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Li-Fraumeni and Related Syndromes: Correlation between Tumor Type, Family Structure, and *TP53* Genotype[1]

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ABSTRACT

A database has been created to collect information on families carrying a germ-line mutation in the *TP53* gene and on families affected with Li-Fraumeni syndromes [Li-Fraumeni syndrome (LFS) and Li-Fraumeni-like syndrome (LFL)]. Data from the published literature have been included. The database is available online at http://www.iarc.fr/p53, as part of the IARC TP53 Database. The analysis of the 265 families/individuals that have been included thus far has revealed several new findings. In classical LFS families with a germ-line *TP53* mutation (83 families), the mean age of onset of breast cancer was significantly lower than in LFS families (16 families) without a *TP53* mutation (34.6 *versus* 42.5 years; $P = 0.0035$). In individuals with a *TP53* mutation, a correlation between the genotype and phenotype was found. Brain tumors were associated with missense *TP53* mutations located in the DNA-binding loop that contact the minor groove of DNA ($P = 0.01$), whereas adrenal gland carcinomas were associated with missense mutations located in the loops opposing the protein-DNA contact surface ($P = 0.003$). Finally, mutations likely to result in a null phenotype (absence of the protein or loss of function) were associated with earlier onset brain tumors ($P = 0.004$). These observations have clinical implications for genetic testing and tumor surveillance in LFS/LFL families.

INTRODUCTION

LFS[3] is a rare autosomal disorder characterized by a familial clustering of tumors, with a predominance of sarcomas, breast cancers, brain tumors, and adrenocortical carcinomas, diagnosed before the age of 45 years (1). Other cancers, such as leukemia, lung cancer, skin melanoma, gastric cancer, pancreatic cancer, and prostate cancer are also present in excess in some families and, in some cases, germ cell tumors, choroid plexus papilloma, and Wilms' tumor have been reported as part of the spectrum (2–5). In 1990, Malkin *et al.* (6) found that a few LFS families had a germ-line mutation in the tumor suppressor gene *TP53*. Since then, analyses of several series of LFS families have shown that ~70% of such families are attributable to germ-line mutations in *TP53* (2, 7–9).

The *TP53* tumor suppressor gene (chromosome 17p13; OMIM #191170) encodes a protein involved in many overlapping cellular pathways that control cell proliferation and homeostasis, such as cell cycle, apoptosis, and DNA repair. The p53 protein is a transcription factor constitutively expressed in most cell types and activated in response to various stress signals (including in particular genotoxic stress; reviewed in Ref. 10). Loss of p53 function is thought to suppress a mechanism of protection against accumulation of genetic alterations. Somatic *TP53* genetic alterations are frequent in a variety of human sporadic cancers, with frequencies varying from 10 to 60%, depending on the tumor type or population group (11). These alterations are compiled in the IARC *TP53* Database and can be searched online (12).[4] Mutations observed in the germ line and in sporadic cases are very similar (13), with a majority of missense mutations (~75%) usually resulting in a defective transcriptional activity. Mutations are scattered throughout the coding sequence of the gene, all codons in the DNA binding domain being mutated at least once (11). However, ~30% of mutations cluster at 8 "hotspot" codons (codons 175, 176, 220, 245, 248, 249, 273, and 282). Thus, *TP53* differs from other tumor suppressors such as *RB1*, *APC*, or *BRCA1*/2, which are inactivated frequently by deletions or nonsense mutations.

Despite a wide research interest in *TP53* and LFSs, not all of the underlying genetic defects responsible for LFS have been found. In several families fulfilling the definition of classical LFS, no defect in *TP53* has been found. On the other hand, some families having a germ-line mutation in *TP53* display some, but not all features of LFS. These families are referred to as LFL (7, 9, 14). To aid the study of these syndromes, we have created a relational database that compiles data from the literature and our own laboratory data (Institute of Cancer Research), to assess all of the patients and families with a *TP53* germ-line mutation, as well as LFS and LFL families, which may be caused by other genes. This database is a redesigned and extended version of the IARC database of germ-line mutations described in Kleihues *et al.* (13). In this report, we describe the new database structure and content, and we use the database to investigate the influence of *TP53* mutation on the type of tumor arising in these families.

MATERIALS AND METHODS

Database Design. A relational database was created to enter information on families with LFS/LFL syndromes and those with a germ-line mutation in the *TP53* gene. The database was designed using Microsoft Access software. Data on members with cancer and obligate *TP53* mutation carriers in each family were entered, as well as family structure, tumor samples, details of the germ-line mutation, mutation detection method, and the publication in which the family is described. The central element of the database is the family information unit to which are connected the individual information unit, the germ-line mutation information units (*TP53* and *CHEK2*, with mutation detection methods), and the reference information unit. The tumor information unit is connected to the individual information unit (see Fig. 1). Indexes were used to annotate data in a standardized way. The current structure of the database allows the addition of new table(s) to enter any novel germ-line mutation data if required. Indeed, an alternative susceptibility gene may be involved in some of the LFS and LFL families for which no germ-line mutation has been found. Details of annotations can be found on the internet.[4]

Family Classification. Family history was defined as follows. LFS (classical LFS) refers to proband with sarcoma at <45 years, and a first-degree relative with tumor at <45 and another close relative with tumor at <45 or

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[3] The abbreviations used are: LFS, Li-Fraumeni syndrome; LFL, Li-Fraumeni-like syndrome; wt, wild-type; mut, mutation; FH, family history of cancer.

