High frequency of de novo mutations in Li–Fraumeni syndrome

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ABSTRACT

Background: Li–Fraumeni syndrome is an autosomal dominant cancer predisposition syndrome caused by germline mutations in the TP53 gene. The frequency of germline de novo TP53 mutations is largely unknown; few unequivocal de novo mutations have been reported.

Methods and results: Of 341 patients with early onset cancer sent for clinical testing to a national reference laboratory, 75 patients had TP53 germline mutations. Five (7%) de novo mutations were identified, as well as an additional 10 TP53 germline mutations likely to be de novo by family history. The frequency of de novo TP53 mutations in this patient sample is at least 7% and may be as high as 20%.

Conclusions: The possibility that de novo germline TP53 mutations are relatively common has implications for testing and the identification of potential Li–Fraumeni syndrome in patients with little or no family history of cancer.

Li–Fraumeni syndrome (LFS) (OMIM 151623) is an autosomal dominant cancer predisposition syndrome characterised by early onset tumours. The syndrome was initially described in 1969 by Li and Fraumeni[1] and eventually characterised by five main cancers including sarcoma, breast cancer, leukaemia, brain tumours, and adrenocortical carcinoma (ACC).[2] In some families, the underlying cause of LFS was later discovered to be due to a germline mutation in the *TP53* gene.[3] The lifetime penetrance of LFS is high[4]; females have an overall higher risk of developing cancer (93% in women *vs* 68% in men by the age of 50) and have an earlier average age of onset (29 years of age *vs* 40 years of age in men).[5]

The frequency of germline *TP53* de novo mutations is unclear. *TP53* de novo mutations are reported to be frequent (up to 20%),[6] uncertain (www.geneclincs.org),[7] and very low (1%).[4 8] Only seven de novo mutations have been documented by: (1) absent family history of early cancer, (2) absent mutation in both parents, and (3) DNA based confirmation of paternity.[6 7 9]

We previously analysed patients from 525 families with family histories sent for clinical testing to the Clinical Molecular Diagnostic Laboratory (CMDL) at The City of Hope. Full sequencing of the *TP53* gene identified 91 families (17.3%) with *TP53* mutations. Sufficient family history information was available for 341 (75 TP53 mutant and 266 TP53 wild type) families. At least 7% of the 75 TP53 mutant probands had de novo germline mutations.

MATERIALS AND METHODS

Sequencing TP53

All coding exons (2–11) and associated splice junctions of the *TP53* gene were analysed in both directions by direct DNA sequencing analysis using the ABI 377 or the ABI 3730 DNA automated fluorescent sequencer (Applied Biosystems, Foster City, California, USA). Sequence analysis was performed in both directions.

Patients

Five hundred and twenty-five probands, predominantly from the USA and Canada, were sent to the Clinical Molecular Diagnostic Laboratory (CMDL) at The City of Hope for *TP53* testing. Sufficient family history information was available for 341 families—that is, at least a three generation pedigree with ages of cancer onset. The 341 probands with pedigree data were analysed further. The average age of first cancer diagnosis in these patients was 22 years. Thirty-six per cent (122/341) of probands had two or more cancers, and in 30% (37/122) of these patients a *TP53* mutation was detected.

Classification as origin or potential origin

For the five confirmed de novo mutations, the mother and the father were both tested for, and found not to carry, the germline TP53 mutation identified in their child. Mutations from 10 other families were considered probable de novo mutations if one or both parents were unavailable for testing and if the patient had no first or second degree relatives (other than offspring) with cancer under the age of 70 years. The AmpFISTR Identifiler PCR Amplification Kit (Applied Biosystems, P/N 4322288) was used to confirm maternity and paternity among the five de novo mutation families. In this well validated, commercially available assay, 15 tetranucleotide repeat loci and a gender determining marker are amplified in a single, robust polymerase chain reaction (PCR) amplification.[10] The data analysis and allele designations were carried out using GeneMapper Software (Version 4.0; Applied Biosystems).

RESULTS

A mutation in the *TP53* gene was detected in 75 of 341 probands with sufficient pedigree information submitted for molecular testing. Among the 75 *TP53* mutations, 15 were possibly de novo, based on a negative family history. Among five of these possible de novo mutations, both parents were available, confirmed by DNA analysis to be the biological parents, and found not to carry the mutation. Therefore, the frequency of confirmed de novo *TP53* mutations in this sample is 7% (5/75).

