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## The contribution of *CHEK2* to the *TP53*-negative Li-Fraumeni phenotype

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### Abstract

**Background:** *CHEK2* has previously been excluded as a major cause of Li-Fraumeni syndrome (LFS). One particular *CHEK2* germline mutation, c.1100delC, has been shown to be associated with elevated breast cancer risk. The prevalence of *CHEK2*\*1100delC differs between populations and has been found to be relatively high in the Netherlands. The question remains nevertheless whether *CHEK2* germline mutations contribute to the Li-Fraumeni phenotype.

**Methods:** We have screened 65 Dutch *TP53*-negative LFS/LFL candidate patients for *CHEK2* germline mutations to determine their contribution to the LFS/LFL phenotype.

**Results:** We identified six index patients with a *CHEK2* sequence variant, four with the c.1100delC variant and two sequence variants of unknown significance, p.Phe328Ser and c.1096-?\_1629+?del.

**Conclusion:** Our data show that *CHEK2* is not a major LFS susceptibility gene in the Dutch population. However, *CHEK2* might be a factor contributing to individual tumour development in *TP53*-negative cancer-prone families.

### Background

Li-Fraumeni syndrome (LFS) is a rare autosomal dominant cancer syndrome predisposing for bone and soft tissue sarcoma, breast cancer, brain tumour, adrenocortical carcinoma and leukaemia [1]. The classical LFS criteria are: a proband with sarcoma aged under 45 years and a first-degree relative with any cancer aged under 45 years,

plus a first or second-degree relative in the same lineage with any cancer under the age of 45 years or sarcoma at any age [2]. In addition, Li-Fraumeni-like syndrome (LFL) criteria have been formulated as a proband with any childhood tumour or a sarcoma, brain tumour or adrenocortical tumour diagnosed under 45 years of age and a first or second-degree relative in the same lineage with a typical

LFS tumour at any age, plus a first or second-degree relative in the same lineage younger than 60 years with any cancer [3]. Less stringent LFL criteria were formulated by Eeles et al. as two first or second-degree relatives with typical LFS-extended tumours (classical LFS tumours plus melanoma, prostate cancer and pancreatic cancer) at any age [4]. The Chompret criteria for *TP53* germline mutation testing have been updated in 2008 as: (1) a proband with a tumour belonging to the LFS tumour spectrum (sarcomas, brain tumours, pre-menopausal breast cancer, adrenocortical carcinoma, leukaemia, lung bronchoalveolar cancer) cancer before 46 years of age and at least one first or second-degree relative with an LFS tumour before 56 years of age or multiple tumours; or (2) a proband with multiple tumours two of which belong to the narrow LFS tumour spectrum and the first of which occurred before 46 years of age; or (3) a patient with adrenocortical carcinoma or a patient with breast cancer before 36 years of age without *BRCA* mutation, irrespective of the family history [5].

In 1990 germline mutations in the *TP53* gene were described in LFS [6]. So far, 419 *TP53*-positive families have been reported (IARC mutation database, R13, November 2008 [7]). At present, in approximately 75% of LFS and 40% of LFL families, a germline *TP53* mutation can be detected [8]; i.e. 25% to 60% of LFS/LFL families do not carry a detectable germline *TP53* mutation, implying the existence of alternative LFS susceptibility genes.

*CHEK2* is a cell cycle checkpoint kinase involved in DNA repair, cell death and cell cycle control by stabilizing the p53 protein [9]. In 1999 Bell et al. first described the possible association of the *CHEK2* gene with Li-Fraumeni syndrome [10]. Subsequent studies have addressed the possible contribution of *CHEK2* germline mutations to LFS and LFL syndrome, but could not confirm *CHEK2* as a major gene involved in LFS [10-18].

In other studies, the specific *CHEK2* c.1100delC frameshift mutation was shown to be associated with an elevated breast cancer risk [19-22] and it has been suggested that it contributes to a hereditary breast and colorectal cancer phenotype [23]. The prevalence of this c.1100delC mutation seems to differ according to ethnic backgrounds and populations and is relatively high in the Netherlands [19,24]. We have investigated the *CHEK2* gene mutation status of 65 index patients from 65 Dutch LFS/LFL families and families suggestive of LFS who had tested negative for *TP53* germline mutations, to determine the contribution of *CHEK2* germline mutations to the phenotype in those families.