[4] Internet address: http://www.iarc.fr/p53.

sarcoma at any age (1). LFL refers to LFL in which we grouped Birch definition (LFL-B; Ref. 7), and Eeles (two definitions; LFL-E1 and LFL-E2). LFL-E1 (first definition of LFL by Eeles; Ref. 14) refers to two different tumors that are part of extended LFS in first- or second-degree relatives at any age (sarcoma, breast cancer, brain tumor, leukemia, adrenocortical tumor, melanoma, prostate cancer, and pancreatic cancer). LFL-E2 refers to sarcoma at any age in the proband with two of the following (two of the tumors may be in the same individual): breast cancer at <50 years and/or brain tumor, leukemia, adrenocortical tumor, melanoma, prostate cancer, pancreatic cancer at <60 years, or sarcoma at any age (15). LFL-B refers to proband with any childhood cancer or sarcoma, brain tumor or adrenocortical carcinoma at <45 years, with one first- or second-degree relative with typical LFS cancer (sarcoma, breast cancer, brain tumor, leukemia, or adrenocortical carcinoma) at any age, plus one first- or second-degree relative in the same lineage with any cancer diagnosed under age 60. FH refers to family history of cancer that does not fulfill LFS or any of the LFL definitions (Birch, Eeles E1, or E2,) and No FH refers to no family history of cancer.

Tumor Classification. In the database, tumors are identified by their site (topography) and their histology (morphology) according to the International Classification of Diseases for Oncology classification (16). Because precise information on tumor histology is rarely given in the original publication, the majority of the tumors are classified as "tumor" or "cancer" in the database. For data analyses, tumors were recoded according to their topography/histology: tumors with the International Classification of Diseases for Oncology codes C18 to C20 were grouped as colorectal cancer, leukemia and lymphoma were grouped together, and sarcomas were divided into soft tissue sarcomas and bone sarcomas. Benign lesions and tumors of unknown origin were excluded. Multiple primary tumors in patients were counted individually.

Dataset Description and Statistical Analysis. For the comparison of tumor spectra in LFS families with and without a mutation, the strict clinical definition of LFS was used. All of the tumors described in those families have been included, whether or not the individuals have been tested for the presence of a *TP53* mutation. Tumors in proband/index cases were included (35% were in probands for LFS-wt and 25% for LFS-mut). Analyses have been repeated excluding tumors in proband cases, and the results are qualitatively unchanged.

For the analysis of genotype/phenotype association, tumors in individuals proven to carry a *TP53* mutation and from families with a history of cancer (LFS, LFL, and FH) have been selected, including proband/index cases (55% of the tumors were in probands). To correct for nonindependence of tumors from members of the same family, robust variance estimators were used. *TP53* mutations were classified into type groups and structural groups as shown in Table 1.

Table 1 *TP53 mutation groups*

Type groups	
Group A	Transitions (G to A or C to T base change) outside CpG sites
Group B	Transitions (G to A or C to T base change) at CpG sites
Group C	Transversions (A to C or T to G, A to G or T to C, A to T or T to A, G to C or C to G, G to T or C to A base change)
Group D	Other mutations (deletions, insertions, tandem mutations, complex changes)
Structure/function groups[a]	
Group 1	Missense mutations in the L2 and L3 loops (codons 164 to 194 and 237 to 250 respectively) that bind the minor groove of DNA
Group 2	Missense mutations in the L1 loop (codons 115 to 135) and S2-S2′-H2 motifs (codons 273 to 286) that binds the major groove of DNA
Group 3	Missense mutations in the non-DNA binding loops (codons 136–140, 147–155, 199–203, 208–213, 220–229, 259–263), in the β-sheet skeleton (codons 110–112, 141–146, 156–163, 195–198, 204–207, 214–219, 230–236, 251–258, 264–272) or in the oligomerisation domain (codons 325–355)
Group 4	Nonsense, frameshift and splice mutations which are likely to result in the absence of the TP53 protein or in a non functional protein

[a] Predicted structural/functional properties of mutant proteins based on structural data from Ref. 40.

Table 1. Analysis of the incidence of tumor types by mutation groups was done using multinomial logistic regression with adjustment of the variance for family membership. Analysis of the number/proportion of each tumor type in each family by mutation type and structure was done using Poisson regression. Age at diagnosis for each tumor was compared through the use of generalized linear models, adjusted for family. Before analysis, age at diagnosis was log-transformed to reduce skewness and heteroscedasticity. ANOVA was used to test for effects of mutation structure type (and their interaction) on mean and total severity score. When a significant overall effect of mutation structure or type was found the following three independent comparisons were made: (*a*) mutations in DNA binding domain: L2/L3 *versus* L1 loops (group 1 *versus* group 2); (*b*) missense mutations: DNA-binding *versus* non-DNA-binding (groups 1 + 2 *versus* 3); and (*c*) all mutations: missense *versus* truncating (groups 1 + 2 + 3 *versus* 4). All of the statistical analyses were done using STATA v7.0 (Stata Corporation, College Station, Texas).

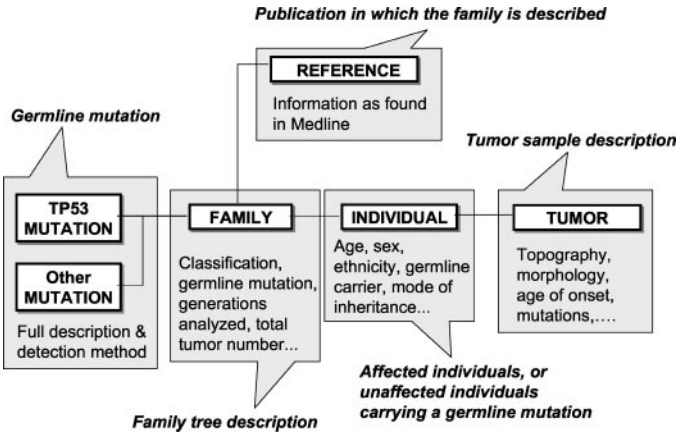

Fig. 1. Structure of the IARC *TP53* germ-line database. The data are organized into a relational database built using Microsoft Access software. The database includes information on families with LFS, LFL, and those with a *TP53* germ-line mutation. Information related to: (*a*) the publication in which the family has been described; (*b*) the family structure; (*c*) the germ-line mutation and its detection method; (*d*) the affected members of the family (or unaffected individuals who are obligate gene mutation carriers); and (*e*) the tumors, is stored in five different tables or "units." The central element of the database is the family information units to which are connected the individual information unit, the *TP53* and other germ-line mutation information unit (with the mutation detection method), and the reference information unit. The tumor information unit is connected to the individual information unit. The current structure of the database allows the addition of new table(s) to enter any novel germ-line mutation data if required.

