

Infrequent Germ-line Mutation of the E-cadherin Gene in Japanese Familial Gastric Cancer Kindreds¹

Satoru Iida, Yoshimitsu Akiyama,
Wataru Ichikawa, Toshiki Yamashita,
Tadashi Nomizu, Zenro Nihei, Kenichi Sugihara,
and Yasuhito Yuasa²

Second Department of Surgery [S. I., W. I., T. Y., Z. N., K. S.] and Department of Hygiene and Oncology [Y. A., Y. Y.], Tokyo Medical and Dental University School of Medicine, Tokyo 113-8519, and Department of Surgery, Hoshi General Hospital, Fukushima 963 [T. N.], Japan

ABSTRACT

Germ-line mutation of the E-cadherin gene was reported in familial gastric cancer (FGC) kindreds from New Zealand. Therefore, we analyzed all of the exons of E-cadherin by PCR-single-strand conformational polymorphism analysis in 16 patients from 14 Japanese FGC kindreds. However, no germ-line mutation was detected, suggesting that a predisposition to FGCs by E-cadherin gene mutation is infrequent in Japanese cases.

INTRODUCTION

Despite a decreasing incidence, gastric cancer remains a major cause of cancer death worldwide (1). Epidemiological studies have shown that there is familial clustering of gastric cancers (2–4). Various reasons, including shared environmental carcinogenesis or inherited gene alterations, have been proposed, but the analysis of such cases has been limited to date.

In contrast, studies on colorectal cancers have revealed many markers that provide evidence of a genetic predisposition. MSI³ at simple repeated sequences has been reported in HNPCC (5, 6), which is associated with defects in mismatch repair genes (7, 8). MSI has also been found in several types of sporadic cancers, including gastric cancers (9–11). MSI may play an important role in the development of gastric cancers, but the

incidences of MSI have been quite different in the reports on MSI in gastric cancers with a family history (12–16). Thus, it is not clear whether or not MSI is also related to FGC.

Recently, in three FGC kindreds in New Zealand, germ-line mutations of the E-cadherin gene were found (17). E-cadherin is a member of a family of transmembrane glycoproteins that are responsible for calcium-dependent cell-cell adhesion and also appear to play a role in organogenesis and morphogenesis (18). Loss or reduction of E-cadherin expression has been demonstrated immunohistochemically in several types of human carcinomas, including gastric carcinomas (19–21). Somatic mutations of the E-cadherin gene have been identified in sporadic, histologically diffuse gastric carcinomas (22–24). In the present study, to determine whether or not germ-line mutation of the E-cadherin gene is also responsible for the predisposition to Japanese FGC, we investigated germ-line mutations of it in Japanese FGC kindreds by PCR-SSCP analysis.

MATERIALS AND METHODS

Subjects. Identification of patients with a family history of gastric cancer was carried out according to the following criteria: (a) at least three relatives should have gastric cancer, and one of them should be a first-degree relative of the other two. Other hereditary tumors, such as cancer family syndrome (Lynch II) of HNPCC (25), should be excluded; and (b) at least two successive generations should be affected. In this study, 14 families satisfying these criteria were collected.

Genomic DNA was extracted from surgically resected tumor tissues and corresponding normal tissues and from EBV-transformed lymphoblastoid cell lines or peripheral blood karyocytes as described previously (26, 27).

PCR-SSCP Analysis. According to the exon-intron boundary sequences (22, 28), 36 sets of primers were designed to amplify all 16 exons, including each splicing site, of the E-cadherin gene. The sequences of the primers used for amplification and sequencing of the E-cadherin gene are available from the authors on request. PCR was performed in 25- μ l reaction mixtures comprising 20–100 ng of template DNA, 5–10 pmol of each oligonucleotide primer pair, 2.5 units of Taq DNA polymerase (Biotech International, Ltd., Bentley, Australia), 2.5 μ l of 10 \times buffer, and 4 μ l of 1.25 mM deoxynucleotide triphosphate (Pharmacia, Uppsala, Sweden). Each PCR comprised 35 cycles of 94°C (1 min), 50°C–66°C (2 min), and 72°C (1 min), with a final 10-min extension at 72°C. PCR-SSCP analysis was performed as described previously (29). Briefly, the PCR products were denatured and then electrophoresed on 12.5% nondenaturing polyacrylamide gels containing 10% glycerol in Tris-glycine buffer [25 mM Tris-HCl and 200 mM glycine (pH 8.3)]. We determined the optimal condition for SSCP analysis in each primer set. After electrophoresis, the gels were stained with silver (Dai-ichi Co., Ltd., Tokyo, Japan).