## Methods

All 65 affected index patients had been assessed and counselled in various clinical genetics centres because of the occurrence of different cancer types related to LFS and had as a consequence been tested for *TP53* germline mutations. On the basis of the available clinical data, the *TP53*-negative families were classified into 3 groups: 1) classical LFS [2], 2) LFL syndrome according to Birch [3] or Eeles [4] and 3) LFS-suggestive, including childhood onset (under 18 years) sarcoma or brain tumours, two or more primary tumours at any age, two first-degree relatives with a tumour at any age, of which at least one relative has a typical LFS tumour or breast cancer under 30 years of age (without *BRCA1* or *BRCA2* mutations) (Table 1). In families with multiple breast cancer cases and individuals with breast and ovarian cancer, *BRCA1* or *BRCA2* mutations were excluded, according to standard procedures. Details are available on request.

DNA from peripheral blood lymphocytes was isolated according to standard procedures. Screening for *TP53* germline mutations was performed by sequence analysis of all coding exons (2-11) including flanking intron-exon boundaries (details are available on request) and multiplex ligation-dependent probe amplification (MLPA) [25] (*TP53* MLPA KIT, MRC Holland). In 34 *TP53*-negative LFS, LFL, or LFS-suggestive families all exons and flanking intron-exon boundaries of the *CHEK2* gene were investigated using denaturing gradient gel electrophoresis (DGGE, see Table 1) [26]. All possible candidate variants, identified as aberrant DGGE fragments, were confirmed by sequence analysis. To avoid amplification of pseudogenes, a long range PCR was performed first for exons 10 to 14, followed by a nested PCR. Data on exons 1-10 were obtained for all patients, on exons 11-14 for 29 of the 34 individuals. All 65 *TP53*-negative individuals were screened for the c.1100delC *CHEK2* mutation and *CHEK2* DNA rearrangements by multiplex ligation-dependent probe amplification (MLPA, see Table 1). Details are available on request (*CHEK2* MLPA KIT, MRC Holland). Mutation analysis was performed using the following reference sequence: *CHEK2* (AF086904.1, GI:3982839, <http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&id=3982839>).

Sequence variants were weighted according to their potential pathogenicity. Three silent sequence variants were seen and not further analysed: c.252A>G, p.Glu84Glu in exon 1, a previously reported silent polymorphism [15], found once, c.1566C>T, p.Pro522Pro and c.1608A>G, p.Pro536Pro, both in exon 14, found in five and seven families, respectively. For these three variants two splice site prediction programs were used, NetGene2 Server <http://www.cbs.dtu.dk/services/NetGene2/> and BDGP Splice Site Prediction/Neural Network <http://www.fruit>

**Table 1: Number of TP53 negative families available for CHEK2 gene analysis divided into 3 groups: LFS, LFL, or LFS-suggestive family history (n = 65), including the cancer type in tested individuals.**

(family) history	Complete CHEK2 mutation analysis (n = 34)	1100delC mutation analysis and DNA rearrangements (n = 31)	Cancer type in tested individuals: B/S/other
1 LFS	1	0	1/0/0
2 LFL	20	15	18/7/10
3 LFS-suggestive	13	16	17/5/7
-childhood onset sarcoma or brain tumour	1	1	0/1/1
-at least 2 primary tumours	3	7	5/1/4
-2 first degree relatives with cancer	5	6	6/3/2
-breast cancer before 30 years	4	2	6/0/0

LFS = Li-Fraumeni syndrome

LFL = Li-Fraumeni-like syndrome according to Birch or Eeles

B = breast cancer

S = sarcoma

Other = other cancers, including adrenal cortical tumour, bladder cancer, brain tumour, colon cancer, kidney cancer, leukaemia, lung cancer, melanoma, non-Hodgkin lymphoma, ovarian cancer and thyroid cancer.

[fly.org/seq\\_tools/splice.html](http://fly.org/seq_tools/splice.html); no alternative splice sites were predicted.

When possible, the presence of a sequence variant detected in an index patient was investigated in other affected relatives. A control group of 150 anonymous Dutch (male and female) blood donors was analysed by DGGE to determine the prevalence of the sequence variants in a general population sample.

