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Genotype phenotype correlation in Li-Fraumeni syndrome kindreds and its implications for management

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Introduction

Li-Fraumeni syndrome (LFS) is a highly penetrant cancer predisposition syndrome. It is defined as an index case (proband) affected by sarcoma before 45 years of age, plus a first-degree relative affected by any cancer before 45 years of age plus an additional first- or second-degree relative affected by any cancer before 45 years of age or by sarcoma at any age [1]. Individuals belonging to LFS families are predisposed to a higher risk of developing young onset cancers, especially childhood cancers and multiple primary cancers. The risks of cancer that are particularly increased in LFS are sarcomas (bone and soft tissue), breast cancer, brain tumours, adrenocortical carcinomas, and childhood leukaemia [2, 3].

Cause

LFS is an autosomal dominant condition and germline mutations in the *TP53* gene account for the majority of LFS families. However, at present, it is unclear whether there are any germline mutations in other genes that may predispose to this syndrome. Germline mutations in *CHEK2* in a few LFS families without *TP53* mutations were suggested as a cause of the syndrome [4], however, later studies suggested that *CHEK2* only predisposed to breast cancers that arose in the context of a family history that matched the LFS phenotype, and not LFS *per se* [5].

The *TP53* gene is recognised as the most common gene mutated in sporadic cancers and is well known as a tumour suppressor gene whereby both copies of the gene have to be inactivated to cause tumour. However, the *TP53* gene is also unique in that occasionally it does not obey the classical Knudson's two-hit hypothesis of cancer development, in that a germline mutation in only one copy can give rise to cancer [6]. This property is partly ascribed a dominant negative effect of some of the mutations in *TP53*, which give rise to the inefficient tetramerization of the protein, which requires correct functioning of both copies of the gene [7–13].

The *TP53* gene also differs from other tumour suppressor genes such as *RB1, APC, BRCA1/2*, in that the mutations commonly seen in this gene are missense mutations. Only 10% of all families with known germline mutations in the *TP53* gene have deletions in this gene [3].

Genotype–Phenotype correlation and its implications on treatment and prognosis

The accepted model for the tumour suppressor gene mechanism is mutation in one allele and loss of the remaining wild type allele, loss of heterozygosity (LOH). However, less than 50% of tumours from members in the LFS families show LOH [14]. LOH is invariably seen in tumours from LFS cases when the germline mutation leads to loss of protein, in other words, the mutation is a

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protein inactivating mutation (nonsense/slicing mutation) [15]. Furthermore, the lowest proportion of LOH is seen in tumours arising in LFS patients with mutations at hotspot codons. Codons, which are hotspots for mutations, are located in the DNA binding region of the *TP53* gene. Mutations at these hotspots are mostly missense mutations and exhibit gain of function properties [16]. These genotypic features could have implications in the management and prognosis prediction of LFS families, in that, tumours with mutant missense *TP53* proteins are thought to be more aggressive or have poor prognosis. Missense mutations within the DNA binding region of the *TP53* gene predispose more commonly to breast cancer and brain tumours. Also, missense mutations within the DNA binding region are associated with breast cancers that occur at a younger age. Mutations outside the DNA binding region are more commonly associated with adrenocortical carcinoma whereas mutations leading to a *TP53* null phenotype are associated with earlier onset tumours, in particular, brain tumours [3].

LFS and breast cancer

Although germline *TP53* mutations account for only a minority of early onset breast cancer or familial breast cancer not attributable to mutations in *BRCA1* or *BRCA2* [17], an analysis of all the cancers in classic LFS families has shown that early onset breast cancer is the most frequent cancer within this syndrome. This is followed by soft tissue sarcomas, bone sarcomas, and brain tumours with the median age at diagnosis as 33, 14, 15 and 16 years respectively. This suggests that women who are *TP53* mutation carriers are a small but significant group with a high risk of developing breast cancer apart from *BRCA* mutation carriers. Breast cancers that occur in LFS families with known *TP53* mutation are of particularly early onset. Similarly, the age of onset of breast tumours in women with germline missense mutations within the DNA binding domain of the *TP53* gene is significantly lower than breast tumours with missense mutations outside the DNA binding domain. These observations could be helpful in the overall management of women in LFS families, particularly for genetic testing and screening [17].

