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Case report

Family with Li-Fraumeni syndrome and no evidence of a germline mutation of the *p53* gene or chromosomal aberrations

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Abstract. Li-Fraumeni syndrome is a rare autosomal, dominant trait of diverse types of cancers in children and young adults, with a predominance of soft tissue sarcomas, osteosarcomas, brain tumours, adrenocortical and breast carcinomas, as well as leukaemias. We present a family with an unusual cancer history fulfilling the criteria of Li-Fraumeni syndrome. Mutational analysis of the *p53* gene in constitutional DNA of several affected members of the family did not show any germline *p53* defect. Cytogenetic studies did not reveal any structural aberrations.

Key words: chromosomal aberrations, direct sequencing, Li-Fraumeni syndrome, PCR-SSCP, *p53* tumour suppressor gene.

Li-Fraumeni syndrome (LFS) was described independently by LI, FRAUMENI (1969) and LYNCH et al. (1973, 1978) as a rare, autosomal, dominantly inherited trait characterized by a high risk of development of diverse types of cancers in children and young adults in affected families. The prevelent types of cancer in LFS are bone and soft tissue sarcomas, brain tumors, breast and adrenocortical cancers as well as leukaemias (BIRCH 1994, VARLEY et al. 1997a, 1997b). In 1990, MALKIN et al. and SRIVASTAVA et al. discovered germline mutations of

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the *p53* tumour suppressor gene in LFS patients. Since then, many reports documenting the causative role of germline *p53* mutations in the development of the disease have been published (VARLEY et al. 1997a, b, SEDLACEK et al.1998). Inherited mutations of the *p53* gene were also found in cancer families that did not conform to all the LFS criteria and were therefore, classified as Li-Fraumeni-like (LFL) (VARLEY et al.1997a). Altogether, it has been estimated that up to 70% of LFS families carry the germline *p53* mutation (KLEIHUES et al. 1997, VARLEY 1997a). In search of another gene defect, germline mutations of the *hCHK2* gene were shown in some families with a wild *p53* gene (BELL et al. 1999). However, in the remaining families genetic changes predisposing to cancer have not been identified yet.

In this report, we present an unusual disease history of a family with cancer phenotype conforming to LFS criteria (LI, FRAUMENI 1969). The family pedigree is shown in Figure 1. According to LFS criteria, the proband is a patient under 45 with a sarcoma; and in the presented pedigree this is individual 1/II with osteosarcoma at 43. A first-degree relative with a cancer under 45 is individual 2/II with ovary carcinoma at 41 and ther first- or second-degree relatives with cancer under 45 include patient 1/I with larynx cancer at 40 and 5/II with cervix carcinoma at 44. This LFS-family pedigree comprises 16 members of three generations (Figure 1). In eight of them the following malignancies have been documented: osteosarcoma, acute myeloid leukaemia (AML), multiple myeloma and cancer of various organs,such as lung, ovary, cervix and larynx. A high incidence of neoplasia occurred in both sexes. A dominant pattern of inheritance was observed in the first and second generation (over 50% of affected members), whereas members of the third generation (present age range 4-18 years) are still healthy. The age of the members of the third generation allows to predict cancer development in the future.

Chromosome analysis was performed on lymphocytes of 10 members of the family. Lymphocytes obtained from heparinized blood were cultivated for 72 hours with or without the mitogen (LF, *Phaseolus vulgaris* extract) in Eagle's medium supplemented with 15% feoetal calf serum, 200 mM L-glutamine, 5 μg/ml insulin and antibiotics (penicillin 100 units/ml, streptomycin 100 μg/ml) in 5% CO_2 atmosphere at 37°C. Harvesting and metaphase spreads were done according to standard procedures (fixation with methanol and acetic acid); and slides were processed for G- and C-banding using the trypsin method (WANG, FEDOROFF 1972). At least 10 mitoses were fully karyotyped from each case, and 50-100 metaphases were counted under the microscope. Chromosome abnormalities were classified according to the International System Nomenclature (MITELMAN 1995). Chromosome findings are summarized in Table 1.

G-banding analysis of chromosome preparations revealed the presence of a normal karyotype in all the studied cases. Cytogenetic analysis revealed no

