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REVIEW ARTICLE

Li–Fraumeni syndrome heterogeneity

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Abstract
Clinical variability is commonly seen in Li–Fraumeni syndrome. Phenotypic heterogeneity is present among different families affected by the same pathogenic variant in *TP53* gene and among members of the same family. However, causes of this huge clinical spectrum have not been studied in depth. *TP53* type mutation, polymorphic variants in *TP53* gene or in *TP53*-related genes, copy number variations in particular regions, and/or epigenetic deregulation of *TP53* expression might be responsible for clinical heterogeneity. In this review, recent advances in the understanding of genetic and epigenetic aspects influencing Li–Fraumeni phenotype are discussed.

Keywords Li–Fraumeni syndrome · Genotype · Phenotype · Epigenome · Pediatrics

Abbreviations

LFS	Li–Fraumeni syndrome
LFL	Li–Fraumeni-like
NGS	Next-generation sequencing
CNV	Copy number variations
WGS	Whole-genome sequencing

Li–Fraumeni syndrome

Li–Fraumeni syndrome (LFS) is a rare predisposing cancer disease transmitted by autosomal dominant inheritance. The variable clinical expressions of this syndrome are an extreme challenge for individualized surveillance [1]. This particular syndrome was described for the first time by Li and Fraumeni in 1969 [2]. Li–Fraumeni disorder predisposes to malignant tumors development. These tumors can appear throughout the life of the patient. Cancer types observed in LFS patients include: soft tissue sarcomas [3, 4], osteosarcoma [5, 6], breast cancer [7, 8], brain tumors, leukemia [9, 10] and adrenocortical carcinoma [11] (#151623 OMIM). However, aggressiveness and the number of tumors vary to a great extending among different patients.

Cumulative incidence for development of at least one tumor at 30 years old is estimated to be 50%, while it is near 100% at 70 years old [12]. Cancer risk at early ages is higher in women due to breast cancer risk. Cumulative incidence in women at 70 years old is 54% for breast cancer, 15% for soft tissue sarcomas, 6% for brain tumors, and 5% for osteosarcoma. For male patients, however, the reported figures are 22%, 19%, and 11% for soft tissue sarcomas, brain tumors, and osteosarcoma, respectively. Fifty percent of patients with a malignant tumor developed a second tumor over the next 10 years [12]. Several patients with many malignant primary tumors have been described in the literature [13].

Approximately 70% of families affected by classical tumors carry germinal mutations in *TP53*. However, 40% of patients with Li–Fraumeni-like (LFL) phenotype (families with other malignant tumors, different from classical tumors) carry *TP53* deleterious mutations. *TP53* mutations, associated to LFS or LFL, are mainly located in the DNA-binding domain. Only few cases harbor *TP53* mutations outside this hotspot location [14, 15].

Pathogenic *TP53* variants do not explain all phenotypic manifestations. Mutations within the cell cycle checkpoint gene *CHEK2* have also been reported in some LFS or LFL families without detectable *TP53* mutations [16–20]. However, there are still relatively few reports of such mutations. Despite the fact that *CHEK2* is no longer considered as a major determinant of LFS, a number of studies support the hypothesis that *CHEK2* gene may act as a factor contributing

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to individual tumor development in families with LFL tumors. In addition to *CHEK2*, mutations in *POT1* (protection of telomeres 1) have also been associated with the risk of developing several tumor types and have been detected in LFL families [21, 22]. *POT1* encodes a nuclear protein that is essential for telomere maintenance. A higher telomeric fragility has been demonstrated in patients affected by pathogenic *POT1* variants [16].

There are still a significant number of LFS/LFL families for whom no underlying genetic determinant has been identified. For this reason, many authors have studied the influence of *BAX* [23], *CDKN1A/p21* (cell cycle arrest mediator) [24], *PTEN* (associated to *PTEN* hamartoma syndrome) [25], *PRDM* and *GAS8* [26] in LFS families without detectable *TP53* mutations. However, none of them has been identified as determinant of LFS.

Advances in next-generation sequencing (NGS) technologies, have allowed the identification of *TP53* pathogenic variants in patients with malignant tumors and without clinical suspicion of LFS. Therefore, tumor development predisposition in those cases seems to be related to these particular variants [27–29]. Consequently, new bioinformatics tools (not clinical data alone) have been suggested to detect suitable patients for genetic studies [30].

