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Li-Fraumeni Syndrome and p53 in 2015: Celebrating their Silver Anniversary

In a typical morning in the Cancer Genetics Clinic at The Hospital for Sick Children in Toronto, the following array of patients and families might be seen: a family of three children, all harbouring a mutation of the succinyl dehydrogenase C gene inherited from their father who had had extensive surgery several years ago for a secreting paraganglioma; three families with Li-Fraumeni syndrome, each with at least one child harbouring a *TP53* gene mutation conferring a lifetime risk of cancer approaching 100% and currently undergoing surveillance for early tumour detection; two children with Li-Fraumeni syndrome undergoing treatment for cancer – one having had three cancer diagnoses before 19 months of age and the other just completing therapy for metastatic adrenocortical carcinoma at age 3; two children with von Hippel-Lindau disease being monitored for persistent pancreatic neuroendocrine tumors and cerebellar hemangioblastomas, respectively; one child with Beckwith-Wiedeman syndrome and Wilms tumor and another child completing therapy for a pleuropulmonary blastoma (PPB).

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This clinic, established in 2000, sees approximately 200 new consults annually with a primary focus on identifying, counseling and offering management plans for families with a high cancer risk, based on family history or the personal cancer history of the index child. Several key questions have been critical to the evolution of this and similar clinics around the world. What makes a family history of cancer significant? What should raise red flags as to the potential risk of cancer for a child? What clinical interventions can be offered to these families? How can emerging genomic/genetic science be used to improve counselling efforts? How can the research/clinical interface be harnessed to advance the care of these families more rapidly and effectively? And who should be referred to a clinic that focuses on cancer predisposition in childhood? As the technology, application and interpretation of next generation sequencing (NGS) evolves, it is likely that clinical definitions will be superseded by incorporation of genetic/genomic information into a patient's profile. Until then, astute clinical attention to cancer history is the best tool to answer these questions: an unusually high number of affected closely related family members, types of malignancies, presence of multifocal /bilateral cancers, multiple primary cancers in one individual, presence of non-malignant features characteristic of signature cancer predisposition syndromes and earlier than typical age of onset.

Until recently, it was widely reported that genetic or hereditary factors accounted for fewer than 5% of incidences of childhood cancer. While many cancer predispositions have been well-defined, both phenotypically and genetically,

cumulatively, they account for only a very small fraction of cancers. It is now recognized that this 'textbook' frequency represents a significant underestimation of the true incidence of childhood cancers associated with a heritable genetic alteration. Two avenues of research have been instrumental in leading to this change: first, studies have reported that, with more astute recognition of family cancer histories, up to 40% of children have personal health or family cancer histories suggestive of a genetic etiologic basis; and second, the emergence of data from large scale (primarily cancer gene panel) next generation sequencing studies report variants in genes known to be associated with cancer risk in at least 10% of children. To gain an appreciation of the potential impact of the study of 'rare' hereditary cancer syndromes on the understanding of fundamental cancer biology, as well as translational impact to the bedside, I will review the progress that has been made in research on the Li-Fraumeni cancer predisposition syndrome (LFS).

Li-Fraumeni Syndrome

Studies of hereditary cancers often lead to identification of genes involved in the multi-step process of carcinogenesis. LFS (OMIM#151623) is a paradigm cancer predisposition syndrome first reported in 1969 [1]. In a retrospective examination of medical charts and death certificates of 648 pediatric rhabdomyosarcoma (RMS) patients in the United States, the authors identified five patients with an extensive family history of cancer that included carcinomas of the breast, lung and pancreas, leukemia and skin cancers. Prospective analysis of 24 families led to publication of a 'classical' definition of LFS as follows: a proband with a sarcoma before the age of 45 who has a first degree relative with any cancer under the age of 45 years and another first or second degree relative with any cancer under the age of 45 years, or a sarcoma at any age [2]. Subsequent analyses by several groups of other cohorts of families suggested that many had an extensive cancer history that resembled LFS but did not conform to the classical definition: these have been termed 'Li-Fraumeni-like syndrome' (LFS-L), for which there are two definitions. The first is defined by a proband with any childhood cancer, or a sarcoma, brain tumor, or adrenocortical carcinoma (ACC) under the age of 45 years who has a 1st or 2nd degree relative in the same lineage with a typical LFS tumor at any age, and an additional first or second degree relative in the same lineage with any cancer diagnosed before the age of 60 years [3]. The second is defined by the presence of 1st or 2nd degree relatives with two different tumors (including bone or soft-tissue sarcoma, breast cancer, brain tumor, leukemia, adrenocortical

