ONLINE MUTATION REPORT

A novel germline mutation in the *MET* extracellular domain in a Korean patient with the diffuse type of familial gastric cancer

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Gastric cancer is one of the most deadly cancers worldwide. Although its incidence has declined in recent years, it is still the most prevalent cancer in Asian countries such as Korea and Japan.¹ Germline mutations of the cell to cell adhesion molecule E-cadherin (*CDH1*) have been reported in patients with the diffuse type of familial gastric cancer.^{2,3} However, the frequency of *CDH1* mutations is low overall⁴ and the observed mutations differ between western and Asian patients.⁵ Truncating mutations (that is, nonsense, frameshift, and alternative splicing mutations) predominate in patients of western origin,^{2,3} whereas only a few missense mutations have been found in patients of Asian extraction.^{5,6} This suggested that *CDH1* does not play a major role in gastric cancer development in Asian countries, and prompted researchers to investigate other gastric cancer causing genes.

One such gene is that for the MET receptor tyrosine kinase. MET transduces motility, proliferation, and morphogenic signals of hepatocyte growth factor/scatter factor (HGF/SF) in epithelial cells.⁷ Similar to other receptor tyrosine kinase genes such as *RET*, the *MET* gene encodes a protein with an extracellular domain (exon 2-13), a transmembrane domain (exon 13), and a tyrosine kinase domain (exon 15-21).^{8,9} *MET* germline mutations have been reported in patients with hereditary papillary renal carcinoma (HPRC).⁷ Most of the *MET* mutations associated with HPRC or sporadic papillary renal carcinomas⁷ were missense mutations in the tyrosine kinase domain,^{10,11} and overexpression of MET has been reported in human diseases such as breast, prostate, gastric, and ovarian cancers.^{12,13}

In the specific context of gastric cancers, a *MET* germline missense mutation was found in a Korean patient suffering from intestinal gastric cancer.¹¹ The mutation, located at the juxtamembrane domain (exon 14), has not been previously reported in papillary renal carcinoma patients.¹¹ In another study, no *MET* germline mutation was found in 18 white and Indian gastric cancer kindreds.¹⁴ In this study, we screened the *CDH1* and *MET* genes in Korean patients suffering from familial gastric cancer and we report a novel germline mutation of the *MET* gene.

METHODS

Twenty-one Korean families affected with familial gastric cancer were investigated for *CDH1* and *MET* germline mutations. Criteria for family inclusion were at least two first or second degree relatives affected with gastric cancer, at least one of whom was diagnosed with cancer before the age of 50.⁵ Blood samples from probands of each of these families were collected from Seoul National University Hospital, and four additional family members of pedigree SNU-G7 were sampled following identification of a mutation in this family. Informed consent was obtained from all participants before testing. Out of 21 probands, nine represented families suffering from diffuse types of gastric cancer, four represented

Key points

- Gastric cancer is the most prevalent cancer in Korea. Germline mutations of the cell to cell adhesion molecule E-cadherin gene (*CDH1*) have been reported in patients with the diffuse type of familial gastric cancer. A *MET* germline missense mutation was previously reported in a Korean gastric cancer patient. In this study, we screened the *CDH1* and *MET* genes in Korean patients suffering from familial gastric cancer
- One subject out of 21 probands from Korean pedigrees afflicted with familial gastric cancers harboured a *MET* missense mutation (P791L) in the extracellular domain (exon 10), an area which has not been extensively studied to date. No *CDH1* germline mutations were found.
- This is the first report of a *MET* germline mutation in a patient with familial gastric cancer. However, as the frequency of both *MET* and *CDH1* mutations is quite low in Korean gastric cancer patients, it seems that neither gene plays a major role in familial gastric cancer in this population.

families suffering from intestinal types, and histological data for the type of the remaining eight were not available. The classification of HDGC (hereditary diffuse gastric cancer) or HIGC (hereditary intestinal gastric cancer) suggested by Caldas *et al*¹⁵ was not possible in these families owing to the lack of histological information.¹⁵

Peripheral blood lymphocytes were isolated from each family member using Ficoll-Paque according to the manufacturer's instructions (Pharmacia Biotech, Uppsala, Sweden). Total genomic DNA was extracted using the TRI reagent (Molecular Research Center, Cincinnati, OH, USA) or the automatic magnetic bead based system (KingFisher, ThermoLabsystems, Finland) according to the manufacturers' instructions. *CDH1* mutational screenings were performed with reported primer sets⁵ using DHPLC (denaturing high performance liquid chromatography; WAVE, Transgenomic, Omaha, USA) as previously described.^{16,17} Coding sequences of exon 2 and exons 4-21 of the *MET* gene were screened by DHPLC using PCR primer pairs published by Duh *et al*.⁸ Primer sequences were not available for exon 3, which was excluded from screening.

Four general positive controls (WAVE DNA sizing control, WAVE low range mutation control standard, WAVE mid range mutation control standard, and WAVE high range mutation control standard; Transgenomic, Omaha, USA) for DHPLC were used to check the reliability of the experiments. The melting temperatures of each exon were optimised by analysing melting curves using WAVEMAKER software (Transgenomic, Omaha, USA). All samples showing abnormal

Figure 1 *MET* germline mutation in exon 10 of patient SNU-G7. (A) Automatic sequencing showing the nucleotide substitution that results in an amino acid change (P791L). (B) Pedigree of SNU-G7 familial gastric cancer family. Solid symbols, affected with gastric cancer; arrows, mutation carriers.