So far, LFS and LFL cases have been commonly classified based on clinical descriptions. New strategies, focused not only on clinical data, but also on molecular alterations, would be more suitable for LFS and LFL classification. Following this idea, nomenclature should also be adapted, and therefore, "*TP53* Cancer Predisposition Syndrome" and "*CHEK2* Cancer Predisposition Syndrome" could be new nomenclatures. All patients with one or more malignant tumors that are clearly related to either of the pathogenic variants (*TP53* or *CHEK2*), might be affected by one of these two proposed entities ("*TP53* Cancer Predisposition Syndrome" or "*CHEK2* Cancer Predisposition Syndrome"), respectively. Sub-classifications could be also possible, but the molecular basis (germline *TP53* or *CHEK2* pathogenic variant) should be the start point to correctly classifying patients in syndromic entities (based on present knowledge). Li–Fraumeni syndrome might be an exclusion diagnosis, when *TP53* or *CHEK2* alterations were not founded and the family or personal story suggests the LF or LFL syndrome.

Li–Fraumeni syndrome dependent on pathogenic variants in *TP53*

Tumors more frequently associated to *TP53* germline mutations are: soft tissue sarcomas, osteosarcoma, breast cancer, brain tumors, leukemia and adrenocortical carcinoma. However, many other different tumor types have also been described: phyllodes tumor, choroid plexus tumors, and melanoma. Additionally, more infrequent tumor types included: lung, digestive tract, thyroid tumors, ovary, colon, lymphoma, and childhood malignant meningioma [31–44].

Up to now, causes of phenotypic differences among families affected by different predisposing mutations to LFS are poorly understood. Furthermore, the potential causes of phenotypic differences among members of the same family are not known. Factors influencing those phenotypic differences will be reviewed below.

TP53 gene

TP53 encodes a tumor suppressor protein which in response to oncogenic mutations or DNA damage triggers a transcriptional program to regulate DNA repair mechanisms, cell cycle progression and apoptosis [45, 46]. TP53 is essential for regulating cell division and preventing tumor formation [47–50]. It also plays a key role in aging [51, 52], cellular metabolism [53, 54], regulation of homeostasis [55] and immune function [46, 56, 57].

Tetramer formation of p53 is essential for its tumor suppressor function. This oligomerization is modulated by the protein concentration of p53, post-translational modifications, and/or interactions with its binding proteins [58]. The active protein conformation induces cell cycle arrest, senescence, and apoptosis through transcriptional regulation of some target genes or non-transcriptional pathways [59]. It is accepted that p53-dependent transcriptional activation occurs by binding to a consensus DNA sequence called the p53 response element in target genes promoters. Fischer M et al. meta-analysis concluded that p53 is not a direct repressor of transcription, but solely activates its target genes [60]. Therefore, p53 acts mainly as conductor conditioning the transcription of several genes: *p21*, *MDM2*, *GADD45*, *BAX*, *XPC, XPE* and *14-3-3*σ [61]. This well-scored transcriptional program performs many of the described TP53/p53 tumor suppressor functions.

Somatic mutations in this central gene are frequently observed in human cancers [62–64] and the knowledge about *TP53*/p53 in tumors has been useful to understand the phenotypic differences in patients with Li–Fraumeni syndrome.

Mutated TP53 gene

TP53 is mutated in more than 50% of human cancers, and disrupted in practically the rest of them [65]. Approximately 80% of *TP53* mutations are single point mutations (the majority of *TP53* well accepted alterations are missense mutations). Moreover, the gene has hotspot mutations [66], in fact, its central domain (nucleotides 102-292) alone accounts for 90% of the changes [66]. Tumor suppressor gene inactivation does not follow the Knudson model for

TP53 (this model implies the inactivation sequence of the two alleles). The p53 protein is especially inactivated by "dominance negative" effect of pathogenic missense variants. The mutated p53 monomers bind and inactivate wild-type p53 monomers. Beside the loss of function (common to all pathogenic *TP53* variants) and the dominant-negative effect on the wild-type p53 activity of pathogenic missense variants, the mutant p53 could also acquire new oncogenic functions, the so-called "gain-of-functions". [67]. Therefore, some missense *TP53* mutations (R282, R175, Y220, R248 and R273) might not only alter the protein function by disrupting the DNA-binding capacity [31], but also can favor a greater oncogenic activity [68]. As a consequence, and speaking about Li–Fraumeni patients, a more aggressive phenotype associated to some pathogenic missense variants (gain-of-function variants) has been observed in large patient cohorts [69, 70]. In this regard, Amadou et al. in a review of 1730 patients found an earlier age of tumor onset in patients with missense mutations (21.3 years), compared to those with all types of loss of function mutations (28.5 years) or genomic rearrangements (35.8 years). Notably, most of children with LFS in this study carried missense mutations [71].

