Letters to the Editor

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Sensitivity and predictive value of criteria for *p53* germline mutation screening

EDITOR—The history of Li-Fraumeni syndrome (LFS) is a good illustration of the delineation of dominantly inherited family cancer syndromes. The identification of this syndrome is the result of the combination of two kinds of evidence, firstly, a number of reports on particular familial aggregations[1 2] and, secondly, systematic family studies of childhood sarcomas.[3–6] Among these studies, the decisive contribution came from Li and Fraumeni[3] who were the first to publish the results of a family study on 641 children with rhabdomyosarcoma which led to the identification of four families in which a sib or a cousin was affected by rhabdomyosarcoma or another soft tissue sarcoma (STS). These families also had several members who were affected by diverse types of malignant tumours, in particular sarcomas and breast cancer at a very young age. This prompted the authors to propose the existence of a new familial syndrome.[7] A prospective study on these families over 12 years provided evidence of a strong predisposition to cancer with a strikingly high frequency of multiple tumours.[8] The term "Li-Fraumeni syndrome" was used for the first time in 1982[9] and the criteria, which subsequently became the classical definition of the syndrome, were proposed by Li and Fraumeni in 1988.[10] These were a proband with a sarcoma before 45 years of age, a first degree relative with cancer before this age, and another close (first or second degree) relative in the lineage with either cancer before this age or a sarcoma at any age. These criteria led to the selection of 24 families which exhibited a wide variety of tumours including bone sarcomas, STS, breast cancer, brain tumours, leukaemia, adrenocortical carcinoma, lymphoma, lung, stomach, pancreas, and prostate cancer, but only the first six types were significantly in excess of the expected proportion among subjects affected by cancer before 45 years in the American population. The follow up of these families confirmed an unusually high predisposition to cancer.[11] Other studies have indicated that a number of other cancers may occur in these families, the most notable being melanoma, germ cell tumours, gastric carcinoma, and Wilms' tumour.[5 12–16]

The definition of the syndrome shifted from clinical and familial criteria to molecular criteria after Malkin *et al*[17] and Srivastava *et al*[18] described the involvement of germline *p53* mutations. The mutations initially found were all missense mutations of exon 7, but further studies, extensively reported by Varley *et al*[19] showed that other regions might also be involved. Studies on series of families with the classical LFS criteria showed that 50 to 70% of these families displayed a *p53* mutation,[19–23] indicating that mutation screening may have overlooked alterations that affect regulatory regions and not *p53* coding sequences or that germline mutation of other gene(s) may be responsible for LFS. Indeed, the study recently published by Bell *et al*[24] showed that heterozygous germline mutations in *hCHK2* occur in LFS. The proportions of *p53* mutations are somewhat lower when less stringent criteria are applied.[20 21]

After ascribing LFS to germline *p53* mutations, different studies were conducted on series of patients with tumours typifying LFS, but not selected on family history, to determine the proportion of gene carriers among them. The studies on patients with bone sarcoma or STS[25–29] showed that up to a third of the group with early onset, an unusual family history, or multiple primary tumours may be carriers. Children with adrenocortical carcinoma were found to have the highest rate (50-80%).[30 31] The frequency of mutations among patients with multiple primary tumours was estimated to be between 7 and 20%.[32–34] Far lower rates were found for patients with brain tumours,[35–39] or early onset/familial breast cancer,[40–43] although the breast cancer risk was clearly high in *p53* mutation carriers. In some of these studies, a selection bias on family history may be suspected. Indeed, a significant proportion of mutations were found among cases with a strong positive family history, the frequency of which appeared to be unusually high.

None of these studies allowed an estimation of cancer risk in mutation carriers, although unaffected carrier relatives are found in family studies. Indeed, LFS selection criteria are so stringent that it is impossible to correct for selection bias. Even looser criteria, such as Li-Fraumeni-like[21 44] (LFL) or Li-Fraumeni incomplete[20] (LFI) do not allow correction for ascertainment bias. This was the reason that we undertook a study at the Institut Gustave Roussy with very loose criteria which offered two advantages: (1) they did not imply the existence of highly penetrant susceptibility genes and therefore potentially allowed the detection of mutations associated with a low cancer risk; (2) correction for selection bias was possible for the estimation of cancer risks in individual subjects. Our main conclusions are: (1) that cancer risks are very high, (2) although unaffected carriers may be observed, there is no evidence for the existence of mutations with particularly low penetrance, and (3) the proportion of de novo mutations is probably substantial.[45]

While the above mentioned were gradually defining with ever greater accuracy the relationship between constitutional mutations and cancer types and risks, an international multidisciplinary group was trying to establish recommendations for predictive testing.[44 46–50] For such testing, it was essential, as a first step, to evaluate individual and familial criteria to undertake the initial search in a family, in terms of sensitivity and predictive value. We report here the results obtained from our study on childhood cancer at the Institut Gustave Roussy and on a study of breast cancer in very young women performed at the Institut Curie in France.