RESULTS

Database Scope and Availability. A new relational database has been created to compile published information on families fulfilling the definition of Li-Fraumeni and related syndromes, and on individuals carrying a germ-line mutation in the *TP53* gene. Data were extracted from peer-reviewed articles published between 1990 and June 2002. Unpublished data on 9 families were also included.[5] Criteria for inclusion were the following: (*a*) individuals carrying a sequenced *TP53* germ-line mutation, affected or not by a cancer; and (*b*) individuals affected by a cancer and belonging to a family defined as LFS or LFL (using LFL-E1, -E2, and -B as defined in "Materials and Methods"), with or without a *TP53* germ-line mutation. Because we mainly concentrated on individuals carrying a *TP53* germ-line mutation, the database did not include LFS or LFL families described in the literature for which *TP53* mutational status has not been investigated.

The database is maintained in Microsoft Access and consists of a set of tables organized in a relational scheme (see "Materials and Methods;" Fig. 1). It is maintained as part of the IARC *TP53* Mutation Database, and a precise description of the information entered in each table can be found on the IARC web site (12).[4] Updates of the database are released every year in a spreadsheet format that can be

[5] R. Eeles and N. Sodha, personal communication.

Table 2 *Content of the IARC TP53 germ-line database*

Family history	Germ-line mutation	Number of families	Number of individuals	Number of tumors
LFS	*TP53*	83	431	520
	CHEK2	2	8	11
	None	14	59	67
LFL	*TP53*	67	330	373
	CHEK2	3	3	6
	CDKN2A	1	5	7
	None	22	78	90
FH[a]	*TP53*	37	114	126
No FH	*TP53*	28	32	47
Unknown	*TP53*	8	8	15
Total	—	265	1068	1262

[a] FH, family history of cancer.

downloaded.[6] It should be noted that only data associated with *TP53* mutations are released. The full content of the database is available upon request.[7] Authors are invited to submit their unpublished germ-line mutation data and clinical information directly.[7] This material is reviewed before inclusion in the database.

Users of the database should be aware of the limitations and possible biases that may affect the analysis of the database. Because data are extracted from published reports from various laboratories, the major sources of bias reside in trends in reporting and publishing mutations, and in the method of mutation detection. These biases are described in Olivier *et al.* (12) and in the online help file.[8]

***TP53* Status and Tumor Spectrum in LFS Individuals.** The dataset included in the database comprises 265 families (Table 2). Two hundred and twenty-six germ-line *TP53* mutations were present in 223 families (1 family had 2 *TP53* germ-line mutations and 1 had 3 *TP53* mutations). Five families had a *CHEK2* (OMIM #604373) mutation, and 1 family had a *CDKN2A* (OMIM #600160) mutation. Thirty-six families did not have any identified mutation. There are a total of 1262 tumors recorded in the database, of which 564 are in individuals with confirmed *TP53* germ-line mutations or obligate carriers of such mutations. Sixty-seven unaffected individuals who were confirmed or obligate *TP53* carriers were recorded.

Among the 99 families fulfilling the classical definition of LFS (see "Materials and Methods") and screened for *TP53* mutation, 83 have a *TP53* mutation (LFS-mut), and 16 have no mutation in *TP53* (LFS-wt). The absence of *TP53* mutation in those families has been confirmed by screening the entire coding region of *TP53* as well as flanking regions, and by using different techniques in 15 of the families. One family has been screened from exon 5 to 9. Thus, it is possible to compare with good confidence the tumor spectrum in LFS families with and without a *TP53* mutation. Fig. 2 shows the tumor site distribution of the cancers observed in individuals from LFS-mut and LFS-wt (expressed as the percentage of all of the tumors in LFS-mut or LFS-wt, *i.e.* 491 and 73, respectively). The two distributions show a higher prevalence of brain tumors (13.2% *versus* 2.7%; $P = 0.006$) in LFS-mut *versus* LFS-wt and the absence of adrenocortical carcinomas in LFS-wt individuals. Tumors from "other" types were found to be twice as prevalent in LFS-wt *versus* LFS-mut patients. However, this latter observation may result from an inclusion bias. In the absence of an identified *TP53* mutation, it is not possible to precisely delineate the limits of the pedigree, and we have included all of the tumors reported in the family tree. Therefore, it is likely that some of these tumors are phenocopies. Nonetheless, the difference observed for brain tumors is still valid if we restrict the analysis to the five main LFS tumor types (breast, brain, bone, soft tissue, and adrenal tumors), with 4.7% brain tumors in LFS-wt *versus* 17.2% in LFS-mut ($P = 0.029$), and if we exclude proband/index cases from the analysis.

The comparison of the age at onset of tumors in LFS-wt and LFS-mut families showed a statistically significant lower average age at onset for breast cancer in LFS-mut families (34.6 *versus* 42.5 years; $P = 0.0035$). No difference in age at diagnosis between LFS-mut and LFS-wt was observed for the other tumor types.