Tumors with missense *TP53* mutations occur earlier in life and are frequently associated to specific histological subtypes [72]. Ognjanovic et al. described that globally, pathogenic missense mutations in exons encoding the DNA-binding domain, were more frequently observed in patients with rhabdomyosarcoma and osteosarcoma while loss of function mutations were more frequent in patients with leiomyosarcoma [72]. In addition, not only the type of variant, but also, the location of the variant may cause certain types of tumors to be more frequent than others [73]. Olivier et al. described that brain tumors were associated with missense *TP53* mutations located in the DNA-binding loop that contact the minor groove of DNA, whereas adrenal gland carcinomas were associated with missense mutations located in the loops opposing the protein–DNA contact surface [73]. The greatest compilation of information regarding the genotype–phenotype relationship is found in the IARC (International Agency for Research on Cancer) *TP53* Database.

The type and location of *TP53* variants may condition a different biological activity of the protein, and facilitate the development of certain tumor types. However, despite having the same genetic alteration in *TP53*, there are significant differences among families, which cannot be explained by the type of mutation.

Polymorphic variants of TP53

The presence of certain polymorphisms within *TP53* sequence may determine LFS clinical presentation since these polymorphisms may modify the oncogenic activity of the p53 protein. A novel p.Gly360Val *TP53* variant (in a linker region near the tetramerization domain) is known to be responsible for a phenomenon called *enhanced transactivation*: transcriptional activation of *TP53* target genes conditions the up-regulation of several p53 response elements and, as a result, the final function of p53 in the cell is modified [74]. The effects of this variant in cancer phenotype among families and members of the same family remain unknown. Otherwise, it was postulated that *TP53* PIN3 polymorphic variant (hg19 chr17: 7579690; a 16 bp duplication in intron 3) may contribute to the phenotypic diversity of germline *TP53* mutations associated with LFS/LFL patients. [75]. Indeed, Marcel et al. reported that the heterozygous *TP53* PIN3 variant supposed a difference of 19.0 years in the mean age at the first diagnosis in *TP53* mutation carriers. The polymorphic variant delayed the appearance of the first tumor [75]. Sagne et al. also observed that cancer tended to occur approximately 15 years later in mutation carriers who also carried the polymorphic variant *TP53* PIN3 [76]. Another example is the p.Pro72Arg allele of *TP53*; Bougeard et al. described that the mean age of tumor onset in Arg allele carriers (21.8 years) was significantly different from the mean age of tumor onset from those with Pro/Pro (34.4 years) [77]. Marcel et al. reported anticipation of 8.3 years when Arg allele was present [75] (Fig. 1). These polymorphic variants could explain the diversity of tumor patterns among members of the same family.

Polymorphic variants in MDM2 gene

Murine double minute 2 (*MDM2*) plays an important role in *TP53* regulation. *MDM2* encodes an E3 ubiquitin-protein ligase that mediates ubiquitination of p53/TP53, leading to its degradation by the proteasome. This gene is itself transcriptionally regulated by p53. Therefore, the encoded protein can promote tumor formation by targeting p53, if it does not function well. In fact, overexpression or amplification of this locus is detected in a variety of different cancers (Fig. 2). It has been proposed that certain polymorphic variants of *MDM2* can condition its function and, therefore, could explain clinical differences among families or members of the same family with LFS. The most outstanding example is *MDM2* SNP309 (hg19 chr12: 69202580; T–>G variation), which has been described as a modifier of tumor phenotype. This particular polymorphism increases the expression of *MDM2* and, as a consequence an attenuation of the p53 pathway is detected [77–80]. Bougeard et al. reported an accelerated phenotype among *MDM2* SNP309 G allele carriers. The mean age of tumor onset in *MDM2* SNP309 G allele carriers (19.6 years) was significantly different from that observed in patients homozygous for the T allele (29.9 years, $p < 0.05$). Their data also supported an amplified effect on the age of tumor onset by the TP53 p. Pro72Arg allele [77]. Ruijs et al. published that among