The average age of first cancer diagnosis for the confirmed de novo mutations was 5.6 years. Four

of the five (80%) patients with de novo mutations had multiple primary cancers (table 1). Four of the five patients also had a childhood cancer, including four with either ACC or choroid plexus carcinoma and two with sarcoma. One patient had bilateral breast cancer in her 20s. The mutations included one splicing mutation and four missense mutations. The missense changes were at amino acids evolutionarily conserved in *TP53* from mammals, chicken, trout, *Xenopus*, invertebrates, and *p73* from human and rat. All five mutations have been reported in the International Agency for Research on Cancer (IARC) database of either germline or somatic changes in cancer.[11]

In addition to the five patients with confirmed de novo mutations, 10 patients had no first or second degree relatives with cancer (other than children or grandchildren) under 70 years of age. None of these 10 patients had a first or second degree relative with an LFS "core" cancer at any age (core cancers are defined as cancers of the breast and brain, adrenocortical carcinoma, and sarcoma, which together account for >77% of cancers seen in LFS probands).[12] However, the parents of these 10 patients were unavailable for testing. The average age of onset of the first cancer was 13.7 years of age (table 2), which is well below the overall average age of onset for all 75 mutation carriers of 21.9 years of age (data not shown). If these patients are included, the frequency of de novo *TP53* mutations could be as high as 20% (15/75).

DISCUSSION

An analysis of 75 patients with *TP53* germline mutations reveals that the de novo mutation frequency is at least 7% and could be as high as 20%. The five patients with demonstrated de novo mutations had a first cancer diagnosis at an average age of 5.6 years, ranging from 4 months to 24 years of age. Four of the five had additional primary cancers. This may reflect a high threshold for sample submission in the absence of family history. The 10 additional patients with candidate de novo mutations had an average of first cancer onset of 13.6 years and 60% had two or more primaries. It follows that patients who develop cancers associated with LFS, especially if they have multiple primary cancers, should be considered for testing even without a family history of cancer.

In the IARC germline *TP53* database (www-p53.iarc.fr), the mode of inheritance is denoted as "new" for several patients. Among a total of seven papers[6–8 13–16] which report these "new mutations," only two papers[6 7] report confirmed de novo mutations (in which both the mother and the father are tested and paternity testing is performed) (tables 3 and 4).

Likewise, Chompret[9] states that germline mutations have been reported and references four studies,[13 17–19] among which only two[18 19] report confirmed de novo mutations.

Reflecting the criteria for Li-Fraumeni syndrome, studies in the literature tend to be biased against patients with a de novo *TP53* mutation. A total of three papers report seven de novo mutations in which both the mother and the father were tested for the mutation and parental identity testing was performed (table 3).[6 7 9 18] Among these seven de novo mutations, three are quite intriguing. Chompret *et al*[9] examined a cohort of 268 patients who were either affected with multiple cancers or had at least one first or second degree relative or first cousin with any cancer before the age of 46.[9] They identified 17 (6.3%) *TP53* mutations, four of which were de novo mutations, as both parents tested negative for the identified mutation and parental identity testing was confirmed in each case. However, probands with three of the four de novo mutations had significant family histories. One patient had a maternal cousin with breast cancer at 29, another had a maternal aunt with ovarian cancer at 30, and one had a maternal cousin with neuroblastoma at 7 years of age.

The 10 patients in this study with putative *TP53* origin mutations had either early onset cancers or multiple cancers, suggestive of a cancer syndrome. The absence of family history in these patients and in the five with confirmed origin mutations, despite the high penetrance of *TP53* mutations in the individual, is in striking contrast to the other 60 families with *TP53* mutations in this study. Unfortunately, the parents of the 10 patients with putative origin mutations are not available for testing. While it is our clinical impression that the cancers in these patients result from de novo mutations in the parent (or perhaps the grandparent), some of these cases may not represent origins of mutations, due to the caveats described below.