Tumor Spectrum, Age at Onset, and Gender Distribution in *TP53* Germ-Line Mutation Carriers. A total of 494 tumors were identified in individuals who were confirmed or obligate *TP53* mutation carriers and who had a family history of cancer (LFS, LFL, or FH). The prevalence, age at onset, and gender distribution of these tumors is shown in Table 3. The most frequent cancer is breast cancer (30.6%), followed by soft tissue sarcoma (17.8%), brain tumor (14%), bone sarcoma (13.4%), and adrenocortical carcinoma (6.5%). Less frequent tumor sites include lung, hematopoetic system, stomach, colorectum, skin, and ovary. The gender distribution for these tumors shows an excess of males for brain tumor, hematopoetic cancers, and stomach cancer, whereas an excess of females was observed for adrenocortical carcinoma and skin cancer. All of the breast cancers were in females. Males and females were equally affected by soft tissue and bone sarcoma, lung cancer, and colorectal cancer. This

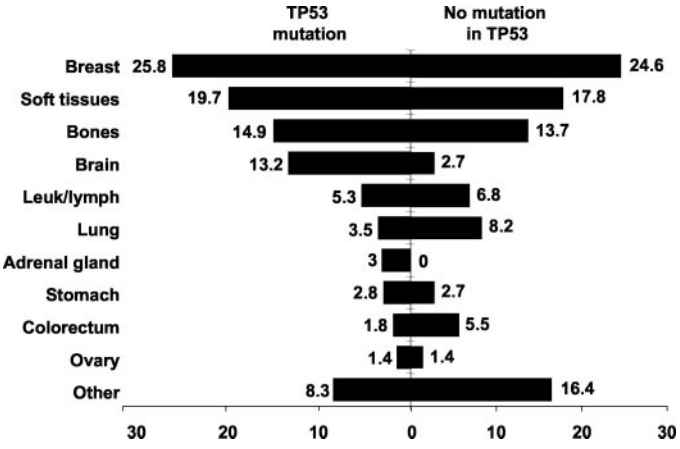

Fig. 2. Tumor spectrum in LFS families with a *TP53* mutated *versus* wt background. Tumors from all individuals belonging to families clinically defined as classical LFS are included. The total tumor number is 73 for LFS families without *TP53* mutation and 491 for LFS families with a *TP53* mutation. The percentage is indicated for each tumor site. *Leuk/lymphoma*, leukemia and lymphoma.

Table 3 *Tumor type, age at onset, and gender distribution in TP53 germ-line mutation carriers from LFS/LFL/FH families*

Tumor type	Number (%)	Median age at diagnosis		% Male (ratio)	
		TP53 carriers	Sporadic[a]	*TP53* carriers	Sporadic[a]
Breast cancer	151 (30.6)	33	63.1	0 (0/151)	0.7
Soft tissue sarcoma	88 (17.8)	14	61.3	54 (43/80)	53
Brain tumour	69 (14)	16	57.4	66 (42/64)	56
Bone sarcoma	66 (13.4)	15	43.3	58 (35/60)	56
Adrenocortical ca.[b]	32 (6.5)	3	41.9	22 (7/32)	51
Lung cancer	17 (3.4)	40	68.7	47 (8/17)	66
Leukemia/lymphoma	15 (3)	27	65.1	67 (8/12)	55
Stomach cancer	12 (2.4)	35	72.6	73 (8/11)	62
Colorectal cancer	8 (1.6)	34	71.6	57 (4/7)	50
Ovarian ca.	7 (1.4)	39.5	64.3	0 (0/7)	0
Skin melanoma	6 (1.2)	43.5	—	0 (0/6)	—
Other	23 (4.7)	—	—	—	—

[a] Data based on cancer registries from United States, France, and United Kingdom compiled in Cancer Incidence in Five Continents v. 7, 1997 (41).
[b] ca., carcinoma.

[6] Internet address: http://www.iarc.fr/P53/Germline.html.
[7] E-mail address: p53database@iarc.fr.
[8] Internet address: http://www.iarc.fr/P53/Help.html.

gender distribution is similar to the one of sporadic cancers observed in the general population with the exception of adrenocortical carcinoma, which occur significantly ($P < 0.001$) more frequently in females than in males in *TP53* mutation carriers compared with sporadic cases in the general population (Table 3). However, most adrenocortical carcinoma cases are from United Kingdom (17 of 32 cases with 2 of 17 in females), where an excess of females has been reported in childhood adrenocortical carcinoma. Thus, it is not clear whether the observed excess of females is related to the presence of a *TP53* mutation or to other genetic or environmental factors (17).

The age at onset of tumors in *TP53* mutation carriers varies with tumor site; however, all of the inherited tumors show an earlier age at onset compared with their sporadic cancer counterparts (Table 3). When looking at the age distribution of the major cancer sites, brain tumor and soft tissue sarcoma show nonsymetric distributions, with a high prevalence of cases before the age of 10 (Fig. 3). The prevalence decreases in the 10–20 years range and increases again after 20 years for brain tumors, whereas soft tissue sarcomas show a constant prevalence after 10 years. Thus, despite similar median age at diagnosis for soft tissue sarcoma and bone sarcoma (Table 3), soft tissue sarcomas occur more frequently in childhood (0–10 years), whereas bone sarcomas are more prevalent in teenagers (11–20 years; Fig. 3). The age distribution for breast cancer and adrenocortical carcinoma reflects the median age at onset for these tumors (34 and 3 years, respectively). There was a trend for later onset brain tumors and soft tissue sarcomas in females compared with males (median age: 28 *versus* 11 years for brain tumors and 19 *versus* 11 years soft tissue sarcomas), although neither was statistically significant.

