

Familial gastric cancer and Li–Fraumeni syndrome

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Gastric cancer occurs in some familial diseases with inherited cancer predisposition. Genetic factors have been correlated with the hereditary diffuse gastric cancer and other familial gastric cancer conditions as hereditary non-polyposis colorectal cancer and Li–Fraumeni syndrome. The present study was aimed at searching for germ line mutations of *TP53* gene in familial gastric cancer with cluster for Li–Fraumeni syndrome or Li–Fraumeni-like syndrome. Twenty-three pedigrees with characteristics for Li–Fraumeni-like syndrome were identified. DNA of the proband was sequenced using polymerase chain reaction/single-strand conformation polymorphism. Among these 23 cases, no germ line mutation of *TP53* was identified, while two single-nucleotide polymorphisms were identified in four patients. In our area, in which a high rate of familial aggregation was demonstrated, the lack of germ line mutation of *TP53* together with the infrequency of mutation of E-cadherin gene seem to limit the role of genetic predisposition in the development of gastric cancer.

Keywords: *TP53*, Li–Fraumeni syndrome, germ line mutation.

INTRODUCTION

The incidence of non-cardia gastric cancer (GC) declines in most countries; however, overall GC remains the second most common cause of cancer death in the world (Parkin *et al.* 2001).

About 10% of GC is correlated with familial clustering and, of these, only 1–3% is hereditary. Germ line mutation of E-cadherin gene is associated with an autosomal dominant clustering and it is responsible for the inherited

susceptibility for hereditary diffuse gastric cancer (HDGC) (Guilford *et al.* 1998; Caldas *et al.* 1999). Besides, GC should be recognized as a component of other hereditary cancer syndromes, such as hereditary non-polyposis colorectal cancer, familial adenomatous polyposis, Cowden and Peutz–Jeghers syndromes and Li–Fraumeni syndrome (LFS).

Li–Fraumeni syndrome (OMIM#151623) is a hereditary disease autosomal dominant associated with *TP53* (chromosome 17p13; OMIM#191170) germ line mutation (Vogelstein *et al.* 2000). Germ line carriers of the *TP53* mutation are at increased risk for multiple primary tumours, such as sarcoma, breast cancer, brain tumours and adrenocortical carcinoma. Other less-common tumours have also been associated with LFS, including

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leukaemia, lung cancer, melanoma, pancreatic cancer, prostate cancer and GC (Birch *et al.* 2001; Olivier *et al.* 2003).

Clinical criteria were firstly described by Li and Fraumeni in 1988 as a proband with a sarcoma aged under 45 years with a first-degree relative aged under 45 years with any cancer, plus an additional first- or second-degree relative in the same lineage with any cancer aged under 45 years or a sarcoma at any age (Li *et al.* 1988). Subsequently in 1994, Birch *et al.* (1994) introduced a new definition of Li–Fraumeni-like syndrome (LFL). It was based on more extensive and updated information of the type of tumours and the ages of onset in families. These criteria considered a proband with any childhood tumour, or a sarcoma, brain tumour, or adrenocortical tumour aged under 45 years plus a first- or second-degree relative in the same lineage with a typical LFS tumour at any age, and an additional first- or second-degree relative in the same lineage with any cancer under the age of 60 years (Birch *et al.* 1994; Varley 2003).

The present study was aimed at searching for germ line mutations of *TP53* gene in familial gastric cancer (FGC) with cluster for LFS or LFL.

METHODS

Patients and pedigree collection

The study considered 238 patients affected by primary GC and operated on at the Division of General Surgery and Surgical Oncology, University of Siena, Italy.

For each patient, a complete oncological family history was obtained as recently described in detail (Roviello *et al.* 2008).

DNA extraction

DNA extraction was restricted to patients who fulfilled the LFS and LFL criteria and aimed at searching a *TP53* germ line mutation. *TP53* germ line mutation was also ruled out in 14 previously described patients with clinical criteria for HDGC. Genomic DNA was purified from peripheral white blood cells or frozen normal gastric mucosa using Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN, USA) in accord with the protocol described in detail.

Polymerase chain reaction/single-strand conformation polymorphism analysis

The *TP53* mutational hotspot (exons 5–8) was amplified by polymerase chain reaction (PCR) from germ line DNA

using the primers from the Operon kit (Operon Technologies, Atlantic City, CA, USA). Polymerase chain reactions were carried out in a volume of 20 µl containing 100 ng genomic DNA, 20 µM of each primer, 10 mM dNTP, buffer 10×, 0.5 unit of Taq polymerase (GE Healthcare, Bucks, UK). Samples were denatured for 5 min at 94°C in a programmable thermocyclers (Bio-Rad Laboratories, Hercules, CA, USA), then amplified by 35 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 1.30 min with a final elongation of 10 min at 72°C. The product of PCR were subsequently diluted 1:1 with denaturing buffer (formamide with 0.025% xylene cyanol and 0.025% bromophenol blue) and heated up to 99°C for 10 min prior to loading onto 0.8× mutation detection enhancement (MDE gel solution, Cambrex Bio Science Milano, Milan, Italy) gels. Gels were run at constant temperature for 12–18 h (8°C) and stained with silver nitrate.