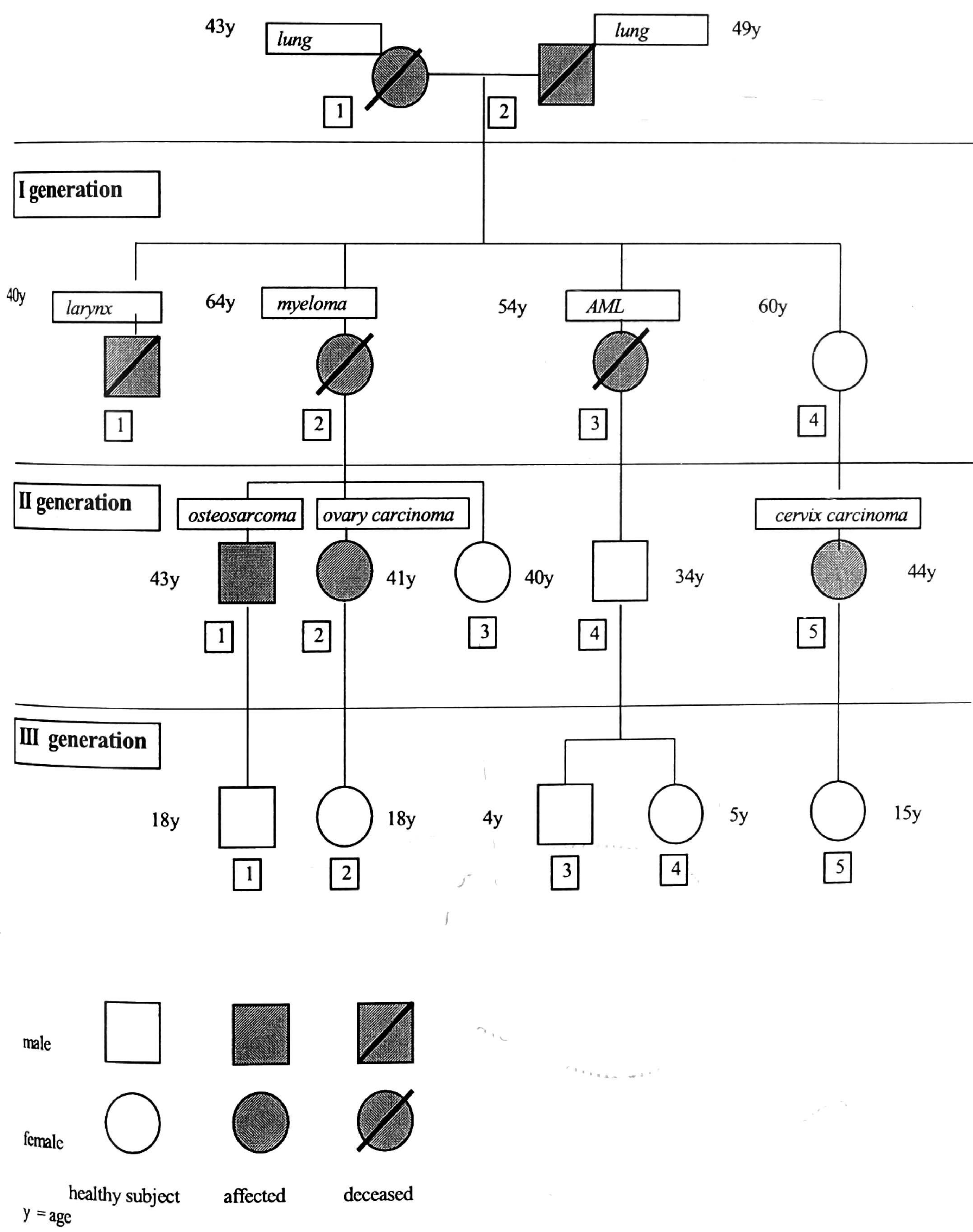

Figure 1. Pedigree of a family with a high cancer incidence

Table 1. Chromosome analysis in generations II and III

Subjects examined		Sex/Age	Constitutional karyotype
1/II	ill	M/43	46, XY.
2/II	ill	F/41	46, XX.
3/II	h	F/40	46, XX.
4/II	h	M/34	46, XY.
5/II	ill	F/44	46, XX.
1/III	h	M/18	46, XY, 1qh+.
2/III	h	F/18	46, XX, 1qh+.
3/III	h	M/4	46, XY, 22ps+.
4/III	h	F/5	46, XX, 22ps+.
5/III	h	F/15	46, XX, 1qh+.

h = healthy

structural chromosomal rearrangement. The only detectable anomaly was enlargement of the heterochromatic mass of chromosome 1 and satellites of chromosome 22. An asymmetry between homologous chromosomes 1 was found in three family members (1/III, 2/III and 5/III), with a difference in the C-positive regions. One copy of chromosome 1 was characterised by increased heterochromatin (qh+). An increase in the length of the satellite on the short arm of chromosome 22 (22ps+) was found in the case of two children (3/III and 4/III). Their father (4/II) had no 22ps+ in his karyotype. The heterogenous chromosome 1(qh+) is a normal asymmetry pattern – a normal variable chromosome feature. The possible role of a heterochromatin variant of chromosome 1 as a predisposition marker in cancer families has been disscused for two decades (ATKIN, BRITO-BABAPULLE 1985, DONEDA et al. 1987, KRISTOFFERSSON 1989, KOPF et al. 1989). Recently, more data have supported the lack of significant differences between the frequency of constitutive heterochromatin heteromorfisms in cancer patients and the control group (KRISTOFFERSSON 1989, KOPF et al. 1989). The meaning of heterochromatin variants of chromosome 22 found in two children (ahed 4 and 5 years) in our study cannot be predicted at this moment. The biological significance of the above heteromorphisms is unknown.

The strategy used to screen for mutations of the *p53* gene was based on PCR (polymerase chain reaction) amplification of all exons (1–11) from genomic DNA and SSCP (single-strand conformation polymorphism) analysis of the products. DNA was isolated according to standard protocols from frozen blood samples. A separate pair of primers was used to amplify each exon, except for exon 5, in which two partially overlapping fragments were studied (TOGUSHIDA et al. 1992). Moreover, exons covering mutational hot spots, ie. exons 5-8, were also directly sequenced. Analysis of constitutional DNA of the patients did not reveal any

germline mutation of the *p53* gene. This result is in agreement with our recently published data on the lack of germline *p53* mutations in high-risk groups in Poland (FISZER-MALISZEWSKA et al. 2000a). We extended this study to a group of more than 50 cancer families with a spectrum of cancers typical for LFS/LFL, including another classical LFS family and several LFL families, and found that the of germline mutations of the *p53* gene in Poland is very low. The only germline *p53* mutation was discovered in a female patient, a member of a family which may be classified both as LFL and hereditary breast-ovary cancer (HBOC) syndrome (FISZER-MALISZEWSKA et al. 2000b). The first candidate gene to screen for defects in LFS/LFL families in Poland seems to be the *hCHK2* gene (BELL et al. 1999). Due to a significant overlap between LFS/LFL and other cancer syndromes (eg., HBOC), other genes associated with them are potential future targets.

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