Received 9/14/98; revised 3/4/99; accepted 3/16/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported in part by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture of Japan (to Y. Y. and Y. A.) and by a research grant from the Uehara Memorial Foundation (to Y. Y.).

² To whom requests for reprints should be addressed, at Department of Hygiene and Oncology, Tokyo Medical and Dental University School of Medicine, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. Phone: 81-3-5803-5182; Fax: 81-3-5803-0125; E-mail: yyuasa.hgen@med.tmd.ac.jp.

³ The abbreviations used are: MSI, microsatellite instability; HNPCC, hereditary nonpolyposis colorectal cancer; FGC, familial gastric cancer; SSCP, single-strand conformational polymorphism.

Table 1 Clinicopathological findings for the 16 FGC cases

| Family | Patients | Age (yr)/sex | Histology ^a | T ^b | Location ^c |
|--------|----------|--------------|------------------------|----------------|-----------------------|
| 1 | G1 | 71/M | I | T ₁ | L |
| 1 | G6 | 57/M | D | T ₂ | L |
| 2 | G2 | 63/M | I | T ₁ | L |
| 2 | G3 | 35/M | D | T ₂ | L |
| 3 | G15 | 58/F | I | T ₂ | L |
| 4 | G13 | 58/M | D | T ₃ | M |
| 5 | G14 | 17/M | D | T ₃ | U |
| 6 | G16 | 65/M | I | T ₁ | M |
| 7 | G17 | 73/F | I | T ₁ | L |
| 9 | G19 | 65/M | D | T ₃ | M |
| 10 | G20 | 60/M | I | T ₁ | L |
| 11 | G21 | 54/M | D | T ₃ | L |
| 12 | G22 | 38/M | D | T ₃ | M |
| 13 | G23 | 66/M | I | T ₂ | L |
| 14 | G24 | 63/M | D | T ₃ | L |
| 15 | G25 | 62/M | I | T ₁ | L |

^a Histological classification was performed according to Lauren's criteria. I, intestinal; D, diffuse.

^b Tumor classification according to the American Joint Committee on Cancer. T₁, invasion of lamina propria or submucosa; T₂, invasion of muscularis propria; T₃, penetration of serosa.

^c L, lower third; M, middle third; U, upper third.

Sequencing. When abnormal patterns were observed on SSCP analysis, the PCR products were cloned into the pT7Blue(R) T-vector (Novagen, Madison, WI) and then sequenced with a cycle sequencing kit (Takara Shuzo Co., Ltd., Kyoto, Japan).

RESULTS AND DISCUSSION

Sixteen patients from the 14 families satisfied the criteria for FGC. The clinicopathological findings in these cases are summarized in Table 1. In 8 of the 14 FGC kindreds, there was at least one case diagnosed before the age of 50 years. Histologically, the 16 FGC cases consisted of 8 cases with intestinal-type carcinomas and 8 cases with diffuse-type carcinomas according to Laurén's criteria (30).

On PCR-SSCP analysis of all of the E-cadherin exons using the 36 sets of primers, no germ-line mutation was detected in any exon of E-cadherin using genomic DNA of the 16 FGC patients. However, two variants were identified. The first variant is located in the third primer set of exon 12 from a healthy individual (Fig. 1A). The PCR product was subcloned and then sequenced. The variant was a C to G transversion at the first base of codon 630, resulting in the substitution of valine for leucine. None of the 16 FGC patients showed a heterozygous pattern as to this polymorphic site.

The second variant is a silent mutation caused by a C to T transition at the third base of codon 692 (Fig. 1B), as described previously (28, 31). Because this nucleotide change destroys a restriction site for *MspI*, the PCR products derived from the normal DNA of the 16 FGC patients were digested with *MspI* and then electrophoresed on 12.5% polyacrylamide gels. At this polymorphic site, 7 of the 16 (43.8%) patients were heterozygous. None of the seven informative cases exhibited loss of heterozygosity in their corresponding cancers.