Sequencing analysis

Samples showing abnormal bands detected by single-strand conformation polymorphism analysis were re-amplified by PCR and products were purified and sequenced on ABI Prism 377 automated sequencer using Big Dye terminator cycle sequencing kit (Perkin-Elmer, Forster City, CA, USA) and primers firstly adopted for both direction.

RESULTS

Familial aggregation

No patient showed clinical criteria of LFS while 23 families (9.7%) demonstrated criteria of LFL. All probands were affected by primary GC, intestinal type in 19 cases and diffuse type in four cases.

Considering the 23 families, 125 first- and second-degree relatives with a positive oncological history were identified. The most frequently observed neoplasia were GC (43 cases; 34.4%), leukaemia (14 cases; 11.2%) and breast cancer (14 cases; 11.2%). Twelve cases (9.6%) affected by brain tumours were also identified. All kinds of tumours were illustrated in Tables 1 and 2.

Genetic mutational screening

TP53 gene hotspot was screened in the 23 patients with the LFL and in the 14 with the HDGC clinical criteria. No genetic changes were found in patients with the HDGC clinical criteria. Among LFL patients, four probands with diagnosed GC showed abnormal bands in amplified region

Table 1. Overall number of associated tumours in the 23 families of gastric cancer patients under study, considering the first- (FDR) and the second-degree relatives (SDR)

	FDR (%)	SDR (%)
Stomach	34 (27.2)	9 (7.2)
Colon-rectum	2 (1.6)	4 (3.2)
Breast	11 (8.8)	3 (2.4)
Bladder	2 (1.6)	–
Pancreas	4 (3.2)	1 (0.8)
Uterus	5 (4)	–
Prostate	–	3 (2.4)
Leukaemia	11 (8.8)	3 (2.4)
Brain	6 (4.8)	6 (4.8)
Lung	2 (1.6)	–
Skin	1 (0.8)	–
Larynx	3 (2.4)	–
Liver	2 (1.6)	–
Kidney	1 (0.8)	–
Tyroide	–	1 (0.8)
Bone	3 (2.4)	–
Not specified	6 (4.8)	2 (1.6)
Total	93 (74.4)	32 (25.6)
	125	

of exon 6 at PCR/single-strand conformation polymorphism analysis. A heterozygous single substitution to nucleotide A > G at position 13372 of uncoding region was evidenced in two patients. Another single-nucleotide polymorphism A > G at position 13308 of coding region was detected in other two patients (Fig. 1). No germ line mutation with pathogenic impact was found.

DISCUSSION

Detailed familial history contributed greatly to the understanding of hereditary cancer syndromes as well as to the evolution of molecular genetic of these familial diseases. Cancer familial history investigation and genetic counselling have an unquestionable role in selecting families with suspicious hereditary cancer syndromes (Lynch & Lynch 2002).

Gastric cancer show familial clustering in about 10% of the cases (Carneiro *et al.* 2008) and only as few as 1–3% of these occur in families with autosomal dominant GC susceptibility (Stone *et al.* 1999; Brooks-Wilson *et al.* 2004; Pedrazzani *et al.* 2007).

Table 2. Clinical characteristics, associated tumours and number of involved generations in families with Li–Fraumeni-like syndrome clinical criteria

	Gastric cancer index	Kind of tumours in related degree	Number of involved generation
1	M-68	Brain (M-54), breast (F-66, F-39, F-75), uterus (56), not specified (F-75, M-80), colon-rectum (M-80)	2
2	M-66	Brain (M-68), stomach (F-81), breast (F-37)	2
3	M-66	Stomach (M-80, F-80, M-45, F-88, F-80, M-60, F-44, M-70), bladder (M-N/A), leukaemia (M-22), brain (M-40), prostate (60), breast (F-24)	4
4	F-55	Stomach (F/NA, M-54), bone (M-84)	3
5	M-30	Colon-rectum (M-80), breast (F-78), leukaemia (F-20)	3
6	F-77	Leukaemia (M-52)	2
7	M-72	Pancreas (F-72), brain (F-56)	2
8	M-63	Not specified (F/NA), leukaemia (F-70), brain (M-45)	3
9	F-74	Leukaemia (F-50), pancreas (M-56)	2
10	F-58*	Tyroide (M-75), colon-rectum (F-55), prostate (81), brain (M-52)	3
11	F-73	Brain (F-83), leukaemia (M-13)	3
12	F-80	Not specified (F-45), bone (F-86), stomach (M-47), colon-rectum (M-67)	3
13	F-73	Not specified (M-50, M-75), breast (F-82), brain (M-56)	3
14	F-76	Stomach (F-74, F-77, M-92), brain (M-60)	2
15	M-51	Not specified (F-89), larynx (F-55), lung (F-40), melanoma (F-30), stomach (M-62), leukaemia (F-26)	3
16	F-71	Stomach (M-65), pancreas (F-58, M-51), lung (F-38), brain (M-58)	2
17	F-79†	Pancreas (M-52), leukaemia (F-81)	1
18	F-71	Bladder (M-48), leukaemia (F-80, M-50), brain (F-60)	3
19	M-66	Stomach (M-72), bone (F-70), gynaecologic (52)	2
20	F-80	Stomach (M-50), liver (F-52, F-60), brain (M-32), not specified (M-50)	3
21	F-78	Gynaecologic (73), brain (F-42)	3
22	M-59	Stomach (M-70), leukaemia (M-56), breast (F-69), gynaecologic (52)	2
23	M-79	Breast (F-50, F-50, F-65, F-66, F-75), brain (F-40), leukaemia (F-70), colon (M-67), larynx (M-70), prostate (55)	3

