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ORIGINAL ARTICLE

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A *TP53*-truncating germline mutation (E287X) in a family with characteristics of both hereditary diffuse gastric cancer and Li-Fraumeni syndrome

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Abstract Mutations in *CDH1*, which encodes E-cadherin, have been associated with hereditary diffuse gastric cancer (HDGC) in Western populations but have not been shown to play a major role in Asians. Recently, a patient with familial gastric cancer (FGC) was shown to harbor a germline mutation in the *TP53* gene, which encodes p53 and has been previously associated with Li-Fraumeni Syndrome (LFS). To determine whether mutations in *TP53* are associated with FGC in Asians, we screened the entire coding region of *TP53* in probands from 23 Korean FGC families. We identified a nonsense (E287X) *TP53* germline mutation in a family whose history is compatible with both HDGC and LFS. Two members of this family (SNU-G2) were afflicted with brain tumors, seven with gastric cancers, two with sarcomas, and one with both gastric cancer and a sarcoma. The E287X *TP53* mutation segregated with the cancer phenotype in the family members from whom DNA samples were available. To our knowledge, this is the first report of a large family with both HDGC and LFS. Our results suggest that *TP53* mutational screening in FGC families should be interpreted with caution because additional *TP53* mutation-carrying HDGC families may also show LFS-related phenotypes.

Keywords *TP53* · FGC · HDGC · LFS · Germline mutation

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Introduction

Germline mutations in *CDH1*, which encodes E-cadherin, were first associated with familial gastric cancer (FGC) when germline mutations were found in a large Maori family with hereditary diffuse gastric cancer (HDGC) (Guilford et al. 1998). Since then, several other groups have reported *CDH1* germline mutations in HDGC families. Western populations show a large proportion of truncating mutations (25–37%) in this gene (Oliveira et al. 2002) whereas East-Asian countries that show very high rates of gastric cancer were found to have only a few missense germline mutations in *CDH1* (Yabuta et al. 2002; Iida et al. 1999; Kim et al. 2000), suggesting that there may be a second causative gene for HDGC. Researchers have sought to identify another genetic event in FGC patients (Lee et al. 2000; Kim et al. 2003); our lab and another Korean group found *MET* germline mutations in one out of 24 Korean FGC patients (Kim et al. 2003) and one out of 85 gastric cancer patients with unknown family histories (Lee et al. 2000). However, no major genetic cause for FGC has yet been identified in an East-Asian population.

Recently, a *TP53* germline missense mutation was found in a European FGC patient without *CDH1* germline mutation (Keller et al. 2004). Thus, we investigated whether *TP53* germline mutations contribute to development of FGC without *CDH1* germline mutations in a Korean population. We screened probands from 23 Korean FGC families for *TP53* mutations and

identified a single nonsense germline mutation in one large HDGC family. A thorough investigation revealed that the family history of this pedigree was also compatible with Li-Fraumeni syndrome (LFS), which is characterized by a family constellation including a proband younger than 45 years with a sarcoma having a first-degree relative younger than 45 years with any cancer and an additional first-degree or second-degree relative younger than 45 years in the same lineage with any cancer or a sarcoma at any age (Li et al. 1988).

Materials and methods

Patient samples

Twenty-three Korean families affected with FGC were investigated for *TP53* germline mutations. These families had previously been screened for *CDH1* and *MET* germline mutations (Yoon et al. 1999; Kim et al. 2003). Criteria for family inclusion were at least two first-degree or second-degree relatives affected with gastric cancer, at least one of which was diagnosed with cancer prior to age 50 (Yoon et al. 1999). Out of 23 probands, 11 represented families suffering from diffuse-type gastric cancer, four represented families suffering from intestinal types, and histological data for the type of the remaining eight families were not available. Blood samples from each proband were collected from Seoul National University Hospital, and affected and unaffected members of family SNU-G2 were sampled following identification of a mutation in this family. Informed consent was obtained from all participants prior to testing. Total genomic DNA was extracted from the blood samples using Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden) and the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturers' instructions.