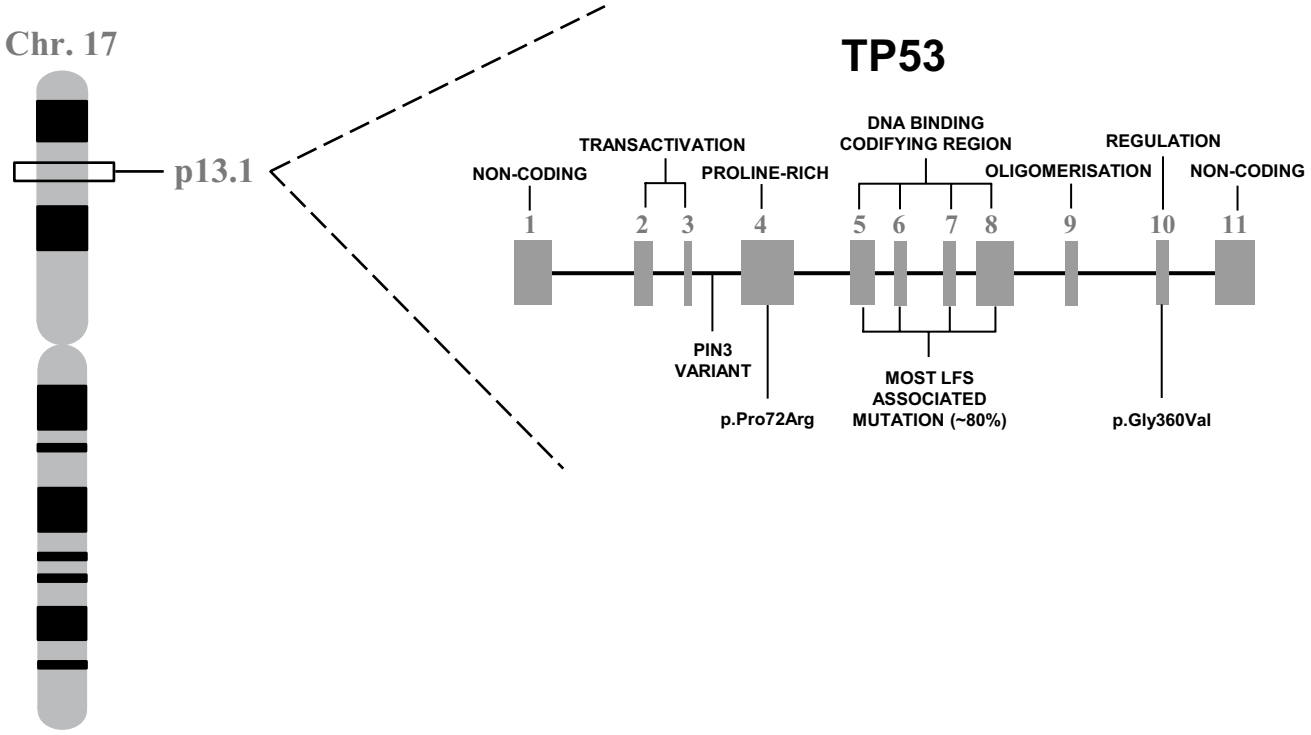

Fig. 1 *TP53* gene is located in 17p13.1 and it is organized in 11 well-defined exons. Codifying protein regions are referred over every numbered exon. Polymorphic *TP53* variants that could explain Li–Fraumeni heterogeneity are p.Gly360Val, p. Pro72Arg and TP53 PIN3 polymorphic variant

the *TP53* germline mutation carriers, a significant difference was seen in the mean age of tumor onset for the SNP309 G allele group, that is, 29.7 years as compared to the SNP309 homozygous T group 45.5 years ($P = 0.005$) [78]. In the same way, Macedo et al. studied the median age at first diagnosis among Li–Fraumeni patients carrying *TP53* R337H mutation. The median age at first diagnosis was earlier in *MDM2* SNP309 GG carriers when compared to other genotypes for both tumors analyzed in their study (adrenocortical carcinoma and breast cancer); however, they did not demonstrate a statistically significant difference [79]. Renaux-Petel et al. published results concordant with these, and also reported other interesting data about *MDM2* 285G and 309G polymorphism interactions. They reported that the *MDM2* 285-309 G–G is a higher risk haplotype in patients with germline TP53 mutations and, therefore, suggesting that the *MDM2* 309G variation is deleterious when its effect is not neutralized by the 285C variation [80].

Unfortunately, not enough information is available in concrete populations to translate to Li–Fraumeni patients polymorphic data with prognosis implications. Nowadays, physicians could not personalize surveillance programs based on polymorphic data. Nevertheless, we consider mandatory to study *TP53* PIN3, *TP53* p. Pro72Arg and *MDM2* SNP309 for all Li–Fraumeni patients. The study of at least these three TP53 polymorphisms (mainly *MDM2*) is the only way to assess their impact on individual and familial diversity of tumor patterns. To do so, national and international contributions integrating all this information joined to *TP53* mutation type and clinical data is the way to follow.

