Short Report

Childhood adrenocortical carcinoma as a sentinel cancer for detecting families with germline TP53 mutations


Li-Fraumeni syndrome (LFS) is a highly penetrant, autosomal dominant disorder where affected individuals carry a 50% risk of developing cancer before 30 years of age. It is most commonly associated with mutations in the tumour suppressor gene, TP53. Adrenocortical carcinoma (ACC) is a very rare paediatric cancer, and up to 80% of affected children are found to carry germline TP53 mutations. Hence, we propose using childhood ACC incidence as selection criteria for referral for TP53 mutation testing, independent of familial cancer history. Under the auspices of the Malaysian Society of Paediatric Haematology–Oncology, four eligible children diagnosed with ACC over a 30-month study period were referred for mutation testing. Three had a germline TP53 mutation. Subsequent TP53 testing in relatives showed two inherited mutations and one de novo mutation. These findings strongly support paediatric ACC as a useful sentinel cancer for initiating a germline TP53/LFS detection programme, particularly in countries where the lack of structured oncogenetic practice precludes the identification of families with LFS features.

Conflict of interest

The authors declare no conflict of interest in this study or in the production of this manuscript.

Li-Fraumeni syndrome (LFS; OMIM 151623) is a rare inherited autosomal dominant disorder with high penetrance and familial clustering of tissue-specific cancers such as bone and soft-tissue sarcomas, adrenocortical carcinoma (ACC), pre-menopausal breast cancers, leukaemia and brain tumours (1, 2). These cancers usually occur in youth, and multiple primary cancers may develop throughout the life of a patient. A cancer patient from an LFS family has a 57% risk of developing a second cancer in the subsequent 30 years following initial diagnosis (3).

LFS was first described by Li and Fraumeni in 1969. Because of the wide range of associated cancers, several clinical definitions have been proposed, evolving into a set of criteria used for referring subjects for mutation testing following the identification of TP53 mutations as the genetic basis of LFS (4). Candidates for TP53 testing should include: (i) proband with a tumour of the narrow LFS spectrum (soft-tissue sarcoma, osteosarcoma, brain tumour, ACC, breast cancer) before 36 years or with any childhood solid tumour, within a family containing at least one first-degree or second-degree relative with a tumour of the LFS spectrum (except breast cancer if the proband has a breast cancer) under 46 years; (ii) patients with multiple primary tumours (except breast cancers), two of which...
belong to the LFS spectrum, the first developing before age 46 years; and (iii) patients with ACC or choroid plexus carcinoma or papilloma before the age of 15 years, or rhabdomyosarcoma before the age of 5 years. About 30% of the subjects referred for TP53 mutation testing using these criteria carry a germline TP53 mutation (5–7).

TP53 is a tumour suppressor gene encoding the p53 protein that controls multiple aspects of cell growth. Mutation-carrying cells are prone to abnormal proliferation due to partial or complete loss of p53 function, while mutant p53 also exerts a dominant negative effect over wild-type p53 (8). The molecular basis for the particular LFS cancer spectrum is still unknown, but TP53 germline mutations are thought to facilitate carcinogenesis by at least two mechanisms: deregulating genetic stability in cells with DNA damage and deficient capacity of excess pools of stem/progenitor cells to enter differentiation and senescence (7).

LFS’s complex multilevel clinical definition hinders its diagnosis, especially outside centres with structured oncogenetic services. As a result, LFS is largely under-recognised in many geographic regions. Although more than 500 families with TP53 mutations have been reported in the literature, only 15 are in continental Asia (China: 4, India: 4, Korea: 4, Malaysia: 2, Singapore: 1) and none in Africa (9).

ACC (OMIM 202300) is one of the most characteristic LFS cancers, and focusing on its trait as a sentinel cancer will help to initiate an LFS detection and screening program. Although representing only about 8% of all cancers detected among TP53 mutation carriers, ACC is present in 27% of the families with documented mutations. More significantly, 80% of the ACC subjects younger than 15 years carry germline TP53 mutations (10). As ACC accounts for only 0.2% of all paediatric cancers (11) and rarely occurs outside TP53 mutation carriers, it can be considered a strong indicator for presence of germline TP53 mutation.