The family history of cancer in children under 18 years treated for all types of solid malignant tumours in the Department of Paediatric Oncology at the Institut Gustave Roussy in Villejuif (France) was investigated between January 1991 and May 1997. Information was collected through a direct interview with a trained counsellor for families of patients treated in the department during the study period. Information was obtained via a mailed questionnaire and completed in most cases by a telephone interview for patients treated before that period and no longer followed up or who had died. To minimise possible biases owing to genetic and environmental heterogeneity, only white children were included in the study.

Family data were collected through the proband's parents. They included information on each of the proband's first and second degree relatives and first cousins. When necessary, additional family members,

previously informed by the proband's parents, were contacted for a telephone interview. Information on relatives included general characteristics (sex, date of birth, malformations, date and cause of death) and the occurrence of any cancer. When cancers had occurred, confirmation of the diagnosis and age at onset were sought in medical and pathology records. Only invasive cancers were considered, excluding non-melanoma skin cancer and in situ carcinoma.

A subgroup of children in whom the frequency of cancer susceptibility genes would be potentially increased was selected on the basis of the occurrence of either of the following criteria: (1) at least one cancer case affecting a first or second degree relative before the age of 46 (familial cases) or (2) multiple primary cancers in the proband regardless of his/her family history (multiple tumour cases). In the original protocol, the family was also included if cancer had occurred only in first cousins. This criterion had to be removed since it dramatically increased the proportion of chance aggregation in the selected sample.

p53 was genotyped in peripheral lymphocytes isolated from fresh blood samples. Direct sequencing was used for the first set of 100 samples. Genomic DNA was amplified as three fragments including respectively exons 2-4, exons 5-8, and exons 9-11 which were fully sequenced. Genotyping was subsequently carried out with a functional assay in yeast (FASAY), as described by Ishioka *et al*[51] and modified by Flaman *et al*[52] when this test became available. Vent DNA polymerase (New England Biolabs) was used to amplify *p53* reverse transcripts before transfection in yeast. Yeast colonies carrying a *p53* mutant allele were identified either as His-auxotroph or as red colonies. *p53* cDNA was extracted from mutant colonies and sequenced. The FASAY has been reported to show over 90% of *p53* mutant alleles[52] as does direct sequencing of amplified *p53* exon scores in our hands.

Women suffering from invasive breast cancer before 36 years, which was diagnosed between January 1990 and August 1995 and followed up at the Institut Curie, were interviewed about their family history and were requested to give a blood sample for the study of genes involved in breast cancer predisposition. Among the 275 women fulfilling these criteria, 119 were interviewed between January 1993 and August 1995 and 116 gave their informed consent for DNA analysis.

The pedigrees were constructed by taking into account first to third degree relatives of the proband on both parental sides. Information concerning the family history of tumours and age at onset of the tumours was verified when possible in medical and pathology records.

Screening for the presence of mutations was performed by analysis of PCR products from genomic DNA with denaturing gradient gel electrophoresis (DGGE). Exons 4, 5, 6, 7, 8, 9, 10, and 11 and respective flanking regions were studied[53] (unpublished data). PCR products exhibiting a variant electrophoretic pattern were directly sequenced on both strands. In order to confirm the loss of biological function of missense mutations detected, a functional assay in yeast was performed according to Flaman *et al.*[52] Any mutation identified was confirmed on a second independent blood sample.

The objectives of defining criteria for recommending *p53* mutation screening are triple: (1) to look for a mutation in situations in which it is likely to be found; (2) to miss as few mutations as possible; (3) not to select subjects who are not carriers. The first objective needs a high positive predictive value, which is the probability that a mutation will be found for given criteria. The second objective needs a high sensitivity, which is the probability that the criteria will be fulfilled, given that the mutation is found. The third objective needs a high specificity, which is the probability that a mutation will not be found given that the criteria are not fulfilled. The positive predictive value can be estimated by the proportion of subjects carrying a germline *p53* mutation among those tested using given criteria. The estimation of sensitivity and specificity requires reference criteria that would allow the ascertainment of carriers and non-carriers from an unselected population. These parameters therefore cannot really be estimated. However, it is possible to estimate the relative sensitivity by the ratio between the number of mutations detected when given criteria are applied and the number of mutations detected in the whole sample. Besides, since a negative result is of no value at this point, specificity is not particularly interesting. At this point, the importance of wording should be emphasised. The sentence "a mutation will be found" is used instead of "a mutation exists", because this would also depend on the sensitivity of the method used to detect mutations, which is not the subject of the present study. The positive predictive value and the relative sensitivity are estimated in relation to the whole sample when more and more stringent criteria are applied on: (1) the number and age of affected relatives, (2) the tumour spectrum (probands and relatives), and (3) the existence of multiple primary tumours.