A chi-square test was used to determine the statistical significance of the proportion of CHEK2 mutation carriers in our study group compared to healthy controls.

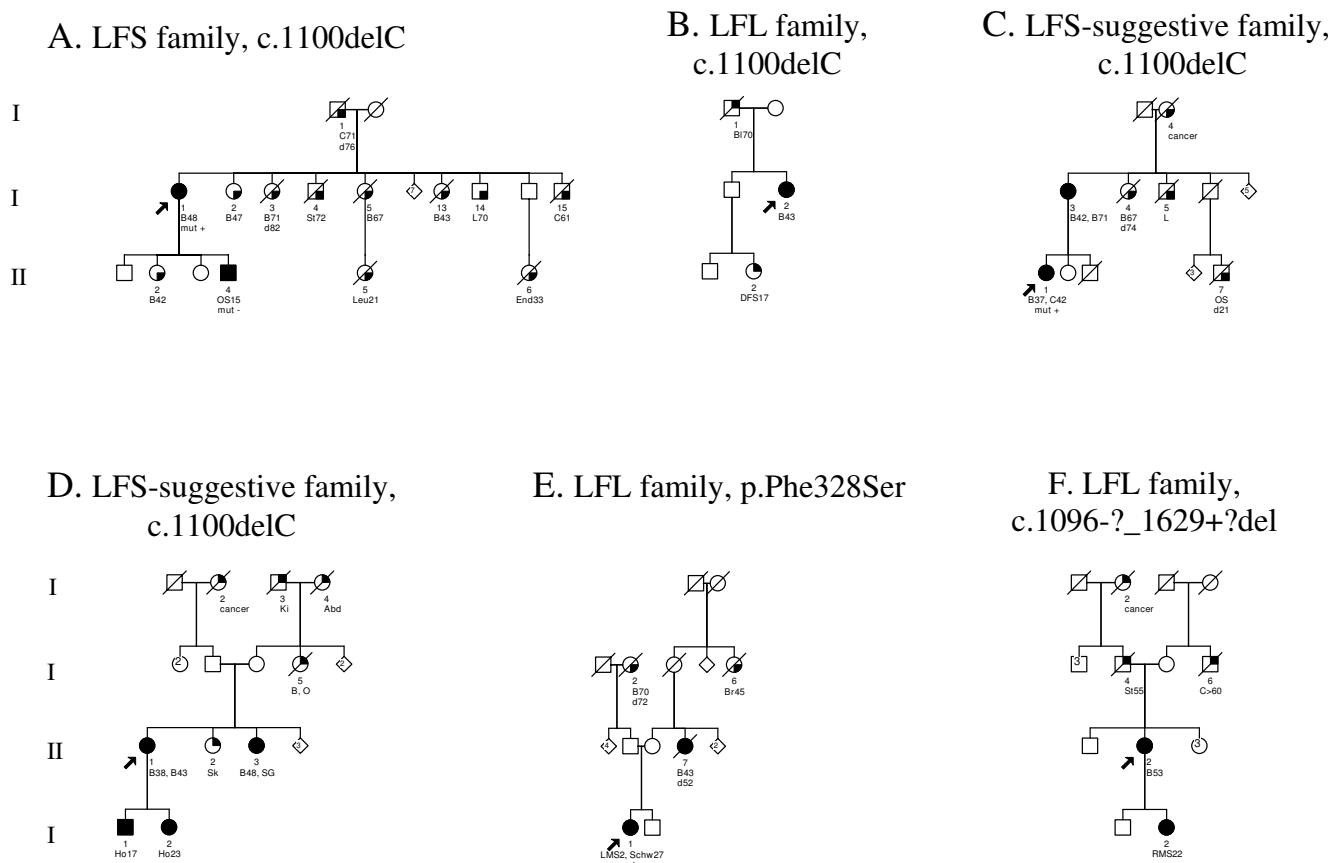
## Results and discussion

Sixty-five TP53-negative individuals from 65 families were screened for the CHEK2 1100delC germline mutation and DNA rearrangements. Thirty-four of these individuals were screened comprehensively by DGGE for CHEK2 mutations. Six index patients were found to carry a possibly pathogenic germline CHEK2 sequence variant.