microRNA regulation pattern

It is known that certain microRNAs are members of *TP53* transcriptional program. It has been proposed that miR-605 (regulator of loop p53-MDM2) could affect the tumor phenotype in LFS [81]. When cellular stress is present, p53 escapes the p53:Mdm2 negative feedback to accumulate rapidly and to induce cell cycle arrest and apoptosis. Xiao et al. demonstrated that miR-605 is transcriptionally activated by p53 and post-transcriptionally represses Mdm2. The activation of p53 upregulates miR-605 expression, via interacting with the promoter region of the gene [81]. Based on the knowledge about p53-miR-605-MDM2 interactions, polymorphic variants in *miR-605* gene and their role in Li–Fraumeni phenotype were studied. Indeed, the variant G allele of *miR-605* (Hg 19 chr10: 53059406) was proposed by Id Said B and Malkin D as modifier of the LFS phenotype. They described a 10-year acceleration in the mean age of LFS tumor onset when miR-605 (Hg 19 chr10: 53059406) is present, supporting their hypothesis [82].

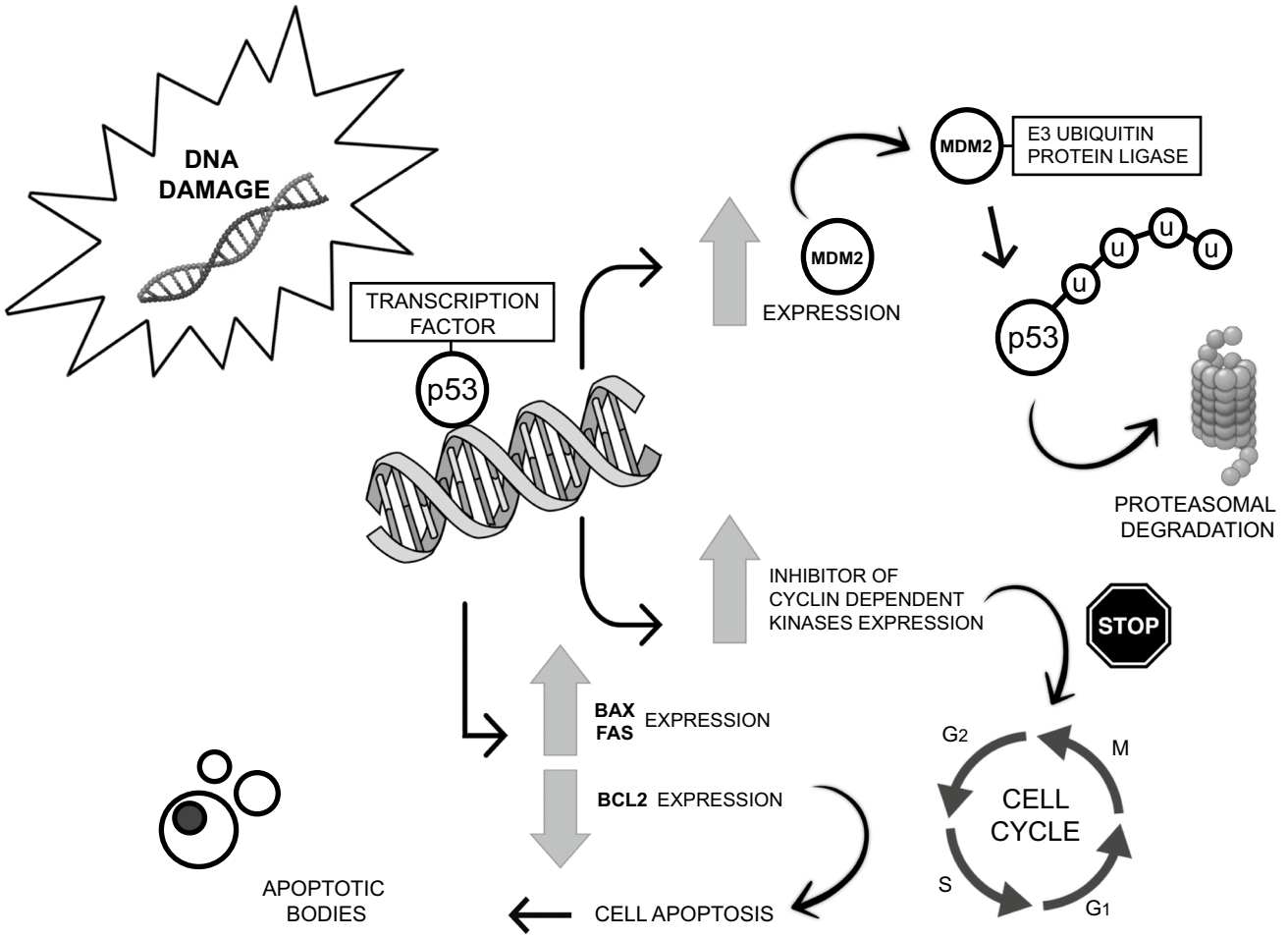

Fig. 2 DNA damage drives p53 activation. Protein p53 develops transcription factor functions that condition cell cycle stop and apoptosis activation and also stimulates *MDM2* transcription. Murine double minute 2 (MDM2 protein) plays an important role in p53/*TP53* regulation. *MDM2* encodes an E3 ubiquitin-protein ligase that mediates ubiquitination of p53, leading to its degradation by the proteasome