The prevalence of germline TP53 mutations and the incidence of LFS in Southeast Asia are still unknown. To increase the disease awareness and initiate an LFS detection program, we have recently launched a project involving paediatric oncologists in Malaysia. We report on our experience of identifying families with germline TP53 mutations using childhood ACC as the sentinel cancer and suggest a surveillance program to efficiently detect early cancer occurrence. It has recently been shown that enrolling TP53 mutation carriers into relatively simple surveillance programmes has been remarkably effective in detecting cancer early and reducing mortality (12).

Patients and methods

This project was carried out under the auspices of the Malaysian Society of Paediatric Haematology–Oncology (MASPHO) involving paediatric oncologists nationwide. Patients with childhood ACC diagnosed using standard diagnostic criteria, irrespective of familial cancer history between January 2009 and May 2011 were referred for this study. Before screening, appropriate counselling was provided and signed informed consent obtained.

Three to five millilitres of peripheral blood was taken from each subject by venepuncture, and genomic DNA was isolated from total leukocytes using a modified phenol–chloroform method. We then performed polymerase chain reaction using primers and protocols available from the International Agency of Research on Cancer (IARC) website to amplify TP53 exons 2 till 11 and their flanking splice site junctions (13) followed by bi-directional sequencing. Electropherograms were analysed for mutations using BioEdit sequence alignment editor (Ibis Biosciences, Carlsbad, CA). Our findings were confirmed by an independent analysis carried out by the IARC. The Medical Ethics Committee of University Malaya Medical Centre approved this study.

Results

In the 30-month study period, four children with ACC aged between 3 and 10 years as well as their family members (n = 23) were recruited.

Familial cancer profiles

Figure 1 shows the pedigree of Family F1 with five cancer cases to date, all on the maternal side. Proband P2 developed ACC at 6 months of age. Figure 2 shows the pedigree for Family F2. The proband was diagnosed with ACC at 3 years of age. There were six cancer cases on the paternal side. In Family F3, the proband was diagnosed with ACC at 4 years of age with no documented familial cancer history. The proband in Family F4 was diagnosed with ACC at the age of 10 years. Her maternal grandmother had breast cancer in her 30s.

Mutation detection

A germline mutation was detected in Proband P1 in Family F1 (previously reported) (14) involving a six base-pair insertion (GGCGTG) in the tetramerization domain between residues 334 and 336 in exon 10 of the TP53 gene. This removes codon 335 (Glu) and introduces two codons (Gly and Val). Her sister, Proband P2 carried the same mutation, as did their mother who had breast cancer at the age of 26 years and two youngest siblings (unaffected). A maternal aunt who developed osteosarcoma at 26 years also had the same mutation. The father and two other children carried wild-type TP53.

In Family F2, the proband had a G > A missense mutation in exon 5 codon 175 of one allele, replacing arginine with histidine and disrupting the DNA binding domain folding in a well-known LFS mutation hotspot. The same mutation was detected in the father and in one paternal uncle (both unaffected).

In Family F3, no mutation was found in the proband. The sample was re-sequenced as well as
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P1 Proband 1 Diagnosed with rhabdomyosarcoma at the age of 8 months
P2 Proband 2 Diagnosed with adrenocortical carcinoma aged 6 months
A Diagnosed with breast cancer at age 38 years
B Diagnosed with breast cancer at age 26 years
C Diagnosed with osteosarcoma at age 26 years

screened using multiplex ligation-dependent probe analysis (MLPA) at the Molecular Genetics Laboratory, The Hospital for Sick Children in Toronto to confirm the absence of a large deletion in \textit{TP53} and no mutation was discovered. Other family members were not tested.

In Family F4, the proband was found to carry a C -> A missense mutation at codon 124 in exon 4, converting cysteine to a stop codon in one allele of \textit{TP53}; a previously unreported mutation. Screened parents showed wild-type \textit{TP53}, suggesting a \textit{de novo} mutation in the child.