The c.1100delC in exon 10 of the CHEK2 gene, a mutation located in the kinase domain of the gene and abolishing the kinase activity of the protein, was detected in four index patients. In one family, a classical LFS family, the c.1100delC was detected in a patient who developed breast cancer at the age of 48 years (Figure 1A), which is in line with the c.1100delC acting as a low penetrance

breast cancer susceptibility allele [19]. Relatives with a 50% chance of being a c.1100delC carrier in this family who had developed breast cancer were not available for testing. However, it is not likely to be the LFS-causing mutation in this family, considering the absence of the c.1100delC in the patient's son who developed a sarcoma at 15 years of age. In an LFL and LFS-suggestive family, the patients identified as carrying the c.1100delC had breast cancer (Figure 1B and 1D); in a fourth family, a LFS-suggestive family, the patient identified with the c.1100delC sequence variant had both breast and colorectal cancer (Figure 1C). No additional material was available for testing to see if and how the mutation segregates in these families. In all four of the c.1100delC families, this sequence variant seemed to be associated with breast cancer or breast and colorectal cancer, rather than LFS. The reported frequency of the CHEK2 c.1100delC in Dutch controls is 1.4%, in Dutch breast cancer patients not selected for family history 2.5% and in Dutch BRCA1/2-negative families with breast cancer 4.9% [19]. In our sample the frequency was 6.2% (4/65), significantly different from that for healthy controls ( $p = 0.006$ ).

Another sequence variant, c.983T>C, p.Phe328Ser in exon 8, localised in the kinase domain of the gene, was detected in a female patient who had developed a leiomyosarcoma at 2 years of age and a schwannoma at 27 years of age (Figure 1E). The family of the index patient fulfilled the LFL criteria (Eeles [4]). The parents of the index



**Figure 1**  
**Pedigrees of germline *CHEK2* sequence variation families.** Square symbols indicate males, round symbols indicate females, line across symbol means deceased individual. Filled symbols indicate affected individuals with diagnosis confirmed by pathology reports. A quarterly filled symbol indicate affected individuals with diagnosis by family history. Tumour type and age at diagnosis of the tumours are indicated below the individual identifiers, d = age of death. The index patient is indicated with an arrow. Abd = abdominal cancer, B = breast cancer, Bl = bladder cancer, Br = brain tumour, C = colorectal cancer, cancer = cancer of unknown origin, DFS = dermatofibrosarcoma, End = endometrial cancer, Ho = Hodgkin lymphoma, Ki = kidney cancer, L = lung cancer, Leu = leukaemia, LMS = Leiomyosarcoma, O = ovarian cancer, Oes = oesophagus carcinoma, OS = osteosarcoma, RMS = rhabdomyosarcoma, Schw = schwannoma, SG = salivary gland cancer, Sk = skin cancer, St = stomach cancer, mut + = mutation detected, mut - = mutation excluded.

patient are both healthy and over 60 years of age. A maternal aunt died of breast cancer at 45 years of age and a sister of the index patient's maternal grandmother died of a brain tumour; no material was available for testing. Under the assumption that these two affected family members were carriers of the mutation, penetrance would be incomplete with non-penetrance exhibited by two older healthy obligate mutation carriers. The hypothesis that the exon 8 mutation caused the complete LFL phenotype in this family is unlikely although a de novo mutation, contributing to the disease in the index patient, cannot be excluded. The p.Phe328Ser missense mutation has not been described in the literature before and was not found in 150 healthy Dutch controls. The phenylalanine in this

position is conserved in mice and frogs but not in either zebrafish or *C. Elegans* (Ensembl, v39-Jun 2006 [27]).

One *CHEK2* DNA rearrangement was found, c.1096-?\_1629+?del, a deletion of exons 10–14 of the gene. The family fulfilled the LFL criteria according to Birch[3] and the index patient developed breast cancer at the age of 55 years. A deletion of this size in the kinase domain of the gene will probably abolish the kinase activity. This deletion has not been described in the literature before. A deletion of exon 9–10 predicting protein truncation at codon 381 was discovered as a founder mutation among patients of Czechoslovakian ancestry with breast cancer [17]. Unfortunately, no material from the daughter who developed a rhabdomyosarcoma at 22 years of age was

available. Four sibs of the index patient were healthy; her father developed stomach cancer at 55 years of age.