tumor, melanoma and prostate cancer) at any age [4]. Some years after discovery of the genetic basis of LFS, and in an attempt to better define the indications for gene testing, the “classic” definition of LFS further evolved by Chompret *et al.* [5] to include the following: 1) a proband with a characteristic LFS component tumor (soft tissue sarcoma, osteosarcoma, brain tumor, breast cancer and adrenocortical carcinoma) before 46 years of age and at least one 1st or 2nd degree relative with an LFS tumor (except breast cancer, if the proband has breast cancer) before age of 56 years, or with multiple tumors; OR 2) a proband with multiple primary tumors (except breast tumors), two of which belong to the LFS component spectrum tumors, the first of which occurred before 46 years; OR 3) a patient with adrenocortical carcinoma or choroid plexus tumor, irrespective of family history [6]. Recent studies have further expanded the criteria to include children with the anaplastic histologic variant of RMS [7], hypodiploid acute lymphoblastic leukemia, or the SHH subtype of medulloblastoma [8], or women with very early onset (<30 years) breast cancer [9]. In gene mutation carriers, the probability of developing cancer approaches 40% by age 20 years, and >90% by age 70 years; furthermore, patients exhibit an 83-fold increased risk to develop multiple tumors. The tremendous phenotypic variation in LFS, specifically in age of onset, multiplicity of tumors, outcome and degree of disease penetrance between families suggests a complex genetic basis, and raises important challenges in designing and implementing effective surveillance approaches for early detection, and opportunities for cancer chemoprevention or therapy.

The Molecular Basis of Li-Fraumeni Syndrome

Based on previous observations of somatic inactivation of the *TP53* tumor suppressor gene in sporadic forms of most cancers associated with the LFS spectrum, as well as reports of a *Trp53* transgenic mouse that developed a multi-cancer phenotype [10], Malkin *et al.* used a candidate gene approach to identify germline heterozygous *TP53* point mutations in five classic LFS families [11]. Missense mutations were identified in the DNA binding domain of the gene in each family. Subsequent studies have confirmed these initial findings, demonstrated approximately 75-80% of LFS families to harbor *TP53* mutations and identified germline mutations across virtually the entire gene.

The distribution of germline *TP53* mutations is similar to that observed in sporadic cancers [12]. Approximately 75% are missense, with nonsense (~9%) and splice-site (~8%) mutations accounting for the bulk of the remainder. Rare intronic variants that abrogate transcription of a functional

p53 product have also been reported. The majority of mutations reside in the highly conserved DNA binding domain with ~25% at several “hotspot” residues (codons 175, 245, 248, 273 and 282); however, mutations in the nuclear localization signal and tetramerization domain have also been reported. One unique mutation in particular, Arg337His, has been reported as a founder mutation in the eastern and south-eastern provinces of Brazil. The population carrier rate for this mutation has been estimated to be as high as 1:300. Unlike other mutations, R337 confers a pH-dependent structural conformational change to the protein leading to its dysfunction. Furthermore, this mutation is overrepresented in children with ACC, although more recent studies suggest that with more careful surveillance more ‘classic’ LFS pedigrees are found. As the breadth of *TP53* gene sequencing became more complete and more families were reported, the association of germline *TP53* mutations is 75-80% for “classic” LFS families, ~40% of LFS-L families and 30% in individuals meeting the revised Chompret criteria [13]. *De novo* mutations have been estimated to occur in 7-20% of patients [14, 15].