Discussion
This study shows the presence of \textit{TP53} mutations in three of four Malaysian children with ACC. Three families showed familial cancer patterns, and mutations were detected in relatives of two probands. In both, the mutation appears to segregate with observed cancer risk. Overall, this study confirms that childhood ACC represents a powerful sentinel diagnosis for familial cancer associated with inheritance of \textit{TP53}.

Of the three mutations reported in this study, only one (p.R175H) has been reported as a classical, hotspot
Choong et al. germline TP53 mutation. The other two have not been previously documented: a six base-pair insertion between codons 334 and 336 of exon 10 (Family F1) and a C > A nonsense mutation at codon 124 of exon 4 (Family F4). In Family F1, the mutation induces both a mutation at residue 335 (Glu to Gly) and the insertion of a new residue (Val) between positions 335 and 336. This mutation falls within the alpha-helix of the tetramerization domain, predicted to alter p53 function by preventing oligomerization into tetramers that have high affinity for target DNA. However, the extent of functional disruption of this mutant protein has not been evaluated experimentally. The mutation at codon 134 in Family F4 introduces a stop codon and precludes the assembly of full-length p53. Nevertheless, it leaves intact the reading frame of a p53 isoform, Delta133p53, which has recently been shown to regulate p53 function (15). Whether the expression of this isoform is retained in the cells of the proband is unknown.

Approximately 50% LFS patients have no detectable p53 mutations in the coding region. In fact, in suspected LFS families with no detectable mutation, the TP53 gene may be disrupted by a deletion or genomic rearrangement encompassing a large portion of the coding sequence. MLPA was performed on proband F3 to investigate this possibility. Shlien et al. recently showed intragenic microdeletions of TP53 in four LFS patients (16). We concede that a mutation residing in the promoter or intronic regions (include splice site mutations) of TP53 as has been reported before (17) may exist in this patient but was undetectable by our techniques.

The results from this study are compatible with those reported in similar trials. In the United States, Wagner et al. reported that three of six patients with paediatric ACC carried TP53 mutations (18), while in the British series of Varley et al. 9 of 11 cases (82%) had TP53 germline mutation (19). Childhood ACC patients from Brazil recorded even higher figures (36/37, 98%) (20), as a particular TP53 mutation (p.R337H) is prevalent as a result of widespread occurrence of the founder effect (21, 22).

Figure 3 elaborates a general strategy for detecting and managing TP53 mutation carriers in paediatric oncology practice. This strategy relies on the clinical diagnosis of sentinel cancer cases and on their referral for TP53 mutation testing. Probands and their families who are tested positive for the mutation or show a significant familial history of cancer should then be recruited into a surveillance programme based on National Comprehensive Cancer Network (NCCN) guidelines as described in Villani et al. (12). The development of a nationwide consortium of paediatric oncologists facilitates the sharing of common resources, staff and infrastructure for assessing pedigrees, genetic counselling, mutation testing and for follow-up of carriers. As individuals with LFS have a high lifetime risk of developing cancer, we believe that a screening programme for germline TP53 mutations should be initiated in Malaysia. The findings of Villani et al. (12) where a biochemical and imaging surveillance programme significantly increased survival in individuals further strengthen this stand. However, because of local constraints, we had to build upon existing clinical practice i.e. the network of paediatric oncologists nationwide and developed shared facilities for standard procedures of counselling and disease surveillance as well as a central laboratory for genetic testing.

Conclusion

This pilot study in Malaysia shows that diagnosis of ACC in children provides an effective approach to detect subjects and families with germline TP53 mutation with a high risk of multiple cancers. The sensitivity of this approach to identify carriers may be further increased by including other childhood cancers that are highly characteristic of the LFS spectrum such as rhabdomyosarcoma before the age of 6 years and choroid plexus tumours. This strategy of germline TP53 mutation detection using the platform of a nationwide paediatric oncology consortium may provide guidance for other countries where there is currently no structured oncogenetic service for identification of families with LFS.
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Acknowledgement

The authors thank David Malkin and Ana Novokmet for the MLPA studies and Pierre Hainaut for helpful comments. This work was supported by the University of Malaya HIR-MOHE research grant initiative.

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