Genotype/Phenotype Correlations in *TP53* Mutation Carriers. Many *in vitro* experiments have shown that not all of the *TP53* mutations are functionally equivalent, some exhibiting gain of function and/or dominant-negative properties (reviewed in Ref. 18). Thus, it is expected that *TP53* germ-line mutants may have distinct biological properties that could promote tumorigenesis in distinct organ sites. To investigate this possibility, we searched for associations between tumor type and mutation type on the dataset of individuals who were confirmed or obligate *TP53* mutation carriers and who had a family history of cancer (LFS, LFL, or FH).

The type and location of *TP53* germ-line mutations observed in those individuals is shown in Table 4 (only one mutation by family is included). The majority of these mutations are missense mutations (72%) and deletions (10%). Most of the deletions are small (1–4 bp) and induce a frameshift (data not shown). Overall, 46% of the missense mutations were located at codons 175, 213, 245, 248, 273, and 282. These codons are classic mutation hotspots in most forms of sporadic cancers and correspond mostly to transition mutations at cytosines within pyrimidine repeats (CpG sites; Ref. 11). Codons 133, 152, and 337 were the next more frequently mutated codons. Mutations at codons 152 and 337 are transitions at CpG sites, whereas mutations at codon 133 were AT>CG and AT>GC. These codons are rarely mutated in sporadic tumors (<0.8% of all somatic mutations; IARC *TP53* Database; R7). Transitions at CpG sites and small deletions are thought to result from endogenous mutagenic processes, *i.e.* spontaneous deamination of methylated cytosine for transitions at CpG and polymerase slippage during replication for small deletions (19, 20). Thus, ~60% of the germ-line mutations described for these families may result from endogenous processes. It is of note that the mutation type did not differ significantly among LFL, LFS, and FH families.

Mutations were classified based on the predicted structural/functional properties of mutant proteins (see "Materials and Methods" and Table 1). The structural categories were selected on the basis of: (*a*) specific tertiary structure motifs; and (*b*) role of these motifs in DNA binding. Group 1 corresponds to missense mutations affecting residues belonging to loops 2 and 3 of the p53 protein, which, together, constitute a folded domain stabilized by the binding of zinc and supporting the residues that bind to the minor groove of target DNA. Group 2 includes missense mutations affecting residues located in the loop-sheet-helix motif that binds to the major groove of target DNA. These two categories reflect the fact that the DNA-binding surface of

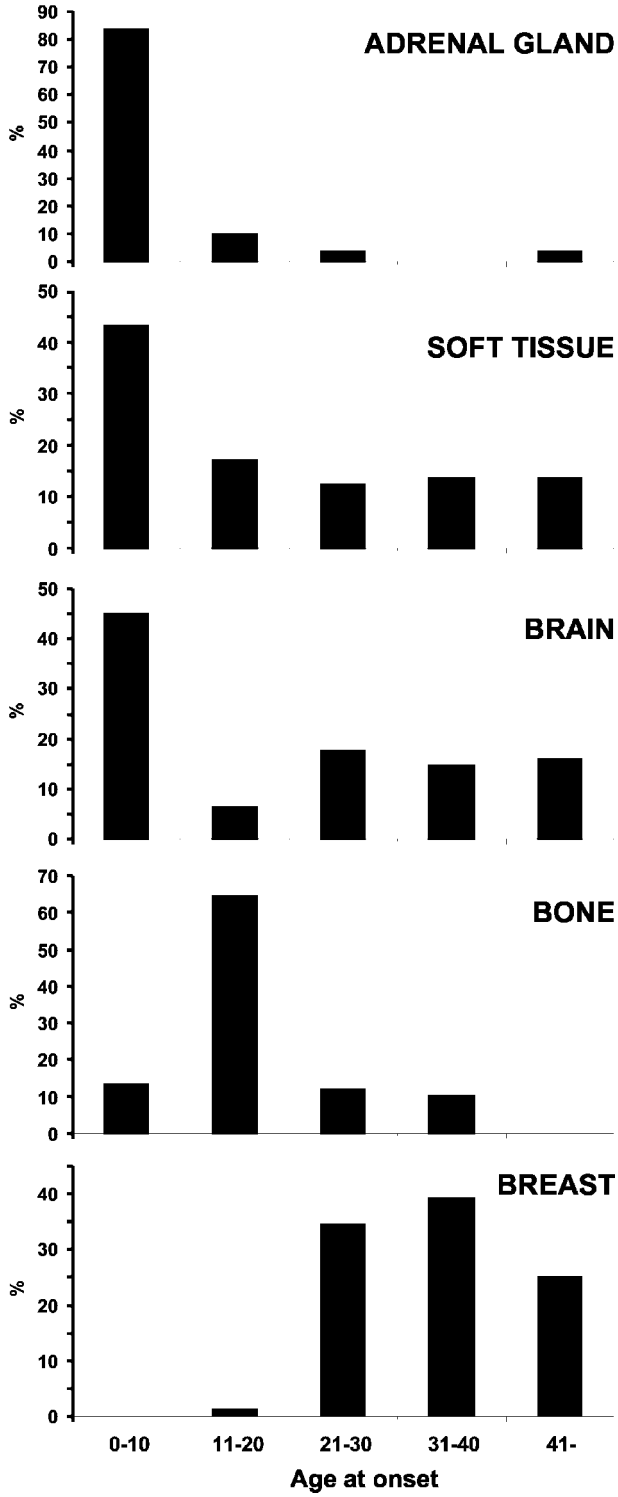

Fig. 3. Age distribution of the major tumor sites in *TP53* mutation carriers. Only tumors from individuals who were confirmed or obligate *TP53* mutation carriers and who had a family history of cancer (LFS, LFL, or FH) are included. The total tumor number is 148 for breast, 62 for brain, 59 for bone, 81 for soft tissue, and 30 for adrenal gland.