Moreover, miR-34A is a key component of the p53 regulatory network. It was shown that p53 regulates the expression of miR-34A, representing an important mechanism of p53 signaling. Members of the miR-34 family were proposed as the most prevalent p53-induced miRNAs and are frequently silenced in variety of tumor entities, suggesting that they are important tumor suppressors [83]. Accordingly, miR-34A is inactivated by hypermethylation across many histologic types of primary tumors from patients with LFS. Malkin D group, described that loss of function *TP53* mutations were significantly associated with hypermethylation at the locus encoding miR-34A ($P < 0.001$) in germline, and this observation was validated in an independent patient cohort ($P < 0.001$) [84]. At tumoral level, miR-34A hypermethylation was associated with decreased overall survival in a cohort of 29 patients with choroid plexus carcinomas ($P < 0.05$) [84].

In conclusion, the systematic study of polymorphic variants in *TP53* and *MDM2* genes could enrich Li–Fraumeni knowledge, as commented above. In the same way, the polymorphism *miR-605* (Hg 19 chr10: 53059406 G allele) and the methylation pattern at the locus encoding miR-34A should be mandatory when a Li–Fraumeni patient carrying a *TP53* mutation is diagnosed. We will be able to enhance our comprehension of this entity sharing this information internationally.

Copy number variations

Copy number variations (CNV) among Li–Fraumeni patients carrying *TP53* mutations are understudied. *TP53* dysfunction causes an increased number of copy number variations due to tumor instability [85–87]. Shlien et al. published that LFS *TP53* mutation carriers present an increased CNV both in tumors and germline [88]. They studied a cohort of 53 individuals from Li–Fraumeni families, 33 were *TP53* mutation carriers and 20 harbored wild-type *TP53* (controls). Controls displayed a median of 2 CNVs per

genome in germline. However, the *TP53* mutation carriers displayed a significant increase in CNVs (a mean of 12.19 CNVs) ($p=0.01$). They also suggested a dose–response relationship between CNV frequency and severity of the LFS phenotype. Interestingly, they showed even greater number of CNVs among those TP53 carriers affected by cancer, than those which have not developed cancer yet. Moreover, they found two genes involved in recurrent duplications among LFS families: *MLLT4* and *ADAM12*. They proposed that CNV frequency, or another high-resolution measure of instability, may help to define the nature and severity of the germline *TP53* mutations found in LFS families [88]. This hypothesis was tested by Ariffin et al. in a family with clinical data of anticipation. They analyzed CNV exceeding 10 kb in size. They concluded that CNV composition did not show significant variation among family members, despite their differences in *TP53* mutation carriage and in cancer status [89]. Furthermore, Silva et al. did not find any difference in the total number of germline CNV present in LFS patients versus controls. However, they noted a highly significant increase (> fivefold) in the rare CNVs (estimated based both on DGV and db Var) in *TP53* DNA-binding domain mutation carriers as compared both to controls and to p.R337H carriers. They proposed that different microarray technologies used by Shlien et al. could be the origin of their hopeful results [90].

Total number of germline CNVs cannot be used to stratify risk assessment for Li–Fraumeni patients based on present knowledge. Nevertheless, deletions or duplications in concrete genome regions could explain some phenotypic differences among families or members of the same family. Larger cohort and homogeneous populations of Li–Fraumeni patients sharing *TP53* mutation should be studied in this way to obtain conclusive results.

Telomeric length variations

The influence of telomere length in final phenotypic differences has been studied among the carriers of germline mutations in *TP53*. Human telomeres are nucleoprotein complexes at chromosome ends, consisting of TTAGGG repeats and associated telomere-binding proteins. In germ cells, telomeres range from 10 to 15 kb in length. Telomeres protect chromosomes from nuclease degradation and chromosome rearrangements and serve as mitotic clocks that monitor the number of cell divisions. A possible link between p53, telomeres, tumor initiation, and anticipation in LFS, has been suggested [91–93]. Based on this hypothesis, Trkova et al. published that the telomere length in peripheral blood cells was shorter among *TP53* mutation carriers than in general population. They did not find progressive telomere shortening among Li–Fraumeni generations. However, they observed a trend (not statistically significant) of earlier onset of cancer in individuals with shorter telomeres and vice versa [94]. Tabori et al. published that telomere length was significantly shorter in affected than in non-affected *TP53* mutation carriers. They concluded that telomere length could explain earlier age of onset of tumors in successive generations of the same family with identical TP53/MDM2-SNP309 genotypes [95]. Not enough information is available in this way to reach to conclusions and to take clinical decisions. More in-depth studies are needed.