We searched for mutations in all of the exons of the E-cadherin gene by SSCP in the Japanese FGC kindreds. How-

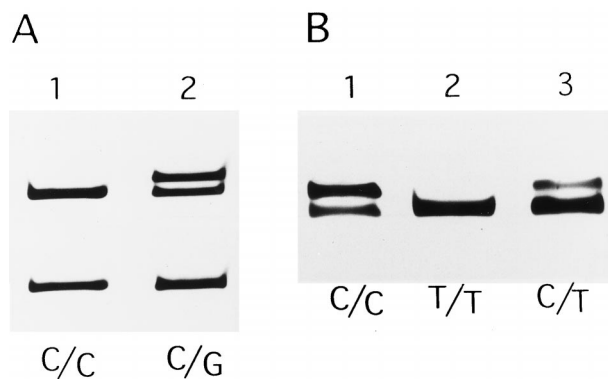


Fig. 1 PCR-SSCP analysis of the E-cadherin gene. A, the third primer set of exon 12. A normal SSCP pattern is shown in Lane 1. A variant band from a healthy individual is seen in Lane 2. This pattern was shown to represent a polymorphism of codon 630 (CTA to GTA) by sequencing analysis. B, the third primer set of exon 13. SSCP patterns shown in Lanes 1 and 2 represent C/C and T/T homozygosity at nucleotide position 2076, respectively. A pattern shown in Lane 3 exhibits C/T heterozygosity. This polymorphism was confirmed by *MspI* digestion.

ever, no germ-line mutation was detected in the 16 patients from the 14 kindreds. It is possible that the SSCP technique may not be sufficient to detect all mutations of E-cadherin, although we determined the optimal SSCP conditions. Nevertheless, our data indicate that the frequency of a predisposition to FGC by an E-cadherin gene mutation may be infrequent in Japanese FGC cases. No loss of heterozygosity was seen in the E-cadherin gene in the cancers from the seven informative cases, supporting less association of E-cadherin with FGC.

It is not known why there is a discrepancy between the New Zealand and Japanese cases. All three of the New Zealand FGC kindreds, which have germ-line mutations in E-cadherin, suffered from diffuse-type gastric carcinomas (17). However, this does not explain the discrepancy because there are also eight cases of the diffuse type among the Japanese cases. Other cancer-related genes may be responsible for the Japanese FGC cases. Some extrinsic factors, such as carcinogens or *Helicobacter pylori* infection, may also contribute to the difference.

There have been several reports on MSI in FGC (12–16), which may be induced by germ-line mutations in one of the mismatch repair genes like HNPCC. However, only a germ-line missense mutation of the *hMLH1* gene has been reported in a German FGC patient thus far (32). We could not detect any germ-line mutation of *hMSH2*, *hMSH3*, *hMSH6*, or *hMLH1* in our four MSI-positive Japanese FGC kindreds (14).⁴ Thus, it is likely that mismatch repair genes are not major causative genes for FGC.

In conclusion, the E-cadherin gene may not be responsible for most Japanese FGC cases. Additional studies are necessary to elucidate the nature of FGC.

After this article was submitted for publication, S. A. Gayther *et al.* (33) reported that they had identified germ-line

⁴ Unpublished observations.

E-cadherin mutations in 3 of 10 diffuse-type and 0 of 8 intestinal-type FGCs of European origin.

ACKNOWLEDGMENTS

We thank doctors for providing the specimens and Y. Takagi for excellent technical assistance.