Function of TP53

The *TP53* gene product is a protein that was originally found to complex to the large T-antigen of SV40 [18]. The cellular tumour antigen TP53 in its normal form, functions as the guardian of the genome. The normal protein becomes activated after it senses DNA damage and decides whether the cell will arrest in response to the DNA damage to enable repair to occur or if it will undergo apoptosis. It does so by either (i) transcriptionally activating downstream genes (e.g. *P21, BAX, MDM2, GADD45*, etc.) involved in DNA damage repair or (ii) by signalling a sensor molecule that confirms DNA damage and leads the cell to programmed cell death (apoptosis). The cell cycle arrest required for DNA damage repair is mediated by activating the *RB* pathway. The TP53 protein also seems to play a direct role *per se* in the process of DNA repair.

Susceptibility to and mechanisms of damage in patients with LFS

In ideal circumstances, the normal TP53 protein guards the genome from further damage after it recognises DNA damage [18]. Double strand break damage is commonly caused by exposure to radiation and if TP53 is malfunctioning, this may not be repaired. Genomic instability is considered to be a hallmark of carcinogenesis and is manifested in most cancers by multiple unbalanced chromosomal aberrations [19]. It is initiated if DNA damage is not repaired (for example when *TP53* is non-functioning or mutated) and the instability could also be potentially amplified [20] in cells with a mutant TP53 protein function and further destabilization could be accelerated [21]. In addition, the mutant TP53 protein functions in association with other oncogenic proteins e.g. RAS by disabling the binding capacity of the normal TP53 protein. This is in effect the dominant negative function of the mutant TP53 protein, which has led researchers to the idea that it is a dominant oncogene as well as a tumour suppressor gene. Genomic instability is memorised and can be transmitted through generations of the cell. The accumulation of cells with damaged DNA can potentially give rise to an immortal clone of cells and eventually cancer. *In vitro* work has demonstrated that breast epithelial cells from LFS patients with germline mutations in *TP53* attain a state of spontaneous immortality in culture and those that do not immortalise spontaneously can be easily made to do so by using agents that have no effect on cells with normal *TP53* [22, 23]. Results from functional studies suggest that cells with mutant *TP53* have lost the control over various checkpoints in the cell cycle [24]. Not only are the cell cycle checkpoints, responsible for genome surveillance, perturbed, but also these cells bear a potential mutator phenotype [25]. However it needs mentioning here that genomic instability could be induced in all cells irrespective of the *TP53* function, suggesting the complex nature of this process.

The extreme sensitivity of cells from LFS cases to DNA damage from any source has implications on the management of patients in LFS families. Firstly, radiation resistance to apoptosis has been observed in EBV immortalised lymphoblastoid cell lines derived from LFS patients [26]. In addition, preclinical models have demonstrated resistance of tumours that lack functional TP53 to DNA damaging agents (chemotherapy and radiation) used for treatment of these tumours. This has been associated with the very important role of TP53 in

inducing apoptosis after exposure to such agents [27, 28]. Secondly, *in vivo* evidence suggests a possible causative role of DNA damaging agents (chemotherapy and radiotherapy) that have been used for treating the first cancer in the development of subsequent second cancers in mutant *TP53* carriers [29]. Evidence exists for the development of multiple primary cancers in LFS cases and their close relatives affected by cancer, subsequent to receiving prior treatment with radiation or chemotherapy as well as independent of any prior treatment received [30–32]. A retrospective analysis of 200 cancer patients from 24 LFS kindreds, the largest reported so far, by Hisada et al. [30], demonstrated that 20% (42/200) of these patients developed more than one cancer and the second cancer developed in these patients up to 30 years after the diagnosis of the first cancer. Specimen for mutation analysis was not available from a third of these families and interestingly germline *TP53* mutations were identified in only 50% of the kindreds from whom specimens were available. The incidence of second cancer was irrespective of either the presence of a confirmed *TP53* mutation in the kindred, or the first cancer being a component of LFS. Additionally, up to 30% of the subsequent cancers that developed in these individuals were not component cancers of the LFS. The risk of developing second cancer was found to be associated with the age at diagnosis of first cancer and those patients who were 20 years or younger at the time of developing their first cancer were at highest risk. Though the risk of second cancer reduced with increasing age at diagnosis of the first cancer it remained significantly higher in patients with ages up to 44 years at the time of diagnosis of the first cancer. Half of the patients who developed multiple cancers in this report did not receive prior radiotherapy or chemotherapy. Most of the second cancers that occurred in patients who received prior radiotherapy occurred within the radiation field and within periods consistent with radiation carcinogenesis. These facts highlight several issues that need addressing in LFS such as (i) possibility of other genes within or outside of the *TP53* pathway that may be possibly involved in predisposition to LFS and additional predisposition to second cancers within the kindred (ii) possibility of a larger spectrum of component cancers in LFS (iii) possibility of carcinogens other than radiotherapy and chemotherapy in predisposition to second cancers in these kindreds. More importantly these facts have implications for the management of LFS kindreds, with or without cancer, from screening through to treatment and prevention.