Of the 2691 children eligible for the family study on 1 January 1998, 239 fulfilled the selection criteria and consented to give a blood sample. Among these 239 children, 211 had at least one first or second degree relative affected by cancer before 46 years of age, 16 had at least two primary malignant tumours, and 12 fulfilled both familial and multiple tumour criteria. Among these cases, 15 mutations were detected, nine in the first group (4.3%), one in the second (a de novo mutation in a child with rhabdomyosarcoma and adrenocortical carcinoma) (6.2%), and five in the third group (4.2%). The complete descriptions of families with mutations are published elsewhere.[45]

Among the 223 children (211 + 12) fulfilling the familial criteria, four levels of nested criteria were defined according to the number and tumour type in the affected relative(s) and are listed in table 1: very loose criteria (223 children), at least one first or second degree relative affected by any cancer; loose criteria (141 children), the tumour type in the affected relative(s) is restricted to sarcoma, brain tumours, breast cancer, adrenocortical carcinoma, haematological malignancies, stomach cancer, melanoma, and germ cell tumours, which are the most prevalent tumours described in LFS; stringent criteria (81 children), the tumour spectrum in relative(s) is restricted to unquestioned tumours, that is, sarcomas, brain tumours, breast cancer, and adrenocortical carcinoma (narrow spectrum); very stringent criteria (21 children), a new criterion is added to the previous ones, at least another first or sec-

Table 1 Definition of the four levels of nested criteria according to the number and tumour type in the affected relative(s) in the study on childhood cancer

Criteria on relatives	*Definition*
Very loose	At least one first or second degree relative affected by any cancer
Loose	Tumour type in the affected relative(s) restricted to sarcoma, brain tumours, breast cancer, adrenocortical carcinoma, haematological malignancies, stomach cancer, melanoma, and germ cell tumours
Stringent	Tumour spectrum in relative(s) restricted to unquestioned tumours, ie sarcomas, brain tumours, breast cancer, and adrenocortical carcinoma (narrow spectrum)
Very stringent	New criterion added to the previous ones: at least another first or second degree relative affected by cancer before 46 years or a sarcoma at any age

Table 2 Positive predictive value and relative sensitivity of criteria on probands and relatives for predictive p53 screening in childhood cancer

	Criteria on relatives							
	Very loose		*Loose*		*Stringent*		*Very stringent*	
Criteria on proband	*Predictive value*	*Relative sensitivity*	*Predictive value*	*Relative sensitivity*	*Predictive value*	*Relative sensitivity*	*Predictive value*	*Relative sensitivity*
Any tumour	6% (14/223)	93% (14/15)	9% (13/141)	87% (13/15)	15% (12/81)	80% (12/15)	43% (9/21)	60% (9/15)
Narrow spectrum	12% (12/102)	80% (12/15)	16% (11/67)	73% (11/15)	23% (11/47)	73% (11/15)	53% (8/15)	53% (8/15)

ond degree relative affected by cancer before 46 years or a sarcoma at any age. Criteria were also defined by stratification on the tumour type in the proband with two levels, a narrow spectrum tumour (102 children) or any tumour.

The results of the combination of criteria for relatives and probands among the 223 familial cases are given in table 2. They show that the positive predictive values for the criterion "any tumour" in the proband are quite low (less than 15%) except in the category "very stringent" criteria in relatives. It is significantly higher when the tumour type in the proband is restricted to the narrow spectrum and attains 23% when stringent criteria are fulfilled among relatives.

The parameters estimated among the 28 (16 + 12) multiple tumour cases (excluding bilateral tumours of paired organs) are shown in table 3. The positive predictive values are much higher than in the group selected on a familial basis. However, this criterion overlaps markedly with the previous ones; in the six carriers of a germline mutation, five fulfil the "narrow spectrum" for the first tumour of the proband, of which four also fulfil the "stringent" criteria on relatives. Thus, adding the criterion "multiple tumours" in the proband to the combination of narrow spectrum in the proband's tumour and stringent criteria for relatives yields 24 new cases, two of which have a germline mutation. This results in an increase in relative sensitivity from 73% (11/15) to 87% (13/15) and a decrease in the predictive value from 23% (11/47) to 18% (13/71). If both tumours (or at least two tumours) are restricted to the narrow spectrum, then only six families are added, one of which carries a mutation, resulting in a relative sensitivity of 80% (12/15) and a predictive value of 23% (12/53). There are very few multiple tumours among relatives (excluding bilateral tumours of paired organs): six families among which three fulfil the stringent criteria in relatives and four fulfil the narrow spectrum in the proband. There are two *p53* mutations, two of which belong to the group defined by narrow spectrum and multiple tumours in the proband and stringent criteria in relatives. Consequently, the predictive value and the relative sensitivity are not modified when multiple tumours are added to the narrow LFS spectrum in relatives (data not shown).