PCR amplification and sequencing

Fragments covering the entire coding sequence of *TP53* were PCR amplified as previously described (Bakkar et al. 2003). PCR reactions were carried out in a volume of 25 ul containing 100 ng genomic DNA, 10 pmol of each primer, 250 μM each dNTP, 0.5 units of Taq polymerase, and the reaction buffer provided by the supplier (QIAGEN, Hilden, Germany). Samples were denatured for 5 min at 94°C in a GeneAmp PCR system 9700 (Applied Biosystems, Inc., Foster City, CA, USA) then amplified by 35 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min with a final elongation of 10 min at 72°C. Exons 2–11 of *TP53* were bidirectionally sequenced in duplicate reactions using the Taq dideoxy terminator cycle sequencing kit and an ABI 3100 DNA sequencer (Applied Biosystems). Samples showing a mutation in direct sequencing were freshly PCR amplified, ligated into PCR-TOPO vectors (Invitrogen), and subcloned using the TA cloning system (Invitrogen) for sequencing and confirmation of the mutation.

Two patients in family SNU-G2 who had been previously reported to harbor *CDH1* germline mutations (Yoon et al. 1999) were rescreened at this gene in an attempt to confirm the mutations by direct sequencing.

Denaturing high-performance liquid chromatography (DHPLC) analysis

Members of family SNU-G2 were screened for the presence of *TP53* germline mutations by DHPLC (WAVE, Transgenomic, Omaha, USA). DHPLC analyses were performed as described by Kim et al. (2003). Melting curves were investigated by WAVEMAKER (WAVE) software to set the optimized temperature for each amplicon. Samples showing abnormal DHPLC results were further examined by automatic sequencing.

Immunohistochemistry (IHC)

Among the family members, two patients had undergone surgical resection for liposarcoma and medulloblastoma, respectively, and their paraffin blocks were available. Immunohistochemistry was performed on the two paraffin-embedded tissue sections using the avidin–biotin peroxidase complex (ABC) method. After antigen retrieval using a citrate buffer solution (Antigen Unmasking Solution, Vector Laboratories, Burlingame, CA, USA) for 15 min in an 800-W microwave oven, mouse anti-p53 monoclonal antibody (clone Bp53.11, DAKO, Carpinteria, CA; dilution 1:50) was applied. Negative controls included the use of nonimmune serum or PBS in place of the primary antibody.

Results

Out of 23 Korean FGC probands tested, we found a nonsense *TP53* germline mutation [E287X mutation in exon 8 (codon 287, GAG → TAG)] in the proband for family SNU-G2, which had been diagnosed as an HDGC family according to the criteria suggested by Caldas et al. (1999). As *TP53* germline mutations are found in about 70% of LFS patients, we thoroughly investigated the history of family SNU-G2 and found, to our surprise, that this family also showed evidence of the sarcoma and brain tumor incidences characteristic of LFS. The proband of SNU-G2 died of gastric cancer, as did his five older siblings (three male, two female) (Fig. 1). The father of the proband died of a brain tumor, his mother died of gastric cancer, and his eldest brother had both a liposarcoma and gastric cancer. The proband's five siblings were not available for genetic analysis due to a lack of tissue samples, but we were able to obtain blood samples from 14 of the siblings'