Several groups have explored the frequency and characteristics of germline *TP53* mutations in patient cohorts in which the cancer was a ‘component’ tumor of LFS, but in whom a family cancer history was not apparent. In unselected patient populations, germline *TP53* mutations have now been reported by several groups in ~55% of children with ACC [16], 50% of children with CPC, 75% of children with anaplastic RMS, up to 60% of children with the SHH molecular subtype of medulloblastoma and 7-8% of women with early onset (i.e., <30 years) breast cancer – all in the absence of a family history of cancer. These frequencies are not insubstantial, and they have important implications for clinical management of the patient as well as potential impact on other family members.

It is impossible to explain the diverse phenotypic heterogeneity within and across LFS families by the simple presence of a heterozygous germline *TP53* mutation. Studies have shown that while the mutation is ‘necessary’ for cellular transformation and development of cancer, it is not ‘sufficient’. Other genetic events are at play in either a cell-specific context or in modifying the effect of the functional perturbation to the p53 protein conferred by the gene mutation. Polymorphisms in *TP53* and genes regulating the p53 pathway modify age of cancer onset. The SNP 309 (T>G) of the mouse double minute 2 (*MDM2*) gene, an ubiquitin ligase directly regulating p53 degradation, is a plausible candidate modifier in hereditary and sporadic p53 mutant tumors [17, 18, 19]. The presence of the G allele at this locus has been associated with increased

MDM2 levels and aberrant p53 regulation, as demonstrated by the abrogation of DNA repair processes, increased mutation rates and reduced apoptosis, which leads to faster and more frequent tumor formation [17]. On average, G-allele carriers developed cancers nine years earlier than homozygous T-allele carriers. In LFS patients diagnosed with soft tissue sarcoma, *MDM2* SNP 309 accounts for an age-of-onset difference of 12 years, while in patients with breast cancer, it accounts for a 10-year difference. A correlation between the *MDM2* SNP 309 polymorphism and the occurrence of multiple tumors in LFS families was also observed, in which G-allele carriers exhibited a greater frequency of independent subsequent cancers [17].

Two *TP53* polymorphisms may also influence age of onset in LFS families. A polymorphism at codon 72 (Arg>Pro variation), located in exon 4, has been analyzed in the context of LFS, as well as sporadic cancer [18, 19]. Age of onset in Arg-allele carriers was lower than in Pro-allele carriers, with a difference at first diagnosis ranging between 12.6 years in French LFS families [18] to 8.3 years in Brazilian LFS families [19]. The non-duplicated allele (A1) of a 16 bp duplication in intron 3, termed PIN3, was associated with a significantly earlier age of onset in LFS families (28 *vs.* 47 years of age) [19]; moreover, cancer occurrence before the age of 35 was only observed in homozygous non-duplicated (A1A1) PIN3 carriers. Duplicated PIN3 allele carriers also have an increased risk of sporadic and inherited breast cancer and colorectal cancer [20, 21], suggesting both age- and cell type-specific associations [22]. A multivariate analysis of these polymorphisms demonstrated a cumulative effect on age of onset and cancer risk when both the *MDM2* SNP309 and *TP53* 72Arg polymorphisms were expressed, suggesting a synergistic interaction between these two polymorphic loci that alters the cancer phenotype of LFS patients [18, 23]. These were exemplified more recently by the marked effect on age of tumor onset in patients who harbor the G-allele of a polymorphism in miR605 – a microRNA that is involved in p53 regulation [24].

Genetic anticipation is observed in LFS, as reflected by progressive reduction in age of onset and increase in the severity and the proportion of affected individuals in successive generations of a family, supporting the hypothesis that additional genetic modifiers contribute to the variable clinical phenotype observed in affected family members. Although the molecular mechanisms of genetic anticipation are not fully understood, variability in telomere length has been identified to be a key contributor. Telomere length analyzed in peripheral blood lymphocytes was significantly shorter in

carriers of germline *TP53* mutations than in normal age-matched controls [25]. In both children and adults, telomeres were significantly shorter in affected *TP53* mutation carriers than in unaffected carriers and wildtype controls both within and across families [26]; as well, telomere attrition was more rapid in *TP53* mutation carriers than controls [26].