Implications for management:

The present

Inherited mutation in the *TP53* gene in LFS families has serious implications for management of individuals in these families.

Screening implications

Mammographic screening for early detection of breast cancer is conventionally initiated at an early age in predisposed women. This screening method has now been shown to confer a survival advantage [33–35]. The concern with using mammographic screening in LFS is the small theoretical risk of inducing malignancy in *TP53* mutation carriers because of the exposure to radiation through mammography [36]. Magnetic Resonance Imaging (MRI) has a higher sensitivity and specificity than mammography [37] in individuals with *BRCA1/2* mutations and does not involve exposure to radiation but is limited by issues such as cost and accessibility. However, its use could be justified and supplemented to specialist breast self examination in very high-risk groups such as LFS where there is a need to avoid mammography and thus radiation exposure to prevent potential DNA damage [38].

Treatment implications

Increased sensitivity to DNA damaging agents (ionising radiation) commonly used to treat the type of cancers seen in LFS can pose a major limitation for *TP53* mutation carriers. In addition to the tumours being radioresistant, these individuals are at a higher risk for developing treatment induced second cancers. The cumulative probability of developing second cancer was found to be as high as 57% as long as after 30 years of diagnosis of the first cancer in one study [30]. For this reason, radiotherapy as a treatment modality should be avoided if there are other feasible treatment modalities. The presence of a very early onset (< 30 years old at diagnosis) breast cancer within the context of LFS would be an indication for urgent germline *TP53* diagnostic testing since if a germline mutation is found, mastectomy rather than conservative surgery and radiotherapy would be preferred. The data on the relationship of the *TP53* status and response to systemic therapy (hormonal therapy, anthracycline and non-anthracycline based chemotherapy) in breast cancer are variable and more data need to be available in germline *TP53* mutation carriers and response to such agents before strong recommendations can be made [39].

Chemoprevention for recurrence of breast cancer and occurrence of contra lateral breast cancer using Tamoxifen is well recognised. It also seems to reduce the incidence of contra lateral breast cancer after an initial breast cancer in *BRCA* mutation carriers [40]. Data from animal studies suggest that it may potentially be similarly effective in LFS [41]. However one would need to balance the increased risk of potentially serious side effects of long-term treatment with Tamoxifen [42], (stroke, deep vein thrombosis and pulmonary embolism) with the expected advantages.

Implications for prevention

Tamoxifen has been shown to be effective in prevention of non-invasive and invasive breast cancer in high-risk

asymptomatic women identified on the basis of family history. It reduced the incidence of breast cancer in women with histories of lobular carcinoma *in-situ* or atypical hyperplasia [42]. Studies involving the use of other chemopreventive agents with a lower side effect profile than Tamoxifen are underway. It is unknown if Tamoxifen or any of these other agents can safely be used in *TP53* mutation carriers at present. Results of studies on chemopreventive agents in *TP53* knockout mice may prove useful in this regard [41].

Prophylactic removal of breast tissue (prophylactic mastectomy) is now commonly being offered and remains a viable option for women with a very high risk of breast cancer. It is now an accepted option in *BRCA1/2* and *TP53* mutation carriers [43]. The residual risk of breast cancer after such preventive but radical surgery is substantially reduced in *BRCA* mutation carriers (by 90%) [44]. However this approach can be problematic in women who have germline *TP53* mutations due to the increased chances of developing additional cancers at other anatomical sites. Although there is significant benefit in women choosing this option in terms of reduction of anxiety and cancer related worry, [45], there may be a less favourable body image and changes in sexual relationships [46] after undergoing such radical surgery, but further studies are needed.

The future

The ideal way to cure cancers that have occurred due to germline *TP53* mutations would be to restore normal TP53 function in cancer cells. Recent results of work in animal models [47] to devise such strategies, are promising, however they are yet to be proven in man [48]. Until such time, advice on having a healthy lifestyle, avoiding environmental carcinogens, and X-rays and having regular check ups by a physician to recognise early signs of any cancer (within or outside the LFS spectrum of cancers) is the current best management in these kindreds.

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