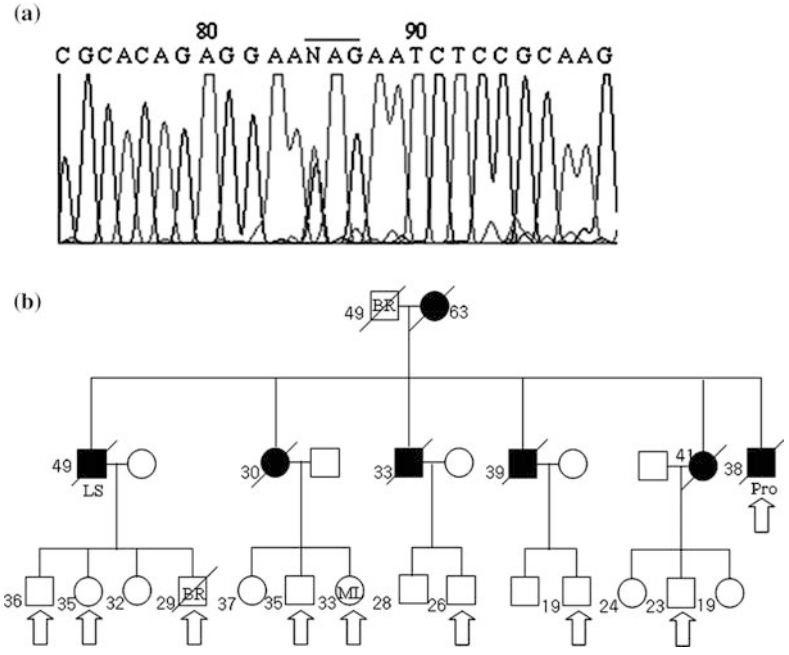

Fig. 1 *TP53* germline mutation in exon 8 of the proband of family SNU-G2. **a** Automatic sequencing showing the nucleotide substitution that results in an amino-acid change (E287X). **b** Pedigree of SNU-G2 with both hereditary diffuse gastric cancer (HDGC) and Li-Fraumeni Syndrome (LFS). *Square* male, *circle* female, *solid symbols* affected with gastric cancer, *diagonal symbols* deceased, *arrow* mutation carrier, *pro* proband, *BR* brain tumor, *ML* metastatic leiomyosarcoma, *LS* liposarcoma. The numbers indicate ages of death (for deceased family members) or age at the time of analysis (for living family members)

children, two of whom were affected with LFS. One had died of a brain tumor while the other had a metastatic lung leiomyosarcoma of unknown origin. For segregation analysis of the mutation within family SNU-G2, the 14 members of the third generation were all screened for the presence of E287X *TP53* germline mutation. We identified eight carriers of the mutation and six non-carriers. Of the eight carriers, two were the individuals affected with LFS whereas the other six individuals showed no symptoms of the disease. None of the non-carriers showed any clinical phenotype related with LFS. Thus, the E287X *TP53* germline mutation appeared to be associated with the cancer phenotype in family SNU-G2. Although this family was previously reported as harboring a *CDH1* missense mutation, we were unable to confirm this mutation by direct sequencing. Immunohistochemical staining for p53 did not show any p53 nuclear protein accumulation in the tumor cells of the liposarcoma or medulloblastoma samples.

Discussion

Recently, a European group found a *TP53* germline mutation in one gastric cancer patient (Keller et al. 2004). In search of a genetic mechanism for hereditary gastric cancer in families without *CDH1* or *MET* germline mutations, we screened the entire coding region of *TP53* in 23 Korean FGC families. We identified a nonsense germline *TP53* mutation in one HDGC family (SNU-G2) that had been previously reported as having a *CDH1* germline missense mutation (Yoon et al. 1999). This unexpected result prompted us to carefully rescreen *CDH1* in this family (SNU-G2) and another *CDH1* mutation-carrying family (SNU-G1001). Repeated experiments with DHPLC and automatic sequencing did not show any *CDH1* germline mutations in these two families. We hypothesize that the previous incorrect results might have originated from experimental artifacts generated during the PCR-SSCP or cloning-sequencing steps. Further analysis of family SNU-G2, in which the *TP53* mutation seemed to segregate with the cancers, revealed that the phenotypes in this family were compatible with both HDGC and LFS.