Among the 116 breast cancer cases fully analysed, a total of three germline *p53* mutations (2.5%) were detected. Two of them are missense mutations (Leu130Phe, Arg175Gly) and one is an in frame deletion (GluAla346del3Asp). The deleterious effects of both missense mutations have been confirmed with FASAY. One mutation (Leu130Phe) was found in a woman who was affected at 31 years and had no family history of cancer and in particular five unaffected sisters aged from 34 to 49 years. The second mutation (GluAla346del3Asp) concerned a case of bilateral breast carcinoma at 29 and 30 years whose family history was clearly indicative of Li-Fraumeni syndrome, including chondrosarcoma at 16 years, leukaemia at 26 years, breast cancer at 20 years, and renal tumour at 36 years (unconfirmed) in the sibship, and the father affected with a soft tissue tumour of an unknown histological nature at 64 years. The third mutation (Arg175Gly) was detected in a woman suffering from osteosarcoma at 18 years and bilateral breast cancer at 27 and 29 years. Her father had developed a rectal carcinoma at 39 years, meningioma at 54 years, and pancreatic carcinoma at 55 years, and her paternal uncle had developed a germ cell tumour at 45 years.

Table 3 Positive predictive value and relative sensitivity in families ascertained on the existence of multiple tumours in the proband child

Criteria on relatives	*Predictive value*	*Relative sensitivity*
None	21% (6/28)	40% (6/15)
Very loose	42% (5/12)	33% (5/15)
Loose	63% (5/8)	33% (5/15)
Stringent	71% (5/7)	33% (5/15)
Very stringent	100% (3/3)	20% (3/15)

Because of the small number of mutations found, we had to consider a smaller number of criteria than in the previous section, and only two levels of nested criteria were defined: loose criteria, at least one first or second degree relative affected by any cancer before 46 years of age or a proband with multiple primary malignant tumours; stringent criteria, the tumour spectrum in relative(s) (or in the proband in case of multiple tumours) is restricted to the narrow spectrum. However, since breast cancer is common in the general population, familial aggregation of breast cancers may be either because of chance or germline *BRCA1/2* mutations. Therefore, two situations were considered, the narrow spectrum tumour is breast cancer (situation A) or another tumour (situation B).

Thirty three cases fulfilled the loose criteria (two mutations), 21 cases the stringent criteria A (no mutation), and two the stringent criteria B (two mutations). The positive predictive values are presented in table 4, but not the relative sensitivities which would be meaningless with only three mutations.

Most of the studies on germline *p53* mutations conducted to date and quoted in the introduction did not permit evaluation of different selection criteria. Some of them concerned families ascertained on the basis of strong familial aggregation (corresponding roughly to our very stringent criteria) and the relevance of looser criteria could not be assessed. Other studies concerned series of tumours with very limited information on family history, so that it was impossible to evaluate criteria. The most well documented studies are by the group of Jillian Birch, based on the Manchester Children's Tumour Registry.[16 21] These authors showed the relatively high predictive value (4/18=22%) of the so called Li-Fraumeni-like criteria, that is, a proband with any childhood cancer or sarcoma, brain tumour, or adrenal cortical tumour diagnosed under the age of 45 years with one first or second degree relative with a typical LFS cancer at any age, plus a first or second degree relative in the same lineage with any cancer under the age of 60 years. There is some degree of overlap

Table 4 Positive predictive value in families of women with breast cancer before 36 years according to criteria

Criteria		*Positive predictive value*	
None		3% (3/116)	
Loose		7% (2/29)	
Stringent	A	0% (0/21)	9% (2/23)
	B	100% (2/2)	

between these criteria and ours; 18 families conform to this definition in our sample, four of which exhibit a *p53* mutation, which is exactly the same number and proportions as those found by these authors. Note that all positive families fulfil our stringent criteria in relatives and narrow spectrum tumour in the proband.

In the present study, we have quantified various criteria which can be a very useful basis for establishing recommendations for conducting *p53* screening.