REFERENCES

- Fuchs, C. S., and Mayer, R. J. Gastric carcinoma. *N. Engl. J. Med.*, 333: 32–41, 1995.
- Zanghieri, G., Di Gregorio, C., Sacchetti, C., Fante, R., Sassatelli, R., Cannizzo, G., Carriero, A., and Ponz de Leon, M. Familial occurrence of gastric cancer in the 2-year experience of a population-based registry. *Cancer (Phila.)*, 66: 2047–2051, 1990.
- La Vecchia, C., Negri, E., Franceschi, S., and Gentile, A. Familial history and the risk of stomach and colorectal cancer. *Cancer (Phila.)*, 70: 50–55, 1992.
- Kikuchi, S., Nakajima, T., Nishi, T., Kobayashi, O., Konishi, T., Inaba, Y., Wada, O., Satou, H., Ishibashi, T., Ichikawa, S., Okamoto, N., Hirata, T., Kubo, T., Sato, N., Miki, K., and Myoga, A. Association between family history and gastric carcinoma among young adults. *Jpn. J. Cancer Res.*, 87: 332–336, 1996.
- Aaltonen, L. A., Peltomäki, P., Leach, F. S., Sistonen, P., Pylkkänen, L., Mecklin, J. P., Järvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Petersen, G. M., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. *Science (Washington DC)*, 260: 812–816, 1993.
- Wu, C., Akiyama, Y., Imai, K., Miyake, S., Nagasaki, H., Oto, M., Okabe, S., Iwama, T., Mitamura, K., Masumitsu, H., Nomizu, T., Baba, S., Maruyama, K., and Yuasa, Y. DNA alterations in cells from hereditary non-polyposis colorectal cancer patients. *Oncogene*, 9: 991–994, 1994.
- Kinzler, K. W., and Vogelstein, B. Lessons from hereditary colorectal cancer. *Cell*, 87: 159–170, 1996.
- Akiyama, Y., Sato, H., Yamada, T., Nagasaki, H., Tsuchiya, A., Abe, R., and Yuasa, Y. Germ-line mutation of the *hMSH6/GTBP* gene in atypical hereditary nonpolyposis colorectal cancer kindred. *Cancer Res.*, 57: 3920–3923, 1997.
- Peltomäki, P., Lothe, R. A., Aaltonen, L. A., Pylkkänen, L., Nystrom-Lahti, Seruca, R., David, L., Holm, R., Ryberg, D., Haugen, A., Brdøgger, A., Borresen, A. L., and de la Chapelle, A. Microsatellite instability is associated with tumors that characterize the hereditary nonpolyposis colorectal carcinoma syndrome. *Cancer Res.*, 53: 5853–5855, 1993.
- Han, H-J., Yanagisawa, A., Kato, Y., Park, J-G., and Nakamura, Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res.*, 53: 5087–5089, 1993.
- Rhyu, M-G., Park, W-S., and Meltzer, S. J. Microsatellite instability occurs frequently in human gastric carcinoma. *Oncogene*, 9: 29–32, 1994.
- Zelada-Hedman, M., Iselius, L., Gunven, P., Weger, A., Norden-skjöld, M., Skoog, L., and Lindblom, A. Genetic rearrangements in sporadic and familial gastric carcinomas detected with microsatellite markers. *Eur. J. Surg. Oncol.*, 20: 667–673, 1994.
- Sasaki, A., Nagashima, M., Shiseki, M., Katai, H., Maruyama, K., Iwanaga, R., Akiyama, Y., Yuasa, Y., and Yokota, J. Microsatellite instability in gastric cancer prone family. *Cancer Lett.*, 99: 169–175, 1996.
- Akiyama, Y., Nagasaki, H., Nihei, Z., Iwama, T., Nomizu, T., Utsunomiya, J., and Yuasa, Y. Frequent microsatellite instabilities and analyses of the related genes in familial gastric cancers. *Jpn. J. Cancer Res.*, 87: 595–601, 1996.
- Ottini, L., Palli, D., Falchetti, M., D'Amico, C., Amorosi, A., Saieva, C., Calzolari, A., Cimoli, F., Tatarelli, C., De Marchis, L., Masala, G., Mariani-Costantini, R., and Cama, A. Microsatellite instability in gastric cancer is associated with tumor location and family history in a high-risk population from Tuscany. *Cancer Res.*, 57: 4523–4529, 1997.
- Shimura, K., Yin, W., Isogaki, J., Saitoh, K., Kanazawa, K., Koda, K., Yokota, J., Kino, I., Arai, T., and Sugimura, H. Stage-dependent evaluation of microsatellite instability in gastric carcinoma with familial clustering. *Cancer Epidemiol. Biomark. Prev.*, 6: 693–697, 1997.
- Guilford, P., Hopkins, J., Harraway, J., McLeod, M., McLeod, N., Harawira, P., Taite, H., Scouler, R., Miller, A., and Reeve, A. E. E-cadherin germline mutations in familial gastric cancer. *Nature (Lond.)*, 392: 402–405, 1998.
- Takeichi, M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science (Washington DC)*, 251: 1451–1455, 1991.