Table 4 *TP53 germ-line mutations in the LFS/LFL/FH families*

Mutation type	LFS/LFL/FH families
All	(n = 190)
Missense	136 (71.6%)
Deletion	19 (10%)
Nonsense	14 (7.4%)
Insertion	5 (2.6%)
Other	16 (8.4%)
Point	(n = 164)
GC:AT at CpG	92 (56%)
AT:GC	18 (11%)
GC:AT not CpG	16 (9.7%)
GC:CG	13 (7.9%)
AT:TA	11 (6.7%)
GC:TA	10 (6%)
AT:CG	4 (2.4%)
Hotspot codons	(n = 150)
248	18 (12%)
273	16 (10.7%)
175	12 (8%)
245	9 (6%)
282	7 (4.7%)
213	7 (4.7%)
133	5 (3.3%)
152	5 (3.3%)
337	4 (2.7%)

p53 contains two distinct parts. Group 3 includes all of the missense mutations outside groups 1 and 2 (located in the non-DNA-binding loops, in the β-sheet scaffold of the p53 protein, or in the oligomerization domain). Group 4 includes mutations expected to confer a "p53-null" phenotype (insertions or deletions with frameshift and nonsense mutations). The prevalence of the most frequent cancers (breast cancer, brain tumor, adrenocortical carcinoma, soft tissue sarcoma, and bone sarcoma) was calculated for each group of mutation as shown in Table 5. There was a statistically significant association between tumor type and mutation group, attributable mainly to brain tumors and adrenocortical carcinomas. Brain tumors were more likely to be associated with group 1 mutations compared with all of the other groups (odds ratio, 2.1; 95% confidence interval, 1.2–3.7; $P = 0.01$) and adrenal tumors with group 3 mutations compared with all of the other groups (odds ratio, 2.9; 95% confidence interval, 1.3–6.4; $P = 0.008$). For brain tumors, most of the effect was in differences between L2/L3 loops *versus* L1 loop ($P = 0.03$). For adrenal cancers, the difference was confined to missense mutations in the non-DNA-binding domain *versus* missense mutations in the DNA-binding domain ($P = 0.003$). No other tumor type exhibited significant variation across structural group, and there was no association between family classification (LFS *versus* LFL *versus* FH) and structural group of mutation. No correlation was found between mutation type (defined by the nature of the base change) and tumor type.

Breast and brain tumors showed significant differences in age at diagnosis. The major difference for breast cancer was between missense mutations in the DNA-binding domain *versus* missense mutations not in the DNA-binding domain (32 years for groups 1 + 2 *versus* 42 for group 3; $P = 0.006$). For brain tumor, the major effect could be seen in the comparison between null and non-null mutations (9 years for group 4 *versus* 25.5 years for groups 1 + 2+3; $P = 0.004$). No association was found between age at onset and mutation type when defined by the nature of the base change.

For brain tumors, 80% of group 1 mutations were at codons 248, 245, and 175. Codon 248 contacts directly with DNA in the minor groove, whereas codons 245 and 175 support and stabilize the L3 loop (Fig. 4). These codons are classical hotspot mutations and correspond to mutant proteins exhibiting loss of transactivation function and dominant-negative effects (on the wt p53 protein) in some cell-types (21). In individuals with adrenocortical carcinomas, 60% of group 3 mutations were at codons 151, 152, 219, and 220 that form a cluster of residues, which oppose with the DNA-binding surface of the protein (Fig. 4). These residues are not classical hotspots in sporadic cancers, but they are conserved among vertebrate, and *in vitro* functional assays have shown that they have impaired transcriptional activities (22, 23).

DISCUSSION

Since the discovery of the involvement of *TP53* in LFS, the classical clinical definition of LFS has been revisited by several groups, because certain families carrying a TP53 mutation do not fulfill this definition (7, 15, 24). In LFS families without a *TP53* mutation, several candidate genes have been screened. Of these, only the *CHEK2* gene was found to be mutant in a limited number of families (25–28). However, from subsequent studies, it has become clear that the presence of a *CHEK2* mutation probably does not predispose to LFS *per se*, but only to the breast cancers that have occurred within the context of families that match the LFS/LFL phenotype (29). Thus, *TP53* mutation remains the only molecular explanation for this syndrome, and a better knowledge of the tumor spectrum associated with *TP53* mutation would help clinicians to identify individuals who should be tested for germ-line *TP53* mutation. Because LFS is a rare syndrome, this question would be more efficiently addressed in collaborative studies. In an attempt to set the ground for such a collaborative effort, we have created an international database based on the published literature of LFS/LFL families and of individuals with *TP53* germ-line mutations. This database has been designed to enter detailed information on family structure, individual characteristics, tumor pathology, mutation description, and detection method. It is available on-line[4] at the site that also provides access to the IARC *TP53* database of somatic mutations.

To investigate how *TP53* mutation influences the tumor spectrum observed in LFS/LFL individuals, we have performed an analysis of the dataset assembled in the 2002 update of the database. The comparison of tumor spectra in LFS families (including only those fulfilling the classical definition) with or without a *TP53* mutation showed that LFS-mut individuals have more brain tumors, have exclusively adrenocortical carcinoma, and have earlier onset breast cancer. No difference was observed for sarcomas between the two

Table 5 *Tumor distribution by structural groups of TP53 mutation in LFS/LFL/FH families*

Structural groups of mutations are defined in Table 1.

		Group 1		Group 2		Group 3		Group 4	
Tumor site	P	% (n = 142)	Age[a]	% (n = 102)	Age	% (n = 108)	Age	% (n = 127)	Age
Breast	0.176	27.4	32	24.5	31	29.6	42	39.4	33
Brain	0.028	21.1	29	9.8	25	14.8	24.5	8.7	9
Soft tissue	0.539	17.6	5	23.5	11	15.7	6.5	17.3	20
Bone	0.575	15.5	15	13.7	13	13.9	13	9.5	15
Adrenal	0.004	4.2	1	1	[b]	13	3.5	7.1	4
Other	0.044	14	34	27.5	38.5	13	34	19	32

[a] Age, the median age at diagnosis is indicated.
[b] Only one tumor in this category.