The functional assay used in the study on childhood cancer detects about 90% of mutations in *p53*.[52] Mutations in exons 2 and 3 are very rare and were not studied in the study on breast cancer A small number of mutations may thus have been missed and the positive predictive value of criteria may thus be slightly higher, which should not modify our conclusions.

At present, searching for *p53* germline mutations is still a cumbersome task and laboratories which perform such screening in France are in favour of criteria yielding a positive predictive value of at least 20%. In our study on childhood cancer, this value is achieved when the following criteria are used: a proband with a narrow spectrum tumour and at least one first or second degree relative affected before 46 years by a narrow spectrum cancer or multiple primary tumours or a proband affected by multiple primary tumours whatever the family history. It should be noted that when very stringent criteria are used, that is, that are very close to those used in previous studies to define LFS, very similar results are obtained with a positive predictive value of 53%.

For cancer occurring in adulthood, our data concern only very early onset breast cancer, which is the most frequent tumour in *p53* carriers (80% of tumours occurring among female carriers after 16 years of age[45]). As it is also common in the general population, we have presented the results separately when breast cancer is the narrow spectrum tumour in the relative who was the determinant factor in the inclusion of the family (criterion A) and when it is another cancer (criterion B). Although this distinction results in loss of precision of estimations owing to a decrease in sample size, it should be noted that no mutation among 21 cases was detected when criterion A was found, whereas both cases with criterion B were *p53* carriers (p=0.02, exact test). Consequently, as in childhood cancer, it seems reasonable to use stringent criteria and to add this restriction on tumour type, that is, exclude breast cancer from the tumour spectrum in relatives.

Although the results obtained by ascertaining early onset breast cancer probands cannot necessarily be extrapolated to all tumours of the narrow spectrum, we expect that the predictive value for the other tumours will be higher than that obtained for breast cancer, given the high frequency of this tumour in the population.

In addition, although we have very few data on adrenocortical carcinoma not selected on family history, it seems logical to add this criterion considering the results of published studies quoted in the introduction.

Finally, it should be noted that although leukaemia is usually reported as belonging to the LFS, this was not included among narrow spectrum tumours in the present study for two reasons. (1) It is absent in our childhood probands because the Department of Paediatrics at the Institut Gustave Roussy treats only solid tumours. (2) Leukaemia is frequent in the general population and the inclusion of families with one relative affected by this cancer would dramatically increase the probability of chance aggregation.

With our knowledge of the interest of the criteria evaluated in this study and considering the state of the art for *p53* mutation screening, the French LFS group decided to recommend a search for *p53* mutation in families fulfilling the following criteria: (1) a proband affected by a narrow spectrum cancer before 36 years and at least one first or second degree relative affected by a narrow spectrum tumour (other than breast cancer if the proband is affected by breast cancer) before 46 years or multiple primary tumours; (2) a proband with multiple primary tumours two of which belong to the narrow spectrum and the first of which occurred before 36 years, whatever the family history; (3) a proband with adrenocortical carcinoma whatever the age of onset and family history. Using such criteria, we expect to find a mutation in about 20% of cases and to miss about 20% (3/15) of the mutations which would be detected using the loosest criteria. These criteria could of course be modified in the near future with more efficient laboratory detection methods and re-evaluated prospectively.

