (94-292 aa) (Pinto et al. 2009) was likely to be disrupted, which was likely to cause the loss-of-function. Moreover, due to the introduction of the premature stop codon, this aberrant transcript was likely to be targeted by the nonsense mediated mRNA decay (NMD) mechanism. Recent studies suggested that the

resistance to radiation, chemotherapy, and anti-androgens arTP53 mutations in prostate cancer are associated with the TP53 mutations indicating that the LFS men may experience more aggressive cancer biology which might have a significant influence on their disease management. The potential of prostate cancer to develop earlier in LFS favours the necessity of an early screening.

The c.323delG deletion was found in the case discussed by the researchers was not reported as a germline variant; however, it was reported in the COSMIC database (COSM437631) as a somatic mutation in the breast tumors. It was also found that the variant laid in the vicinity of other pathogenic variants (Vahteristo et al. 2001) that were known to be associated with the Li-Fraumeni syndrome (LFS). LFS was well described in the paper of a representative family with a history of osteosarcoma, brain cancer, Ca breast, Ca liver and leukemia (Akouchekian et al. 2016). The pattern of inheritance in this family suggested it as a monogenic disorder, autosomal dominant and the D281E mutation in TP53 at exon 8 was the main candidate to be the cause of LFS in this family.

According to the revised Chompret criteria for LFS clinical diagnosis (Gonzalez et al. 2009; Tinat 2009), in this case, as the proband's

family history and the medical history of cancer was indicative of the Li-Fraumeni Syndrome, further post-test genetic counselling was carried out and genetic screening for the detected variant in *TP53* gene was recommended in a non-directive way to the other family members. With informed written consent, fifteen family members (n=15) were screened by Sanger sequencing. In segregation the analysis of the kindred, the same mutation in the p53 gene on chromosome 17, position 7579364del C, c. 323del G, p. Gly 108 Val fs Ter 15 (Fig. 5), was detected in eight of the family members. Among them, two second degree relatives of the proband (Fig.4; III-3, III-13) were affected with osteosarcoma and brain tumor respectively. Along with the proband, both of them later succumbed to the disease. In addition, 6 unaffected males of varying age (II-3, III-1, III-6, III-8, III-9 and III-12; Fig. 4) were the carriers for the same heterozygous mutation. Remaining seven members were found to be negative for the mutation conferring p53 to be wild type and made functional protein products. A spare pedigree chart was further drawn to comprehend the prevailing status of the family with both the phenotypic and genotypic aspects (Fig. 4). In the LFS families, the variability in age of the onset for various cancer types in the family members was previously reported in