The advent of high-resolution genome-wide approaches has refined the identification of genomic alterations in LFS, expanding on opportunities to explore novel potential mechanisms driving the variable clinical phenotype observed in LFS families. Shlien *et al.* conducted a high-throughput genotyping microarray analysis in which copy number variable (CNV) regions were assessed for 53 individuals from families harbouring germline *TP53* mutations. Compared with normal healthy controls, *TP53* mutation carriers exhibited a significant increase in CNV regions in their genome [27]. While this phenomenon reflects increased instability across the genome of germline *TP53* mutation carriers, it was also interesting to note that CNVs encompassed various known cancer genes, suggesting a selection of cells carrying a unique mutator phenotype in LFS that increased cancer risk. It was also observed that an increased number of CNVs progressed in somatic cells of the tumor, indicating that the CNV formation is a dynamic process that accompanies tumor progression. Interestingly, the role of CNVs was also associated with an aggressive phenotype in the offspring, where CNVs from one parent were commonly inherited with a germline *TP53* mutation from the other parent. The mechanisms by which CNVs are formed are not fully understood, although non-allelic homologous recombination (NAHR) and microhomology-mediated events, such as microhomology-mediated break-induced replication (MMBIR), are thought to underlie these genomic changes. The emerging use of whole genome and whole exome sequencing to explore the nuances of the genetic basis of LFS will undoubtedly shed further light on the role of genetic/genomic and epigenetic modifiers.

The Molecular:Clinical Interface

Are these studies of clinical relevance or do they entail simply an academic (and expensive) exercise? To examine this question, one must revisit the challenges still facing the LFS patient community. To address the question of predictability of tumor age of onset and type, one can envision that algorithms taking into account multiple levels of 'omic' data will be designed to more accurately estimate these clinical outcomes. To complement such studies, it will be important to identify tumors as early as possible, in order to provide the

patient the greatest survival advantage with the least amount of treatment and tumor-related morbidity. The feasibility of this approach was exemplified with the publication in 2011 of a clinical surveillance protocol (termed the “Toronto Protocol”) utilizing a battery of blood tests, as well as imaging studies, including abdominal/pelvic ultrasound, colonoscopy and breast imaging modalities (in young and older adults), and the innovative use of rapid whole body MRI for early tumor detection [28]. Initial results demonstrated a remarkable survival advantage in *TP53* mutation carriers who underwent surveillance compared to those who did not, and this advantage is seen to be durable with longer followup. In addition, as numerous centres around the world adopted this approach, it became evident that the protocol was feasible across many geopolitical jurisdictions. It is clear, therefore, that the joint adoption of germline *TP53* testing of at-risk children and adults, identification of modifier gene effects to perhaps more accurately localize tumor formation and the use of innovative surveillance techniques can change the natural course of this disease and effectively address some of the inherent challenges in the care of LFS patients.

A more complex issue that has not yet been addressed is whether tumors could be prevented in the first place or whether knowledge of the molecular basis of LFS tumors might lead to opportunities for chemoprevention or at least more targeted therapies for *TP53* mutation carriers. One clinical trial using metformin is ongoing (sponsored by the National Institutes of Health), but the primary measurable outcomes are to evaluate the metabolic and biological effects in carriers, rather than the much longer outcome measure of reduced tumor incidence in this population. The use of *Trp53* murine and zebrafish models of LFS may prove a more efficient way to identify and subsequently develop chemopreventive and therapeutic agents. Such agents could then be translated into early phase clinical trials with some confidence of promising effect. In the next few years, the direction of research in LFS, and of many other cancer predisposition syndromes, will focus on prevention and therapy – with hopes that a safety net to eliminate the threat of Damocles’ sword of cancer risk will be offered to these patients.

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