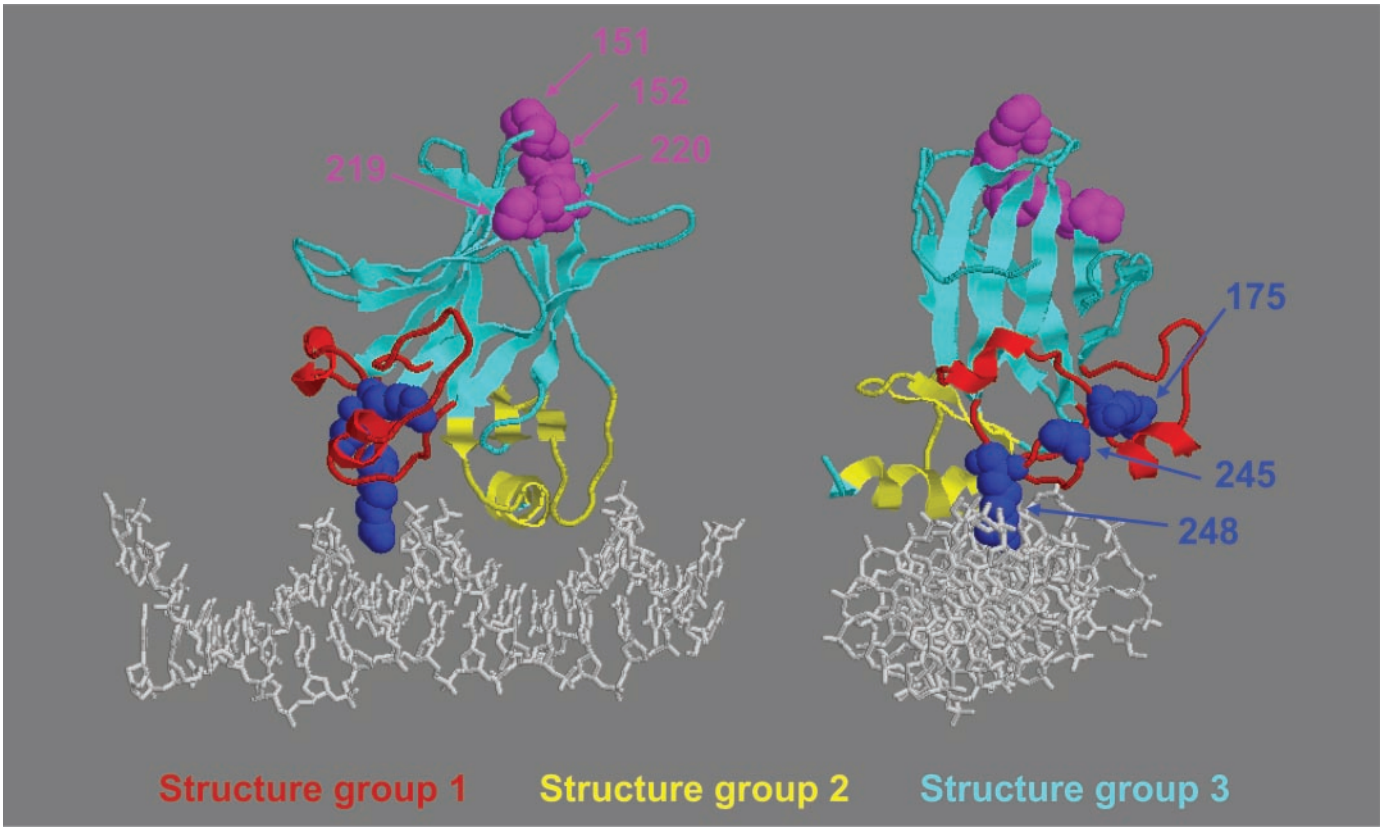

Fig. 4. Hotspot codon positions associated with adrenal gland carcinoma (*ADR*) and brain tumors (*BT*). Three-dimensional view of the central DNA-binding domain of the p53 protein in complex with DNA. Structural groups of residues are in different colors: group 1 residues, which correspond to L2 and L3 loops (binding in the minor groove of DNA helix) are in *red*; group 2 residues, which correspond to L1 loop and S2-S2′-H2 motifs (binding in the major groove of DNA helix) are in *yellow*; group 3 residues, which correspond to the non-DNA-binding loops, the β-sheet skeleton, or the oligomerization domain, are in *cyan*. The codon position of the mutations associated with adrenal gland carcinoma (*ADR*) and brain tumors (*BT*) are indicated in *pink* and *blue*, respectively.

groups. The latter observation was expected, because the definition of LFS is based mainly on the presence of sarcomas. Also expected was the absence of adrenocortical carcinomas in LFS-wt, because they have been shown to occur exclusively in *TP53* germ-line mutation carriers (30). The difference observed for brain tumors suggest that their presence in a family with sarcoma(s) may be a good predictor for the presence of a *TP53* mutation. Moreover, these observations support the idea of an active role of *TP53* mutation in the development of brain and adrenal gland tumors.