So far, 8 studies have been published on *CHEK2* analysis of a total of 196 *TP53*-negative LFS families or families suggestive of LFS (Table 2) [10-17]. Of the seven variants presented, only the c.1100delC, the p.Ile157Thr and the p.Arg145Trp mutation are of reported functional significance. Bell et al [10] found the p.Ile157Thr in an index patient with three primary cancers; no other family members were tested. Allinen et al. [11] screened the *CHEK2* gene in 21 LFS/LFL families and only found the p.Ile157Thr mutation; since it was found in healthy controls as well, they concluded that it does not contribute to an LFL-associated breast cancer risk. Some authors found an association between the p.Ile157Thr mutation and risk of female breast cancer [28-30], others found no association [31]. The p.Arg145Trp, leading to a destabilised protein, was described in a Li-Fraumeni-like kindred; it was only tested in one family-member with a sarcoma at 20 years and breast cancer at 42 years. It was not found in 200 controls [13].

In our study six index patients were found to carry a *CHEK2* sequence variant by screening 65 *TP53*-negative index patients, with no evidence that the sequence variants found caused the complete LFS phenotype in their

families. Our data are in line with the hypothesis that the *CHEK2* c.1100delC might be associated with an elevated breast cancer risk [19,20], and possibly with a breast and colorectal cancer phenotype [23] or more generally a multi-organ cancer susceptibility [32]. We propose that the germline *CHEK2* sequence variants contribute to tumour development in the index patients. Without these tumours, the families would not have fulfilled the established LFS/LFL criteria and *TP53* germline mutation testing would not have been indicated. In this way, the individual *CHEK2* sequence variants may contribute to the Li-Fraumeni phenotype seen in these families.

Because only 75% of classical LFS families and 40% of LFL families have germline *TP53* mutations, research groups have looked at candidate genes like *Bcl10* [33], *CDKN2* [34,35], *TP63* [12], *PTEN* [34,36], *CHEK1* [10,14] and *BAX* [37]; no possible alternative LFS genes were found.

Two polymorphisms, p.Arg72Pro (*TP53* gene) and SNP309 T>G (*MDM2* gene), have been shown to have a modifying effect, resulting in an earlier age of onset of cancer in *TP53* mutation carriers [38,39]; there is even a synergistic effect when both polymorphisms are present. These are proposed examples of modifying factors or low penetrance gene mutations that play a role in age of onset and tumour clustering in cancer-prone families [40]. In

**Table 2: Literature on *CHEK2* analysis in LFS and LFS-related families**

	number of families tested: LFS/ LFL/suggestive	total <i>CHEK2</i> analysis	<i>CHEK2</i> 1100delC analysis + DNA rearrangements	mutations found
Allinen et al. [11]	1/20/0	21	0	p.Ile157Thr
Bell et al. [10]	4/18*	22	0	c.1100delC p.Ile157Thr
Bougeard et al. [12]	0/4/0	4	0	-
Lee et al. [13]	10/49*	59	0	p.Arg145Trp p.Arg3Trp p.Ile157Thr
Siddiqui et al. [16]	1/13/1	0	15	-
Sodha et al. [15]	5/21/0	26	0	IVS5-11G>A c.483-485delAGA
Vahteristo et al. [14]	1/6/32	39	0	c.1100delC
Walsh et al. [16]	3/7/0	10		-
our results	1/35/29	34	31	p.Phe328Ser c.1100delC c.1081-?_1771+?del

\* = LFL and LFS-suggestive combined, subdivision not further mentioned

our present study group, we investigated the possible modifier effects of these polymorphisms but found no association with an earlier age of tumour onset [41] (p.Arg72Pro, data not shown). We did find a larger proportion of homozygotes for the G-allele of *MDM2* SNP309 in our *TP53*-negative group, suggesting a modifier effect on the *TP53* negative Li-Fraumeni phenotype.

## Conclusion

Our data illustrate that *CHEK2* is not a major LFS susceptibility gene in the Dutch population. The *CHEK2* gene might be a factor contributing to individual tumour development in families that are subsequently recognised as having a Li-Fraumeni phenotype. Although many genes have been excluded as alternative LFS genes, many more modifiers or low penetrance susceptibility genes might occur in families showing a Li-Fraumeni phenotype.

## Competing interests

The authors declare that they have no competing interests.

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