In this context, we note that two additional, ultimately spurious, putative de novo mutations were found during the course of our clinical testing. In one family, there was a history of cancer on the paternal side. Neither the mother nor the father carried the mutation; the affected child had a *TP53* germline mutation. Testing indicated non-paternity, revealing a pattern consistent with a sibling of the father. In another family, a child had an LFS core cancer at 2 years of age and both parents were negative for the mutation. However, testing of the father revealed an ambiguous pattern inconsistent with first degree relation, resulting from a past bone marrow transplantation from a donor brother. Analysis of sperm confirmed that the father carried the germline mutation, although, in his 30s, he had never had cancer (his bone marrow transplant was for a non-cancer condition). It is worth noting that, because of the high frequency of female breast cancer, the penetrance of *TP53* mutations in males is considerably lower than that in females (93% *vs* 68%, respectively, by age 50).[4] This can result in an over-estimation of de novo mutations if the father appears to be

Table 1 Confirmed *TP53* origin mutations

Kindred	Proband cancer (age of onset, years)*†	Criteria met	Mutation	Amino acid	Consequence	Conservation‡	Truncating?
31	2 LFS core cancers (<1, teen)	Chompret	IVS6+2 T>C	Splice donor	Splice	N/A	Yes
300	2 LFS core cancers (<1, <5)	Chompret	13216 T>A	His179Gln	Missense	P4	No
491	2 LFS core cancers (<5, <5)	Chompret	13338 A>C	His193Pro	Missense	P4	No
562	LFS core cancer (<5)	Chompret	13167 A>G	Tyr163Cys	Missense	P4	No
541	2 LFS core cancers (20s, 20s)	No clinical criteria	13203 G>A	Arg175His	Missense	P4	No

LFS, Li–Fraumeni syndrome.
*LFS core cancers include cancers of the breast and brain, adrenocortical carcinoma, and sarcoma. The core cancers account for >77% of LFS proband associated cancers.[12]
†Age of onset is given as a range to prevent identification of patient.
‡P0 = not conserved in mammals; P1 = conserved in all mammals; P2 = P1+vertebrates (chicken/trout/Xenopus); P3 = P2+human and rat *p73*; P4 = P3+invertebrates.

Table 2 Family history of patients with putative *TP53* origin mutations*

			Family history		Mutation type				
Kindred	Proband cancer† (age of onset)	Criteria met	Relative(s)	Cancer† (age of onset)	Mutation	Amino acid	Consequence	Conservation‡	Truncating?
78	3 LFS core cancers (20s, 20s, 20s)	Chompret	2° ancestor	Non-LFS core (70s)	13419 A>C	Tyr220Ser	Missense	P3	No
288	LFS core cancer (<5)	Chompret	None	None	14070 G>A	Arg248Gln	Missense	P4	No
358	LFS core cancer (20s)	Chompret	1° descendant	LFS core (<10)	IVS 8-1 G>T	–	Splice	N/A	Yes
362	LFS core cancer (teen)	no clinical criteria	3° ancestor	LFS core (50s)	14070 G>A	Arg248Gln	Missense	P4	No
368	2 LFS core cancers (<5, teen)	Chompret	3° ancestor, 3° ancestor	LFS core (40s), Non-LFS core (40s)	12138 ins C	Pro72ter	Nonsense	N/A	Yes
370	LFS core cancer (<5)	Chompret	None	None	14494 del8ins5§	Cys275ter	Nonsense§	N/A	Yes
381	Non-LFS core cancer (teen), 2 LFS core cancers (20s, 20s)	Chompret	None	None	14486 C>T	Arg273Cys	Missense	P4	No
410	2 LFS core cancers (<14, <14)	Chompret	None	None	17602 C>T	Arg342ter	Nonsense	N/A	Yes
442	2 LFS core cancers (<5, <5)	Chompret	None	None	14049 C>T	Ser241Phe	Missense	P4	No
527	2 LFS core cancers (20s, 30s), 4 non-LFS core cancers (50s, 50s, 50s, 50s)	Chompret	2° descendant, 2° descendant	LFS core (<1), LFS core (unknown age)	13071 A>T	Asn131Ile	Missense	P2	No

LFS, Li–Fraumeni syndrome.
*One or both parents were unavailable for testing, but did not have cancer; these putative origins are unconfirmed.
†LFS core cancers include cancers of the breast and brain, adrenocortical carcinoma, and sarcoma. The core cancers account for >77% of LFS proband associated cancers[12]
‡See table 1.
§Deletion of TGCCTGTC/insertion of AGGTG, resulting in an in-frame deletion of 3 nucleotides and the creation of a stop codon at position 275.

a non-carrier and has not been tested for the mutation. Note also that identity testing with only a few polymorphisms might have spuriously confirmed parentage by chance in both of the cases described above.