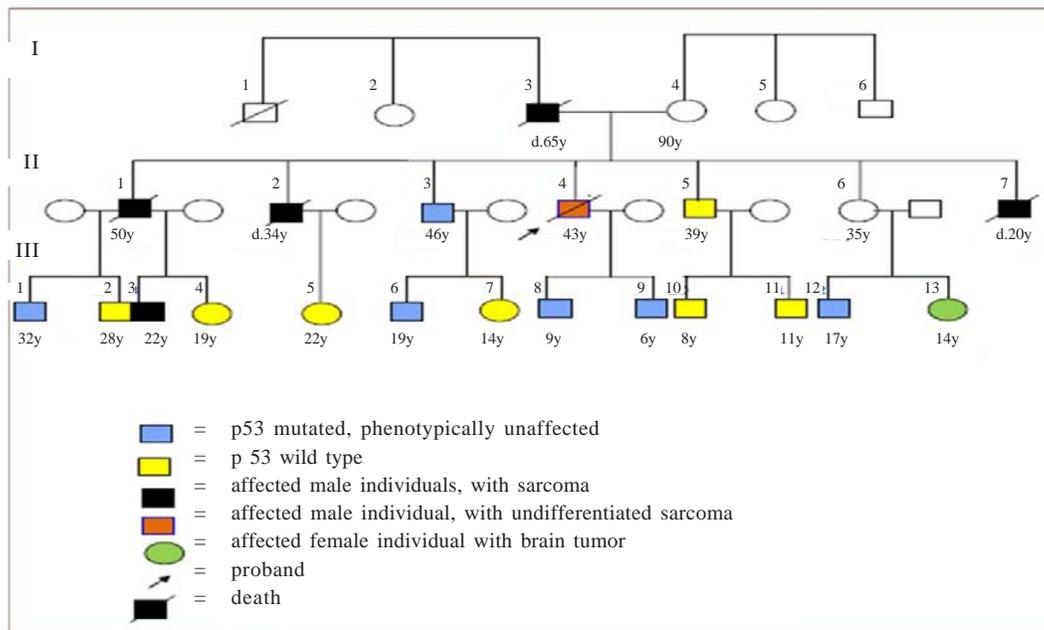


Fig. 4. Post -genetic test pedigree chart

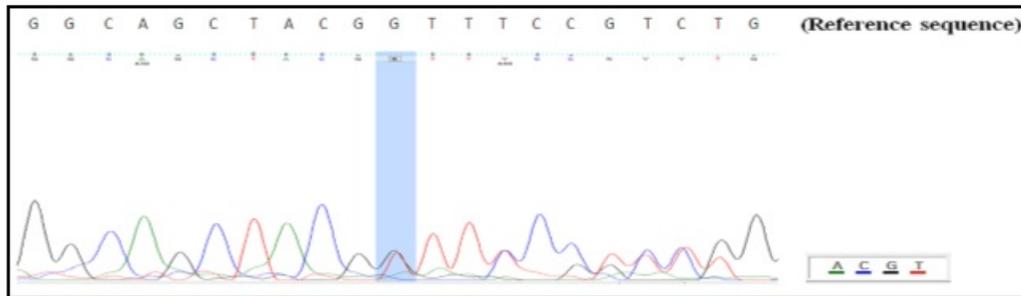


Fig. 5. Sanger sequencing data (electropherogram) from the individual showing heterozygous mutation in p53

other studies (Malkin 2011). The risk of certain malignancies as sarcoma, female breast cancer and hematopoietic cancers was more than 100 times greater in the asymptomatic mutation carriers than those seen in the general population. The asymptomatic family members should undergo periodic surveillance/examination as per the NCCN recommendations for an early detection of the cancer and management of the disease (Schneider et al. 1993).

Genotype-phenotype analysis of LFS families has shown that the germline missense mutation within the DNA binding domain of the *TP53* has a more penetrant cancer phenotype, higher cancer incidence and an earlier age of the onset, compared with the families carrying the protein truncations or other inactivating mutations (Schneider 1993; Birch 1998; Bougeard et al. 2008; Muller and Vousden 2014). Germline alterations of *TP53* leading to the loss-of-function are more associated with the late onset of the disease (Bougeard et al. 2008). Additional factors such as the effects of the modifier genes or alterations in other genes also influenced the LFS phenotype (Malkin 2011).

CONCLUSION

In the proband, screening for the 94 genes associated with the cancer led to the identification of a truncating mutation (p.Gly108 ValfsTer15) in the *TP53* gene. Four paternal first-degree relatives were affected with the sarcoma and two paternal second-degree relatives were affected with the LFS associated cancers, namely, osteosarcoma and brain tumor. The age of the onset of cancer in this family was also variable; in the proband (Fig. 3; II-4) the age for the

diagnosis for sarcoma was 43 years, however, one brother (Fig. 3; II-7) succumbed to sarcoma at the age of 20 years. The age of the onset of sarcoma in the other two brothers (Fig. 3; II-1 and II-2) and the father (I-3) was unknown; however, they all died at different ages: 34 years (Fig. 3; II-2), 50 years (Fig. 3; II-1) and 65 years (Fig. 3; I-3).