The analysis of the tumor spectrum in LFS, LFL, and FH individuals proven to have a germ-line *TP53* mutation or obligate *TP53* mutation carriers showed that the classical LFS tumors (sarcoma, breast, brain, and adrenal) represented 80% of all of the tumors in these individuals. A group of "less-prevalent" tumors including lung carcinoma, hematopoetic cancers, stomach cancer, colorectal cancer, ovary carcinoma, and skin melanoma, accounted for 15% of the tumors. How *TP53* mutations contribute to these "less-prevalent" tumors is not clear. These tumors are frequent in the general population, and their presence in a LFS family may be because of chance. On the other hand, we showed that in the context of a germ-line *TP53* mutation, these tumors occur at earlier age at onset. In a recent study, Birch *et al.* (31) reported the association of Li-Fraumeni cancers with *TP53* mutation by age groups. Their analysis included 28 families ascertained by standard criteria (classical LFS and LFL B). They used data from United Kingdom national cancer statistics for comparison with prevalence in the general population. They found leukemia and, to a lesser extent, stomach cancer to be associated with *TP53* mutation. Thus, as reported recently by Nichols *et al.* (32), germ-line *TP53* mutations could predispose to a wider spectrum of cancers than described previously.

The age distribution of the most common tumor types in families with *TP53* mutations indicates that the risk of developing a specific cancer varies with age. Before 10 years of age, adrenal gland tumors, soft tissue sarcomas, and brain tumors are the most prevalent cancers. In teenagers, the prevalence of these three cancers decreases, whereas the most common cancer is bone sarcoma. After the age of 20, the main cancers are breast cancers and, again, brain tumors, which show a biphasic age distribution. The comparison with the general population indicates that, in the cases of breast cancer, soft tissue sarcomas, and adrenal gland carcinomas, the peak of incidence is at younger ages in *TP53* mutation carriers. For bone sarcomas, the trend in *TP53* mutation carriers reflects the age-distribution in the general population. For brain tumors, the biphasic effect may correspond to tumors of different histological types, thus also reflecting their age distribution in the general population (33).

In the series of *TP53* mutation carriers analyzed here (confirmed or obligatory mutation carriers with LFS, LFL, or FH), we found a significant association between the tumor type and the structural (and potentially functional) class of the mutation. Although the restriction of the analysis to known and obligate carriers reduces the power of the series, this is the most stringent type of analysis, because phenocopies are known to occur in LFS families (34). Missense mutations in the L2 and L3 loops that bind to the minor groove of DNA were associated with brain tumors ($P = 0.029$), whereas those outside the DNA-binding surface (in the non-DNA-binding loops, β-sheets, and oligomerization domain) were associated with adrenocortical carcinoma

($P = 0.004$). Mutations expected to result in a p53-null phenotype were not associated with any specific type of tumor, but were associated with earlier onset tumors, in particular for brain tumors. This suggests that these null mutations have a particularly severe effect or, alternatively, they could predispose to tumors of different histological types with a worse prognosis. However, current data on histologies are too sparse to reach any definite conclusion. In a series of 34 LFS and LFL families, Birch *et al.*, (35) have reported an association of brain tumor and breast cancer with missense mutations in the DNA-binding domain (35). Our analysis of a larger series of families (which include the 34 families reported by Birch *et al.*) revealed a new association between adrenocortical carcinoma and group 3 mutations, and confirmed the association of brain tumor with genotype, refining it to the L2/L3 loops of the p53 protein, which bind to the minor groove of DNA. For breast cancer, although we did not find an association with a particular structure-group of mutations, we found that mutations in the DNA-binding domain were associated with tumors of earlier onset.

It is possible that tissue-specific factors influence the expression of p53 mutant properties. A striking example of this has been described recently for the inherited *TP53* mutation, R337H, which predisposes exclusively to childhood adrenocortical carcinoma (36). A functional assessment of this mutant has shown that its activity is pH-sensitive, *i.e.* inactive (mutant-like) at pH >7.7 and active (wt like) at pH <7.7 (37). Thus, the protein may adopt a mutant phenotype only under particular physiological conditions. Although this does not explain the tissue specificity of the R337H mutant, this example illustrates the dependence of p53 protein function on the cellular context in which it is expressed. In our analysis, adrenocortical carcinoma was associated with codons located in the opposite side of the DNA-binding surface of the protein. These residues are three conserved prolines not frequently mutated in sporadic human cancers (codons 151, 152, and 219) and a conserved tyrosine (codon 220) frequently mutated in head and neck cancers (IARC *TP53* Database[4]). Functional assays have shown that these mutants have defective transcriptional activities (22, 23, 38). The particular clustering of residues may suggest that mutation in this region of the protein lead to specific loss or gain of function, which would promote preferentially (but not exclusively) adrenocortical carcinoma. However, additional studies on a larger number of cases would be necessary to verify this finding. The excess of brain tumor in individuals with group 1 mutations is interesting, because the main hotspot codon for sporadic brain tumors is at codon 273, corresponding to a group 2 mutation. Group 1 and 2 mutations correspond to the two functional segments of the DNA-binding domain of the p53 protein (see Fig. 4). Overall, these results indicate that alteration of direct protein-DNA contacts is important in the pathogenesis of brain tumors and that the consequence of this alteration may differ, depending which functional segment is affected.

The analysis performed here has clinical implications for the management of families with germ-line *TP53* mutations. The finding that breast cancers occur 8 years earlier in LFS families with *TP53* mutations than in those families without mutations argues in favor of an earlier clinical surveillance program for such families, starting in the early twenties. The precise screening schedule is controversial, but many centers would advocate annual clinical examination. Magnetic imaging of the breast is being investigated for this purpose in clinical trials (39). For adrenocortical tumors, some centers advocate annual abdominal ultrasonography in children. Our findings suggest that such a surveillance program would be particularly important in families with structural group 3 *TP53* mutations. Additional exploitation of molecular *TP53* data for clinical purposes awaits a global approach to integrate structural biology, functional assessment of mutants, and the correlation between mutation and the clinical and pathological parameters of the resulting cancers.

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