LFS is a rare autosomal hereditary cancer syndrome characterized by a combination of tumors, predominantly sarcomas, breast cancers, brain tumors, and adrenocortical carcinomas (Olivier et al. 2003). Other less-common cancers have also been associated with LFS, including leukemia, lung cancers, melanoma, gastric cancers, pancreatic cancer, and prostate cancer (Olivier et al. 2003; Birch et al. 2001). According to the IARC (International Agency for Research on Cancer) database, gastric cancer has been reported in up to 2.8% of LFS families (Olivier et al. 2003). With this low frequency of gastric cancers in LFS, it is easy to overlook the possibility of LFS in families that appear to segregate gastric cancer. As no clinical symptom of sarcoma had been seen in family SNU-G2, we had previously reported this family, containing six gastric cancer patients and one brain tumor patient, as affected with HDGC. Based on our mutational and clinical analysis, however, we suggest that this family may additionally suffer from LFS.

TP53 germline mutations have been identified in about 70% (Olivier et al. 2003) of LFS patients; more than 70% of the mutations are missense mutations (Chompret 2002; Olivier et al. 2003), and up to 90% of the missense mutations are located in exons 5–8 (Chompret 2002), which is thought to comprise the DNA-binding domain. The cancer risk in mutation carriers has been reported to be as high as 73% in males

and almost 100% in females due to the breast cancer risk (Chompret 2002). The E287X mutation found in family SNU-G2 was located in exon 8 near one of the hotspot codons, 282. *TP53* null mutations are a poor prognostic indicator in lung cancer (de Anta et al. 1997), and mutations in exons 7 and 8 are also predictive of poor prognosis (Huang et al. 1998; Mitsudomi et al. 2000) although the latter has been disputed (Vega et al. 1997). Thus, it is possible that the HDGC/LFS phenotype in family SNU-G2 may be due to an aggressive nonsense mutation (E287X) in exon 8. Consistent with the previous report, no p53 nuclear protein accumulation was detected (Fenoglio-Preiser et al. 2003). It has been reported that p53 IHC fails to detect nonsense, splice, or null mutation gene products (Mitsudomi et al. 2000). It was previously reported that familial breast cancers could occur simultaneously with LFS, as breast cancer is a major component of LFS (Martin et al. 2003). Although a *TP53* germline missense mutation was previously reported in a case of LFS presenting with a non-FGC (Sugano et al. 1999), this is the first report of a large family with a *TP53* germline mutation associated with both HDGC and LFS. However, it is not clear whether the gastric cancer common in family SNU-G2 is due to LFS or whether it was inherited irrespective of LFS. The proband's father died of a brain tumor, which is one of the major cancers in LFS, whereas the proband's mother died of gastric cancer. Thus, it is possible that in this family, the LFS originated from the paternal allele and the HDGC came from the maternal allele. In support of this, it has been suggested that the less prevalent tumors in LFS, such as gastric cancers, might occur by chance; it is not clear whether the *TP53* germline mutations contributed to these cancers (Olivier et al. 2003). As gastric cancer is the most common cancer in Korea (Shin et al. 2004), the mother of the proband and/or some members of family SNU-G2 might suffer from sporadic, nonheritable gastric cancers. On the other hand, it is possible that the gastric cancer might result from LFS in family SNU-G2. We could not confirm these possibilities because samples were not available from the parents or siblings of the SNU-G2 proband. Among 14 offspring in the third generation (descended from the siblings of the proband), one each was affected by a brain tumor and leiomyosarcoma. These two cancer phenotypes have been related with LFS, and these two members harbored the *TP53* E287X germline mutation. As the other six members of the third generation carrying the mutation are younger than 35, they should be closely watched for further development of both LFS-related tumors and gastric cancer.

In summary, we identified a E287X *TP53* germline mutation in a large family whose criteria are compatible with both HDGC and LFS. This is the first report of simultaneous concurrence of HDGC and LFS and suggests that researchers should be cautious in screening FGC families for mutations in *TP53*, as *TP53* mutation-carrying FGC families may also develop LFS-related phenotypes.

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