*Triple primary cancer (stomach, breast and ovarian cancer).

†Double primary cancer (stomach and kidney cancer).

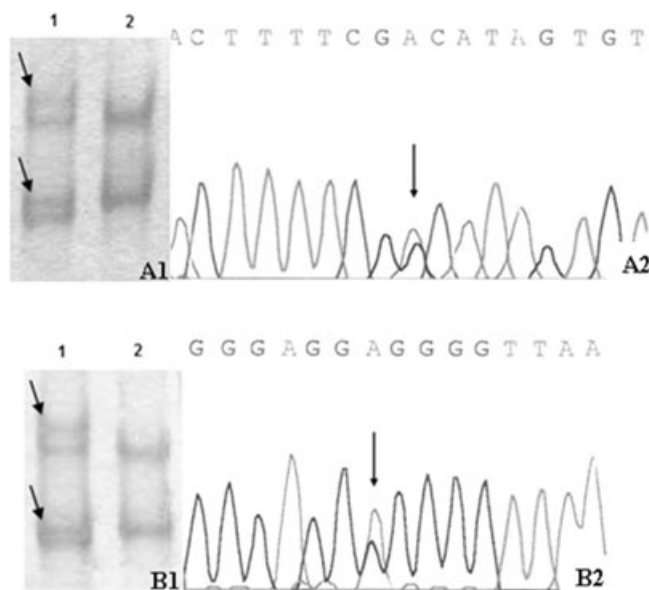


Figure 1. Single-strand conformation polymorphism images (A1, B1) and sequencing results (A2, B2) of the identified single-nucleotide polymorphisms.

We recently reported a high rate of familial aggregation for GC (30%) in Tuscany, a high-incidence Italian area (Bernini *et al.*, 2006; Roviello *et al.* 2007), and the first germ line mutation of E-cadherin gene in an Italian family (Corso *et al.* 2007; Roviello *et al.* 2007). In our series, the prevalence of familial clustering for GC appears extremely high instead of a single pathogenic germ line mutation.

The present results demonstrate that clinical criteria for LFL occur in 9.7% of GC patients. Li-Fraumeni syndrome is a rare autosomal hereditary cancer syndrome characterized by a combination of tumours, predominantly sarcomas, breast cancers, brain tumours and adrenocortical carcinomas (Olivier *et al.* 2003). Other less-common tumours have also been associated with LFS, including leukaemia, lung cancer, melanoma, pancreatic cancer, prostate cancer and GC (Birch *et al.* 2001; Olivier *et al.* 2003).

According to the International Agency for Research on Cancer database, GC has been reported in up to 2.8% of LFS families. *TP53* germ line mutations have been identified in about 70% of patients with clinical criteria of LFS; more than 70% of the mutations are missense changes, and up to 90% of these are located in exons 5–8 (Chompret 2002; Olivier *et al.* 2003; Oliveira *et al.* 2004).

Germ line missense mutation of *TP53* gene was previously reported in a case of LFS presenting as a non-FGC (Sugano *et al.* 1999). Later, the presence of a nonsense germ line mutation of *TP53* gene was demonstrated in a case in which LFS and HDGC clinical criteria were asso-

ciated (Kim *et al.* 2004). Keller and colleagues identified a single *TP53* germ line missense mutation in a group of 34 European FGC patients. This pedigree showed three cases of GC, one case of liver carcinoma and one case of leukaemia in a 17-year-old girl (Keller *et al.* 2004). Among these, Oliveira *et al.* (2004) screened 10 families with familial aggregation of GC and one of these harboured a *TP53* germ line missense mutation in hotspot cluster. Kusano and colleagues reported no E-cadherin and *TP53* mutations in seven families with FGC, concluding that germ line mutations in these genes are infrequently involved in the carcinogenesis of Japanese FGC (Kusano *et al.* 2001).

In our area, in which a high rate of familial aggregation was demonstrated, the lack of germ line mutation of *TP53* together with the infrequency of mutation of E-cadherin gene seem to limit the role of genetic predisposition in the development of GC.

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CONFLICT OF INTEREST STATEMENT

None declared.

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