Oxidative stress cell level

So far, there is just one published study in the literature that compares levels of oxidative stress between *TP53* carriers and controls. Macedo et al. reported an increase in cellular oxidative stress among patients with the p53R *TP53* variant (p.Arg337His). Specifically, an increase in erythrocyte GPx activity and carbonyl levels in plasma (indicator of protein oxidative damage) in mutation carriers compared to non-carriers. In addition, a significant increase in malondialdehyde levels (indicative of increased lipid peroxidation) has been demonstrated in *TP53* p.Arg337His mutation carriers. Thus, the cellular oxidative damage level could also partially explain the different phenotype among LFS families and members of the same LFS family. To the best of our knowledge, this phenomenon has not been studied in large patient cohorts [96].

Epigenetic regulation of TP53 expression

The *TP53* promoter is highly regulated. Different mechanisms participate in a delicate control. A direct binding of several transcription factors in *TP53* promoter is well described. Saldaña-Meyer et al. reviewed the *TP53* epigenetic regulation extensively [97]. *TP53* human promoter has several conserved transcription factor binding motifs.

Different transcription factors bind *TP53* promoter and upregulate its expression. They are Myc/Max, USF, AP-1, ETS2, NFκB, RREB-1, ETS2, YY, NF, HOXA5, p53/p73, pituitary homeobox 1 (hPitx1) and ISGF3 (formed by Stat1, Stat2 and IRF-9). Moreover, kinase C δ (PKCδ) although does not bind the *TP53* promoter, promotes *TP53* transactivation. Nevertheless, Pax and BCL6 transcription factors inhibit the *TP53* promoter. ETS1 also binds on the human *TP53* promoter, but its effects are not well described [97]. A particular transcription factor is E2F1 which binds *TP53* promoter and has a direct role in the induction of mutant p53 [97].

Furthermore, *TP53* human promoter has a CTCF binding site downstream of a CpG island. CTCF influences transcriptional regulation of TP53. In fact, when knocking-down CTCF, the human *TP53* gene loses its expression supporting

its relevant contribution to *TP53* expression regulation [97, 98].

Otherwise, the *TP53* gene promoter regulation by DNA methylation remains controversial. Present knowledge points to the lack of methylation over the *TP53* core promoter. Therefore, other mechanisms might be involved (methylation of genomic regions different from promoters) [97].

Finally, microRNAs can negatively regulate *TP53* gene expression and if deregulated can promote cancer. The best known examples are: miRNA-125a and miRNA-125b which represses p53 post-transcriptionally. MicroRNA-504, microRNA-25, miRNA-30d and LincRNA-p21 interfere as well with p53 functions [97]. The anti-sense RNA Wrap53 is necessary for the proper transcription of *TP53* [97].

TP53 is regulated by multiple transcription factors and microRNAs, which are epigenetically regulated. Thus, a certain pattern of epigenetic regulation of all these regulatory genes could condition a wild-type and mutated p53 cellular level, variable from one individual to another, which might explain phenotypic differences among members of the same Li–Fraumeni family. No studies were developed either studying plasma levels of these regulatory elements or methylation pattern of their codifying genes among Li–Fraumeni patients. It could be a way to explore in the future.

Epigenetic regulation of genes regulated by TP53

Genetic and epigenetic alterations may be involved in the phenotypic variability of LFS. p53 regulates several pathways, including the thymine DNA glycosylase (TDG) pathway, which regulates the DNA methylation of several genes. Fortes et al. compared the DNA methylation pattern of genes related to the TDG pathway among germline *TP53* mutations carriers, patients with wild-type *TP53*, and healthy individuals. Finally, no significant differences were found. However, increased TDG expression was detected in patients with p.R337H *TP53* mutation affected by adrenocortical carcinoma. Further studies in larger patient cohorts are necessary to evaluate the clinical impact of epigenetic alterations on genes potentially involved in LFS variability [99].

Other elements to consider

The presence of mutations in certain RecQ DNA helicases (like BLM (Bloom syndrome (BS) protein) and WRN (Werner syndrome protein)) would affect *TP53* function. The Harris CC group suggests that p53 mediates the cooperation of p53 and BLM to induce apoptosis. Therefore, certain variants in these genes might affect, at least partially, the function of *TP53* [100].