- Shiozaki, H., Tahara, H., Oka, H., Miyata, M., Kobayashi, K., Tamura, S., Iihara, K., Doki, Y., Hirano, S., Takeichi, M., and Mori, T. Expression of immunoreactive E-cadherin adhesion molecules in human cancers. *Am. J. Pathol.*, 139: 17–23, 1991.
- Schipper, J. H., Frixen, U. H., Behrens, J., Unger, A., Jahnke, K., and Birchmeier, W. E-cadherin expression in squamous cell carcinomas of head and neck: inverse correlation with tumor dedifferentiation and lymph node metastasis. *Cancer Res.*, 51: 6328–6337, 1991.
- Mayer, B., Johnson, J. P., Letil, F., Jauch, K. W., Heiss, M. M., Schildberg, F. W., Birchmeier, W., and Funke, I. E-cadherin expression in primary and metastatic gastric cancer: down-regulation correlates with cellular dedifferentiation and glandular disintegration. *Cancer Res.*, 53: 1690–1695, 1993.
- Oda, T., Kanai, Y., Oyama, T., Yoshiura, K., Shimoyama, Y., Birchmeier, W., Sugimura, T., and Hirohashi, S. E-cadherin gene mutations in human gastric carcinoma cell lines. *Proc. Natl. Acad. Sci. USA*, 91: 1858–1862, 1994.
- Becker, K. F., Atkinson, M. J., Reich, U., Becker, I., Nekarda, H., Siewert, J. R., and Höfler, H. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. *Cancer Res.*, 54: 3845–3852, 1994.
- Muta, H., Noguchi, M., Kanai, Y., Ochiai, A., Nawata, H., and Hirohashi, S. E-cadherin gene mutations in signet ring cell carcinoma of the stomach. *Jpn. J. Cancer Res.*, 87: 843–848, 1996.
- Lynch, H. T., and Smyrk, T. Hereditary nonpolyposis colorectal cancer (Lynch syndrome). *Cancer (Phila.)*, 78: 1149–1167, 1996.
- Blin, N., and Stafford, D. W. A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res.*, 3: 2303–2308, 1976.
- Goelz, S. E., Hamilton, S. R., and Vogelstein, B. Purification of DNA from formaldehyde-fixed and paraffin-embedded human tissue. *Biochem. Biophys. Res. Commun.*, 130: 118–126, 1985.
- Berx, G., Cleton-Jansen, A-M., Nollet, F., de Leeuw, W. J. F., van de Vijver, M. J., Cornelisse, C., and van Roy, F. E-cadherin is a tumour/invasion suppressor gene mutated in human lobular breast cancers. *EMBO J.*, 14: 6107–6115, 1995.
- Oto, M., Miyake, S., and Yuasa, Y. Optimization of nonradioisotopic single strand conformation polymorphism analysis with a conventional minislabs gel electrophoresis apparatus. *Anal. Biochem.*, 213: 19–22, 1993.
- Laurén, P. The two histological main types of gastric carcinoma: diffuse and so called intestinal-type of carcinoma. *Acta Pathol. Microbiol. Scand.*, 64: 31–49, 1965.
- Risinger, J. I., Berchuck, A., Kohler, M. F., and Boyd, J. Mutation of the E-cadherin gene in human gynecologic cancers. *Nat. Genet.*, 7: 98–102, 1994.
- Keller, G., Grimm, V., Vogelsang, H., Bischoff, P., Mueller, J., Siewert, J. R., and Höfler, H. Analysis for microsatellite instability and mutations of the DNA mismatch repair gene *hMLH1* in familial gastric cancer. *Int. J. Cancer*, 68: 571–576, 1996.
- Gayther, S. A., Goringe, K. L., Ramus, S. J., Huntsman, D., Roviello, F., Grehan, N., Machado, J. C., Pinto, E., Seruca, R., Halling, K., MacLeod, P., Powell, S. M., Jackson, C. E., Ponder, B. A. J., and Caldas, C. Identification of germ-line E-cadherin mutations in gastric cancer families of European origin. *Cancer Res.*, 58: 4086–4089, 1998.

Clinical Cancer Research

Infrequent Germ-line Mutation of the E-cadherin Gene in Japanese Familial Gastric Cancer Kindreds

Satoru Iida, Yoshimitsu Akiyama, Wataru Ichikawa, et al.

Clin Cancer Res 1999;5:1445-1447.

Updated version Access the most recent version of this article at:
<http://clincancerres.aacrjournals.org/content/5/6/1445>

Cited articles This article cites 32 articles, 12 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/5/6/1445.full#ref-list-1>

Citing articles This article has been cited by 7 HighWire-hosted articles. Access the articles at:
<http://clincancerres.aacrjournals.org/content/5/6/1445.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://clincancerres.aacrjournals.org/content/5/6/1445>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.