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1 Bottomley RH, Condit PT. Cancer families. *Cancer Bull* 1968;**20**:22-4.
2 Lynch HT, Mulcahy GM, Harris RE, Guirgis HA, Lynch JF. Genetic and pathologic findings in a kindred with hereditary sarcoma, breast cancer, brain tumors, leukemia, lung, laryngeal, and adrenal cortical carcinoma. *Cancer* 1978;**41**:2055-64.
3 Li FP, Fraumenii JF. Rhabdomyosarcoma in children: epidemiologic study and identification of a cancer family syndrome. *J Natl Cancer Inst* 1969;**43**: 1365-73.
4 Birch JM, Hartley AL, Marsden HB, Harris M, Swindell R. Excess risk of breast cancer in the mothers of children with soft tissue sarcomas. *Br J Cancer* 1984;**49**:325-31.
5 Strong LC, Stine M, Norsted TL. Cancer in survivors of childhood soft tissue sarcoma and their relatives. *J Natl Cancer Inst* 1987;**79**:1213-20.
6 Birch JM, Hartley AL, Blair V, Kelsey AH, Harris M, Teare MD, Morris Jones PH. Cancer in families of children with soft tissue sarcomas. *Cancer* 1990;**66**:2237-48.
7 Li FP, Fraumeni JF. Soft tissue sarcomas, breast cancer and other neoplasms: a familial syndrome? *Ann Int Med* 1969;**71**:747-52.
8 Li FP, Fraumeni JF. Prospective study of a cancer family syndrome. *JAMA* 1982;**247**:2692-4.
9 Pearson ADJ, Craft AW, Ratcliffe JM, Birch JM, Morris-Jones PH, Roberts DF. Two families with the Li-Fraumeni cancer family syndrome. *J Med Genet* 1982;**19**:362-5.
10 Li FP, Fraumeni JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, Miller RW. A cancer family syndrome in twenty-four kindreds. *Cancer Res* 1988;**48**:5358-62.
11 Garber JE, Goldstein AM, Kantor AF, Dreyfus MG, Fraumeni JF, Li FP. Follow-up study of twenty-four families with Li-Fraumeni syndrome. *Cancer Res* 1991;**51**:6094-7.
12 Hartley AL, Birch JM, Marsden HB, Harris M. Malignant melanoma in families of children with osteosarcoma, chondrosarcoma and adrenal cortical carcinoma. *J Med Genet* 1987;**24**:664-8.
13 Hartley AL, Birch JM, Kelsey AM, Marsden HB, Harris M, Teare MD. Are germ cell tumours part of the Li-Fraumeni syndrome? *Cancer Genet Cytogenet* 1989;**42**:221-6.
14 Hartley AL, Birch JM, Tricker K, Wallace SA, Kelsey AM, Harris M, Morris Jones PH. Wilms' tumor in the Li-Fraumeni cancer family syndrome. *Cancer Genet Cytogenet* 1993;**67**:133-5.
15 Varley JM, McGown G, Thorncroft M, Tricker KJ, Teare MD, Santibanez-Koref MF, Houlston RS, Martin J, Birch JM, Evans DGR. An extended Li-Fraumeni kindred with gastric carcinoma and a codon 175 mutation in *TP53*. *J Med Genet* 1995;**32**:946-50.
16 Varley JM, McGown G, Thorncroft M, Santibanez-Koref MF, Kelsey AM, Tricker KJ, Evans DGR, Birch JM. Germline mutations of *TP53* in Li-Fraumeni families: an extended study of 39 families. *Cancer Res* 1997;**57**:3245-52.