The elements that might condition the tumor phenotype in LFS are detailed in Table 1.

Environmental components

Phenotypic differences are detected among Li–Fraumeni patients from different geographical origins. Environment could affect tumor development among *TP53* carriers, therefore, life style, diet and environmental exposures joined to all above said, probably condition the final phenotype. An environmental component may be responsible for the differences observed among families from different origins that share *TP53* mutation [89]. None large cohort studying its influence has been published. Moreover,

Table 1 Elements that may condition phenotypic differences, among patients carrying the same *TP53* variant

	Regulatory element	References
Genetics		
Polymorphic variants in *TP53* gene	*TP53* p.G360V *TP53* PIN3 *TP53* p.Pro72Arg	Id Said et al. [74] Marcel et al. [75] Bougeard et al. [77]
Polymorphic variants in *MDM2* gene	*MDM2* SNP309 G allele	Bougeard et al. [77]
Polymorphic variants in microRNAs	microRNA 605 (rs2043556 GG) variant	Id Said et al. [82]
Genomics		
Copy number variations (CNVs)	Presence of rare CNVs	Silva et al. [90]
Telomeric length	Telomeric length shortening	Tabori et al. [95]
Epigenomics		
TP53 transcriptional and post-transcriptional regulation	Diversity among individuals in regulation pattern	Saldaña-Meyer et al. [97]
microRNA-34	miR-34A methylation pattern	Samuel et al. [84]
Metabolomics		
Oxidative stress cell level	Protein oxidative damage level Lipid oxidative damage level	Macedo et al. [96]

founding mutations are very common in certain regions and exceptional in others, and this makes comparative studies difficult.

Anticipation?

A decrease in the age at cancer onset and an increase in more LFS-specific cancers in successive generations have been suggested [101, 102]. The genetic mechanisms proposed to explain this heterogeneity include accumulation of copy number variations (CNVs) with successive generations, and progressive telomere shortening [88]. Ariffin et al. studied a dataset of 269 pedigrees of *TP53* germline mutation carriers. Although, they reported a decrease in age at first cancer onset in multigeneration pedigrees, their observations did not fit with a classical model of anticipation. Nevertheless, only pedigrees with three or four generations showed a delayed age at first cancer onset in the older generations of *TP53* mutation carriers. Then, they suggested that the founder patient of such pedigrees may carry, in addition to germline *TP53* mutation, rare independent genetic modifiers that attenuate the risk of early cancer. These genetic variants might allow cancer-free survival until postreproduction age of founders. Based on these observations, they proposed the term "genetic regression" instead of anticipation [103]. To understand this phenomenon, they looked for CNVs larger than 10 kb and for telomere length shortening among kindred affected by LFS-specific cancer, but did not discover significant differences. Moreover, they did not find neither more frequent *MDM2*-SNP309 G allele nor *TP53* PIN3 among affected children compared with their previous generations [103]. Otherwise, this group used whole-genome sequencing (WGS) analysis among family members and identified interesting rare single-nucleotide variants (SNVs). A curious example was a father (non-carrier TP53) who transmitted a rare SNV to two out of four *TP53* mutation carrier children. Children with *TP53* mutation and the rare SNV developed an early cancer but not the two *TP53* mutation carrier children who not carried that rare SNV. Such rare SNV may be considered as candidate-modifier genes that may modulate age at cancer onset. Deeper studies looking for these variants could be important [103]. In fact, Franceschi et al. reported recently an affected child who inherited the *TP53* mutation from his affected mother (breast cancer in adulthood age) and received from their non-affected father 25 predicted deleterious variants including a nonsense mutation in *ERCC3*. They proposed that those inherited mutations are possible candidate modifiers linked to *TP53* [104]. Undiscovered genetic variants could determine also Li–Fraumeni heterogeneity among members of the same family.

Conclusions

It is very difficult to elucidate the genotype–phenotype relationship in LFS. Based on the evidence described in the present review, not only would the genotype condition phenotypic peculiarities, but also the epigenome seems to play a key role, although to date, studies in this field are scarce.

The current knowledge of LFS makes it difficult to state individual recommendations adapted to the risk at all levels of clinical care (genetic counseling in assisted reproductive treatments, pediatric or medical oncology). Therefore, it is urgent to increase the understanding of this devastating entity. The systematic and coordinated study of all the elements involved in LFS is the only way to move forward.

Compliance with ethical standards

Conflict of interest The authors declare to have no conflict of interest.

Ethical approval This work is not a research involving human participants and/or animals.

Informed consent Not applicable.

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