There are a number of caveats that could affect the measured frequency of de novo mutations. The classic criteria for LFS or LFS-like syndrome (Birch and Eeles) cannot be made in a patient with sporadic disease.[20–22] However, recent criteria advocated by Chompret *et al* do account for sporadic disease.[9 23] Incomplete pedigrees which are missing relatives with cancer could result in an overestimation of the number of possible de novo mutations. More likely, families with de novo mutations are under-represented because of the general sense that de novo mutations are uncommon in LFS. Thus, one affected individual in a family is unlikely to be sent for testing unless that individual is very young or has multiple cancers. Of the 15 documented or possible de novo mutations described in this study, 11 affected patients had cancer in childhood. Three of the four patients older than 18 had multiple cancers. One had an LFS core cancer in the late 20s and a son with an LFS core cancer at age <10 years. In addition, all but three with childhood cancer and a *TP53* mutation had a second cancer. Since the majority of patients with germline *TP53* mutations do not have cancer in childhood and do not have multiple cancers before 30, the sporadic patients submitted for analyses are likely to represent the minority of the phenotypes displayed by a minority of all the patients with de novo *TP53* germline mutation. Thus, it is prudent to recognise that a patient with sporadic cancer of onset <50 may have a de novo *TP53* mutation.

A high frequency of de novo mutations is not surprising given that fitness is limited. Recognition of families with *TP53* germline mutations can be difficult given the wide spectrum

Table 3 Confirmed de novo *TP53* mutations in literature

No.	Mutation	Amino acid	Consequence	Conservation*	Truncating?	Proband cancer (age in years)	Family history	Parental identity testing	Reference
1	G>T	Arg273Leu	Missense	P4	No	ACC (1)	Parents (no cancer)	Yes	Chompret *et al*[9]
2	A>T	Asp281Val	Missense	P4	No	Osteosarcoma (18)	Parents (no cancer); maternal cousin (breast, 29)	Yes	Chompret *et al*[9]
3	G>A	Gly245Ser	Missense	P4	No	Choroid plexus carcinoma (4)	Parents (no cancer); maternal aunt (ovarian, 30)	Yes	Chompret *et al*[9]
4	Ins4	ins4/exon5†	Splice	N/A	Yes	Meduloblastoma (10)	Parents (no cancer); maternal cousin (neuroblastoma, 7)	Yes	Chompret *et al*[9]
5	C>T	Arg196ter	Nonsense	N/A	Yes	Rhabdomyosarcoma (1)	Parents (no cancer)	Yes	Bendig *et al*[6]‡
6	G>A	Arg248Gln	Missense	P4	No	ACC (7)	Parents (no cancer)	Yes	Bendig *et al*[6]‡
7	C>T	Arg248Trp	Missense	P4	No	Embryonal rhabdomyosarcoma (2)	Parents (no cancer)	Yes	Ayan *et al*[7]‡

*See table 1.
†Splice mutation is an insertion of G within intron 4 in the splice acceptor site resulting in alternative splicing.
‡These references appear in the International Agency for Research on Cancer germline mutation database as "new" mutations.

Table 4 Non-confirmed de novo *TP53* mutations in the literature or in the International Agency for Research on Cancer (IARC) database