17 Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH. Germline p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. *Science* 1990;**250**:1233-8.
18 Srivastava S, Zou Z, Pirollo K, Blattner W, Chang EH. Germline transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature* 1990;**348**:747-9.
19 Varley JM, Evans DGR, Birch JM. Li-Fraumeni syndrome - a molecular and clinical review. *Br J Cancer* 1997;**76**:1-14.
20 Brugières L, Gardes M, Moutou C, Chompret A, Meresse V, Martin A, Poisson N, Flamant F, Bonaïti-Pellié C, Lemerle J, Feunteun J. Screening for germ line p53 mutations in children with malignant tumors and a family history of cancer. *Cancer Res* 1993;**53**:452-55.
21 Birch JM, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM, Harris M, Morris Jones PH, Binchy A, Crowther D, Craft AW, Eden OB, Evans DGR, Thompson E, Mann JR, Martin J, Mitchell ELD, Santibanez-Koref MF. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. *Cancer Res* 1994;**54**:1298-304.
22 Birch JM, Heighway J, Teare MD, Kelsey AM, Hartley AL, Tricker KJ, Crowther D, Lane DP, Santibanez-Koref MF. Linkage studies in a Li-Fraumeni family with increased expression of p53 protein but no germline mutation in p53. *Br J Cancer* 1994;**70**:1176-81.
23 Frebourg T, Barbier N, Yan YX, Garber JE, Dreyfus M, Fraumeni JF, Li FP, Friend SH. Germ-line p53 mutations in 15 families with Li-Fraumeni syndrome. *Am J Hum Genet* 1995;**56**:608-15.
24 Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DCR, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE, Haber DA. Heterozygous germ line *hCHK2* mutations in Li-Fraumeni syndrome. *Science* 1999;**286**:2528-31.
25 Toguchida J, Yamaguchi T, Dayton SH, Beauchamp RL, Herrera GE, Ishizaki K, Yamamuro T, Meyers PA, Little JB, Sasaki MS, Weichselbaum RR, Yandell DW. Prevalence and spectrum of germline mutations of the p53 gene among patients with sarcoma. *N Engl J Med* 1992;**326**:1301-8.
26 Porter DE, Holden ST, Steel CM, Cohen BB, Wallace MR, Reid R. A significant proportion of patient with osteosarcoma may belong to Li-Fraumeni cancer families. *J Bone Joint Surg Br* 1992;**74**:883-6.
27 Iavarone A, Matthay KK, Steinkirchner TM, Israel MA. Germ-line and somatic p53 mutations in multifocal osteosarcoma. *Proc Natl Acad Sci USA* 1992;**89**:4207-9.
28 McIntyre JF, Smith-Sorensen B, Friend SH, Kassell J, Børresen AL, Yan YX, Russo C, Sato J, Barbier N, Miser J, Malkin D, Gebhardt MC. Germline mutations of the p53 tumor suppressor gene in children with osteosarcoma. *J Clin Oncol* 1994;**12**:925-30.
29 Diller L, Sexsmith E, Gottlieb A, Li FP, Malkin D. Germline p53 mutations are frequently detected in young children with rhabdomyosarcoma. *J Clin Invest* 1995;**95**:1606-11.
30 Wagner J, Portwine C, Rabin K, Leclerc JM, Narod SA, Malkin D. High frequency of germline p53 mutations in childhood adrenocortical cancer. *J Natl Cancer Inst* 1994;**86**:1707-10.
31 Varley JM, McGown G, Thorncroft M, James LA, Margison GP, Forster G, Evans DG, Harris M, Kelsey AM, Birch JM. Are there low-penetrance TP53 alleles? Evidence from childhood adrenocortical tumors. *Am J Hum Genet* 1999;**65**:995-1006.
32 Malkin D, Jolly KW, Barbier N, Look AT, Friend SH, Gebhardt MC, Andersen TI, Børresen AL, Li FP, Garber J, Strong L. Germline mutations of the p53 tumor-suppressor gene in children and young adults with second malignant neoplasms. *N Engl J Med* 1992;**326**:1309-15.
33 Shiseki M, Nishikawa R, Yamamoto H, Ochiai A, Sugimura H, Shitara N, Sameshima Y, Mizoguchi H, Sugimura T, Yokota J. Germ-line *p53* mutation is uncommon in patients with triple primary cancers. *Cancer Lett* 1993;**73**:51-7.
34 Russo CL, McIntyre J, Goorin AM, Link MP, Gebhardt MC, Friend SH. Secondary breast cancer in patients presenting with osteosarcoma: possible involvement of germline p53 mutations. *Med Pediatr Oncol* 1994;**23**:354-8.
35 Chung R, Whaley J, Kley N, Anderson K, Louis D, Menon A, Hettlich C, Freiman R, Hedley-White ET, Martuza R, Jenkins R, Yandell D, Seizinger BR. *TP53* gene mutations and 17q deletions in human astrocytomas. *Genes Chrom Cancer* 1991;**3**:323-31.
36 Kyritsis AP, Bondy ML, Xiao M, Berman EL, Cunningham JE, Lee PS, Levin VA, Saya H. Germline p53 mutations in subsets of glioma patients. *J Natl Cancer Inst* 1994;**86**:344-9.
37 Chen P, Iavarone A, Fick J, Edwards M, Prados M, Israel MA. Constitutional p53 mutations associated with brain tumors in young adults. *Cancer Genet Cytogenet* 1995;**82**:106-15.
38 Felix CA, Slavc I, Dunn M, Strauss EA, Phillips PC, Rorke LB, Sutton L, Bunin GR, Biegel JA. p53 gene mutations in pediatric brain tumors. *Med Pediatr Oncol* 1995;**25**:431-6.
39 Li YJ, Sanson M, Hoan-Xuang K, Delattre JY, Poisson M, Thomas G, Hamelin R. Incidence of germ-line p53 mutations in patients with gliomas. *Int J Cancer* 1995;**64**:383-7.
40 Børresen AL, Andersen TI, Garber J, Barbier-Piraux N, Thorlacius S, Eyfjörd J, Ottestad L, Smith-Sorensen B, Hovig E, Malkin D, Friend SH. Screening for germ line *TP53* mutations in breast cancer patients. *Cancer Res* 1992;**52**:3234-6.
41 Prosser J, Porter D, Coles C, Condie A, Thompson AM, Chetty U, Steel CM, Evans HJ. Constitutional p53 mutations in a non Li-Fraumeni cancer family. *Br J Cancer* 1992;**65**:527-8.
42 Sidransky D, Tokino T, Helzlsouer K, Zehnbauer B, Rausch G, Shelton B, Prestigiacomo L, Vogelstein B, Davidson N. Inherited p53 gene mutations in breast cancer. *Cancer Res* 1992;**52**:2984-6.
43 Warren W, Eeles RA, Ponder BA Easton DF, Averill D, Ponder MA, Anderson K, Evans AM, DeMars R, Love R, Dundas S, Stratton MR, Trowbridge P, Cooper CS, Peto J. No evidence for germline mutations in exons 5-9 of the p53 gene in 25 breast cancer families. *Oncogene* 1992;**7**:1043-6.
44 Eeles RA. Germline mutations in the TP53 gene. *Cancer Surv* 1995;**25**:101-24.
45 Chompret A, Brugières L, Ronsin M, Gardes M, Dessarps-Freichey F, Abel A, Hua D, Ligot L, Dondon MG, Bressac-de Paillerets B, Frebourg T, Lemerle J, Bonaïti-Pellié C, Feunteun J. Prevalence of p53 germline mutations in childhood cancer and estimation of cancer risk for carrier individuals. *Br J Cancer* 2000;**82**:1932–7.
46 Li FP, Correa P, Fraumeni JF. Testing for germline p53 mutations in cancer families. *Cancer Epidemiol Biomarkers Prev* 1991;**1**:91-4.
47 Li FP, Garber JE, Friend SH Strong LC, Patenaude AF, Juengst ET, Reilly PR, Correa P, Fraumeni JF. Recommendations on predictive testing for germ line p53 mutations among cancer-prone individuals. *J Natl Cancer Inst* 1993;**84**:1156-60.
48 Eeles RA. Predictive testing for germline mutations in the p53 gene: are all the questions answered? *Eur J Cancer* 1993;**29**:1361-5.
49 Li FP, Fraumeni JF. Collaborative interdisciplinary studies of p53 and other predisposing mutations in Li-Fraumeni syndrome. *Cancer Epidemiol Biomarkers Prev* 1994;**3**:715-17.
50 Eng C, Schneider K, Fraumeni JF, Li FP. Third international workshop on collaborative interdisciplinary studies of p53 and other predisposing mutations in Li-Fraumeni syndrome. *Cancer Epidemiol Biomarkers Prev* 1997;**6**:379-83.
51 Ishioka C, Frebourg T, Yan YX, Vidal M, Friend SH, Schmidt S, Iggo R. Screening patients for heterozygous p53 mutations using a functional assay in yeast. *Nat Genet* 1993;**5**:124-9.
52 Flaman JM, Frebourg T, Moreau V, Charbonnier F, Martin C, Chappuis P, Sappino AP, Limacher JM, Bron L, Benhattar J, Tada M, Van Meir EG, Estreicher A, Iggo RD. A simple p53 functional assay for screening cell lines, blood, and tumors. *Proc Natl Acad Sci USA* 1995;**92**:3963-7.
53 Hamelin R, Jego N, Laurent-Puig P, Vidaud M, Thomas G. Efficient screening of p53 mutations by denaturing gradient gel electrophoresis in colorectal tumors. *Oncogene* 1993;**8**:2213-20.