Screening of 15 family members of the proband for the identified *TP53* mutation revealed that 8 individuals also carried this mutation. Among these 8 individuals, two were affected with cancer; the proband's nephew (Fig. 3; III-3) passed away at age of 22 years due to osteosarcoma and niece (Fig. 3; III-13) succumbed to brain tumor at the age of 14 years. However, the 6 male family members: II-3, III-1, III-6, III-8, III-9 and III-12 were asymptomatic. Also, the sister (Fig. 3; II-6) of the proband, who was an asymptomatic at the time, was likely to be a carrier of the *TP53* mutation as both her children, III-13 and III-12, tested positive for the mutation. In the LFS families, the risk of developing any invasive cancer (excluding skin cancer) is fifty percent by the age of 30 years and is ninety percent by age 70 years. The most commonly occurring cancer in LFS is the early onset (<30 years) of breast cancer (25%), followed by sarcomas (45%), brain tumor (13%) and other cancers such as adrenocortical carcinoma, leukemia, lymphoma, melanoma and colorectal cancer. The identified truncating variant (p.Gly108 ValfsTer15) in this LFS family was likely to cause the loss-of-function, which may have contributed to the variable phenotypes and the age of onset of cancer in the family members and the penetrance was also noted to be reduced or incomplete as the genotype had not been expressed as the dis-

ease phenotype in every affected individual. Other factors might have also contributed to the observed intra-familial variability.

Based on this paper, the researchers suggest that genetic counseling and *TP53* mutation testing should be considered in patients with sarcoma, especially when there is a history of cancer in the close relatives. A careful follow-up is required in the presence of a Germline *TP53* mutation as there could be a substantial risk for a second malignancy. Cancer screening in individuals with LFS, using techniques such as rapid-sequence whole body MRI in combination with other screening tests (Villani et al. 2011) helps to detect cancer in the early stage. This in turn can improve the survival, particularly in the population with a high risk predisposition to cancer.

Outcome and Follow up

In the researcher's critique, the genetic background of the family members along with risk of predisposition disease prevalence unfolded the presence of similar cancer syndrome. Genetic counselling supported the family with timely screening for molecular evaluation and provided the family members with the flawless knowledge about the genetic test and the outcome. Based on the detectable germline mutation reported, it was concluded that the family was 'at-risk' and predictive and/or predisposition testing were recommended as per the NCCN guidelines for a proper monitoring and surveillance. Women members of this family were suggested to undergo a screening test for an early onset breast cancer. The researchers recommended the family to lead a healthy lifestyle with special attention in case of having symptoms like bone pain, headache and even abdominal discomfort. Individuals of this family were also counselled to avoid exposure of any radiation as p53 function which is prone to DNA damage caused by the radiation (UV, X-ray). The hospital (Health Care Global) also initiated many good training programs to escalate awareness on LFS all around.

ABBREVIATIONS

- LFS: Li-Fraumeni syndrome
- NGS- Next Generation Sequencing
- PET-CT-Positron emission tomography-computed tomography

- MRI-Magnetic resonance Imaging
- CHEK2 - Checkpoint Kinase -2
- COSMIC: Catalogue of somatic mutations in cancer
- aa- amino acid
- NCCN: National Comprehensive Cancer Network

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ANNEXURE

Table 1: Chompret criteria for TP53 Germline mutation screening

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|-------------|--|
| Criterion 1 | Proband with tumor belonging to the LFS tumor spectrum, before the age of 46 years (the spectrum comprises of soft-tissue sarcoma, osteosarcoma, pre-menopausal breast cancer, brain tumor, adrenal cortical carcinoma, leukaemia, or lung cancer) and at least one first-degree or second-degree family member clinically diagnosed with an LFS-related tumor (except breast cancer if the individual has breast cancer) before the age of 46 or with multiple tumours. |
| Criterion 2 | The proband with multiple tumors excluding multiple breast tumors, two of which belonging to the LFS tumor spectrum and the first of which diagnosed before age of 46years. |
| Criterion 3 | The proband who is diagnosed with adrenal cortical carcinoma or a tumor in the choroid plexus, regardless of any family history LFL or Li Fraumeni-like syndrome meets certain criteria that are not adjoined by the classic Chompret criteria (10-11). |
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