No.	Mutation	Amino acid	Consequence	Conservation*	Truncating?	Proband cancer (age in years)	Family history	Parental identity testing	Reference
1	G>A	Arg248Gln	Missense	P4	No	Osteosarcoma, breast, breast (17, 28, 28)	Daughter (rhabdomyosarcoma, 5); parents (no cancer)	No	Toguchida *et al*[19]
2	C>T	Ser241Phe	Missense	P4	No	Hepatoblastosarcoma, osteosarcoma (3 months, 8)	Parents (no cancer)	No	Toguchida *et al*[19]
3	G>T	Glu336ter	Nonsense	N/A	Yes	Multifocal intrafollicular granulosa cell tumour of the ovary (23)	Parents (no cancer)	No	Nogales *et al*[14]‡
4	G>A	Trp91ter	Nonsense	N/A	Yes	Breast (29)	Parents (no cancer)	No	Lalloo *et al*[8]‡
5	Splice†	intron 5 (11 bp del)	Splice	N/A	Yes	Osteosarcoma, testicular/abdominal (12, 22)	Parents (no cancer); MFB (lung, 72); MFS (ovarian, 30); MFS (skin, 80); MMFF(1/2)B (bone, 70)	No	Felix *et al*[13]‡
6	G>A	Cys242Tyr	Missense	P4	No	Osteosarcoma, breast (14, 28)	No 1° relative w/ca	No	McIntyre *et al*[17]
7	?	Arg175His	Missense	P4	No	Not provided	No 1° relative w/ca	No	McIntyre *et al*[17]
8	A>T	Arg209ter	Nonsense	N/A	Yes	Not provided	No 1° relative w/ca	No	McIntyre *et al*[17]
9	A>T	Glu271Val	Missense	P4	No	Not provided	No 1° relative w/ca	No	McIntyre *et al*[17]
10	G>A	Arg273His	Missense	P4	No	Gastric carcinoma (38)	Mother (no cancer); father (hepatocellular carcinoma, 63–had viral hepatitis); son (hepatosarcoma, 11)	No	Sugano *et al*[16]‡
11	A>T	Gln144Leu§	Missense	P1	No	ACC, osteosarcoma of the maxilla, Bowden's disease (1, 26, 31)	Mother (no cancer); father (chondrosarcoma of the maxilla, 27); brother (ACC, 21 mo)¶	No	Patrikidou *et al*[15]‡
12	C>T	Pro278Leu	Missense	P4	No	Liposarcoma, breast, breast, histiocytoma, histiocytoma, adenocarcinoma (40, 42, 45, 48, 49, 50)	Daughter (no cancer); five brothers (no cancer); two sisters (no cancer); father (no cancer); MFS (no cancer); son (multiple tumours); son (multiple tumours)	linkage	Speiser *et al*[18]

ACC, adrenocortical carcinoma; MFB, mother's father's brother; MFS, mother's father's sister; MMFF, mother's mother's father's father; 1° relative w/ca, first degree relative with cancer.
*See table 1.
†Deletion is ctgattcctca in intron 5 (position −25 to −15).
‡These references appear in the IARC germline mutation database as "new" mutations.
§The reference states that the mutation is a change from CAG to CTG, but mistakenly calls the CAG glycine instead of glutamine.
¶The patient's father and brother did not carry the mutation. The mother was not tested.

of cancer types that can occur in LFS families. Several clinical criteria have been constructed in order to diagnose LFS families. Chompret is the only criterion for *TP53* testing which accounts for the possibility of de novo mutations: (1) proband with multiple primary tumours, two of which are sarcoma, brain tumour, breast cancer, and/or ACC, with the initial cancer occurring before 36 years of age, regardless of family history; (2) proband with ACC regardless of age of onset or family history. Four of the five confirmed de novo mutation carriers and nine of the 10 probable de novo mutation carriers meet Chompret criteria. The patients who did not meet Chompret criteria were diagnosed with early onset breast cancers.

In conclusion, herein we report a frequency of de novo mutations of 7–20%. One caveat of the study is that the authors did not know the ages of patients' relatives without cancer, making it possible that some of the "potential" de novo mutations may actually be mutations inherited from relatives who have not yet developed cancer. However, it is possible that the true frequency of de novo *TP53* mutations is even higher than 20% due to the ascertainment bias of a clinical sample set (that is, patients with a negative family history are less likely to be sent for *TP53* testing). A high frequency of de novo *TP53* mutations has major clinical implications for recognition of and screening for LFS patients. It may be prudent at least to consider genetic testing for *TP53* germline mutations in individuals who have cancer below age 30, especially breast cancer, sarcomas, brain tumours, leukaemia, ACC, and choroid plexus carcinoma, or multiple cancers at any age, especially if at least one is of the above types.

Detail has been removed from these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Key points

- The frequency of de novo TP53 mutations is at least 7% and may be has high as 20%.
- The average age of first cancer diagnosis for the confirmed de novo TP53 mutations was ~6 years of age.
- 80% of patients with de novo TP53 mutations had multiple primary cancers.
- A high frequency of de novo TP53 mutations has major clinical implications for recognition of and screening for LFS patients.
- It may be prudent to consider TP53 germline mutation testing for any individual with cancer <30 (especially breast cancer, sarcomas, brain tumours, leukaemia, adrenocortical carcinoma, or choroid plexus carcinoma, or multiple primaries)

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