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Identification of a transcriptionally compromised allele of *c-MYC* in a North American family

EDITOR—Chromosomal translocations that target *c-MYC* at 8q24 are found in all Burkitt's lymphomas (BL), AIDS related non-Hodgkin's lymphoma (AIDS-NHL), mouse plasmacytomas (PCTs), in many examples of diffuse large cell lymphoma (DLCL), and in multiple myeloma (MM). Indications are that *c-MYC* is under strict control and when deregulated results in unchecked cellular proliferation and hyperplasia. Non-random chromosomal translocations found such as t(8;14), t(8;22), or t(2;8) in these lymphoid neoplasias places *c-MYC* under the control of strong immunoglobulin enhancers, which leads to overexpression.[1 2] In addition, *c-MYC* is amplified in many tumours including breast, prostate, gastrointestinal, ovarian, MM, myeloid leukaemia, and melanoma suggesting that the overall transcriptional level is probably a key transforming element associated with *c-MYC*.[3] Besides genetic lesions, other epigenetic factors such as activation of growth factor receptors may also lead to constitutive expression of *c-MYC*. Thus, considerable efforts have been made systematically to identify the *c-MYC* transcriptional apparatus (promoters and enhancers) in an effort to control *c-MYC* expression. While *c-MYC* transcription potentially initiates from one of three promoters, P0, P1, and P2 which reside in the exon 1 region, the P2 promoter normally accounts for 75-90% of cytoplasmic *c-MYC* RNAs. To date, more than 20 transcription factors have been found to reside in the proximity of exon 1 of *c-MYC*.[1] Actually, *c-MYC* was one of the first genes to exhibit transcriptional blockage. RNA polymerase II initiation complexes were shown to pause on P2 before activation.[4–6] Upon chromosomal translocation, the insertion of IG enhancer elements renders a shift in promoter usage from P2 towards P1 and loss of transcriptional blockage.[7]

In concert with the intensely regulated transcriptional machinery, the sequences surrounding *c-MYC* appear to be strictly conserved across species boundaries and little in the way of sequence variation or allelic polymorphisms has