DHPLC patterns were subsequently cloned using the TA cloning system (Invitrogen, Carlsbad, CA, USA) and bi-directionally sequenced using the *Taq* dideoxy terminator cycle sequencing kit on an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

RESULTS AND DISCUSSION

No *CDH1* germline mutations were found in 21 probands from Korean pedigrees afflicted with familial gastric cancers. These 21 probands without *CDH1* germline mutations were then screened for *MET* germline mutations by DHPLC analysis. One subject (SNU-G7) out of 21 harboured a *MET* missense mutation (C to T) at exon 10 (codon 791), which produced a non-conservative amino acid substitution of proline to leucine (P791L, fig 1A). Residue 791 is conserved in mice, rats, and humans, suggesting that mutation of this residue could be pathologically relevant. To examine whether this sequence change is a naturally occurring polymorphism, we screened 181 unrelated, healthy, Koreans by DHPLC. None harboured the variation. Segregation analysis was not possible in this pedigree because there was only one living affected family member (SNU-G7, fig 1B). This person's grandfather and father both died from gastric cancer without operations.

Subject SNU-G7 initially presented with indigestion and intermittent epigastric pain of seven months' duration. Gastrofibroscopic examination showed a gastric cancer lesion on the high body of the stomach. Tumour metastasis to the perigastric lymph node was not detected. SNU-G7 is the oldest in her generation and shows no symptoms of papillary renal carcinoma. Out of her four younger sibs, two inherited the *MET* mutation and two did not. The *MET* mutation carriers are 43 and 38 years old at the time of this report, raising the possibility that these patients will develop gastric cancer in the years to come. Therefore, these people should be carefully monitored so that the disease may be diagnosed in its early stages.

In addition to the single seemingly pathological mutation, we also identified four polymorphisms in exons 2, 7, 20, and 21 in both familial gastric cancer patients and normal controls.

Out of the four, two (D1304D and A1357A) had previously been reported⁷ and two (N375S and Q648Q) were novel. The D1304D polymorphism was found in 12 of 21 (57%) gastric cancer patients and in 23 of 80 (29%) cancer free controls. A1357A was found in three of 21 (14%) gastric cancer patients and 19 of 80 (24%) normal controls. N375S and Q648Q were found in 5% (1/21) and 14% (3/21) of gastric cancer patients, respectively, and 9% (7/80) and 21% (17/80) of normal controls, respectively.

Gastric cancer is the most common cancer in Korea, yet studies have suggested that truncating mutations in *CDH1* do not significantly contribute to the development of diffuse gastric cancers in Asians. Recently, *MET* has been proposed as a candidate gene for gastric cancer.¹¹ Germline mutations in this gene, found in the tyrosine kinase domain, have been associated with HPRC. These tyrosine kinase domain mutations may constitutively activate the protein by altering its substrate specificity or catalytic activity.^{7, 13} In contrast, an oncogenic *MET* mutation (P1009S) reported in gastric cancer patients was localised to the juxtamembrane domain.¹¹ In this study, we identified a novel germline *MET* mutation in a Korean gastric cancer patient in the extracellular domain (exon 10), an area which has not been extensively studied to date.^{7, 11} This is one of two *MET* mutations that have been identified in gastric cancer patients, both of which (P1009S and P791L) were identified in Korean gastric cancer patients and localise to upstream sites (the juxtamembrane and extracellular domains, respectively). The observation that *MET* mutations associated with HPRC or sporadic papillary renal carcinomas localise to the tyrosine kinase domain suggests that there may be different pathological mechanisms responsible for *MET* induced gastric cancer and papillary renal carcinomas. *MET* mutations in gastric cancers may affect dimerisation/oligomerisation of *MET* molecules on the cell surface while missense mutations in *MET* in HPRC alter the conformation leading to constitutive activation of the *MET* protein in the absence of ligand. Germline *MET* mutations in papillary renal carcinomas and gastric cancers are summarised in fig 2.

Because many previous studies have not screened *MET* extracellular domain sequences,^{11, 14} it is possible that other

Figure 2 A spectrum of *MET* germline mutations in papillary renal carcinomas and gastric cancers. Numbered boxes indicate the exons of *MET*. (1) *MET* germline mutations reported by Schmidt *et al*⁷; (2) reported by Schmidt *et al*⁸; (3) reported by Schmidt *et al*¹⁰; (4) reported by Olivero *et al*⁹; (5) reported by Lee *et al*.¹¹ *Identified in this work.

patients harbour *MET* mutations in this domain. Alternatively, it was suggested that *MET* mutations might be specific to people of Asian or Korean ancestry.¹⁴ In support of this, no *MET* germline mutations were found in 18 white or Indian gastric cancer kindreds.¹⁴ However, although all *MET* germline mutations found in gastric cancer patients to date have been associated with Koreans, further investigation will be required to determine the ethnic specificity of these mutations.

In addition to ethnic specificity, it is important to examine the type specificity of various mutations. *CDH1* germline mutations were found in diffuse gastric cancer patients, while the previously identified *MET* mutation (P1009S) was found in an intestinal gastric cancer patient. This led researchers to suggest a correlation between *MET* mutations and intestinal gastric cancer.¹⁴ However, patient SNU-G7 suffered from the diffuse type of gastric cancer, suggesting that further research is needed to address the association between *MET* mutations and the gastric cancer types.

Family history was not available for the patient described by Lee *et al*¹¹ (harbouring the P1009S *MET* mutation),¹⁴ making this the first report of a *MET* germline mutation in a patient with familial gastric cancer. However, as the frequency of both *MET* and *CDH1* mutations is quite low in Korean gastric cancer patients, it seems that neither gene plays a major role in familial gastric cancer in this population. Further studies on the mechanism of *MET* and identification of other genes involved in gastric cancer development will be required.

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