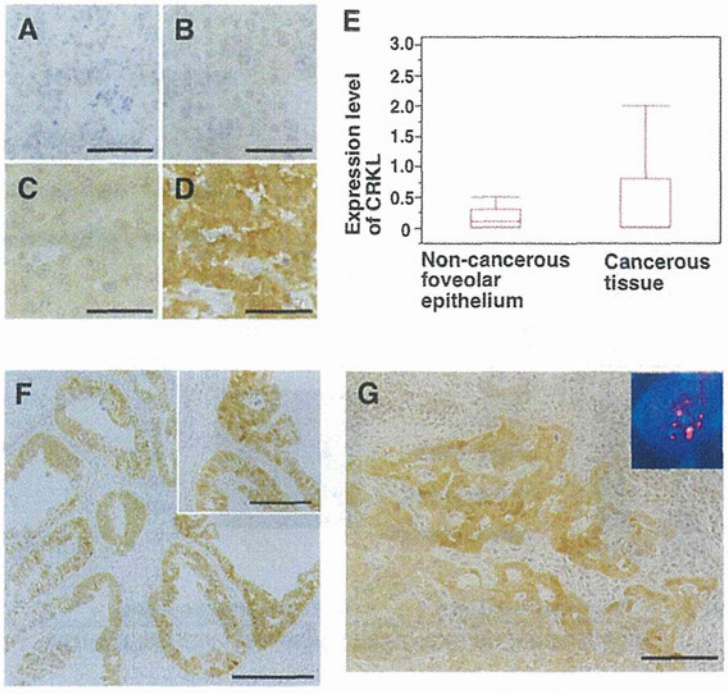


**Figure 3 Immunohistochemical detection of CRKL protein in primary gastric cancer.** TMA block sections were subjected to an immunohistochemical analysis using anti-CRKL monoclonal antibody (Y243; 1:100 dilution), Histofine Simple Stain Max-Po (Multi), and 3,3'-diaminobenzidine tetrahydrochloride. Intensity values of 0, 1, 2, and 3 are shown in **(A)**, **(B)**, **(C)**, and **(D)**, respectively. Bar = 50 μm. **(E)** Box-plot analysis of CRKL protein expression in gastric tissue. A statistically significant difference in the CRKL expression level was detected between non-cancerous gastric foveolar epithelium ($n = 41$) and gastric cancerous tissue ($n = 360$). **(F)** Representative result of the CRKL immunohistochemical analysis. A gastric cancer with a high CRKL expression level is shown. Bar = 500 μm. The inset is a magnified image. Bar = 50 μm. **(G)** Representative gastric cancer case showing both a high CRKL expression level and *CRKL* gene amplification. The high CRKL expression level (value = 2.6) was detected using an immunohistochemical analysis. Bar = 100 μm. The inset shows the amplification of *CRKL* (red) in the cancer cells. The *CRKL* signal (red) and the control signal for chromosome 22 (green) were detected using a FISH analysis. Nuclei are stained with DAPI.

### Preparation of CRKL targeting peptide

In this study, we used the peptides, which has been reported to be disrupted complexes between BCR-ABL and CRKL depend on the SH3 domain of CRKL in CML cells [26]. Peptides used in the experiments are followed: CRKL-targeting peptide; KKW KMR RNP FWI KIQ RC – CGI RVV DNS PPP ALP PKR RRS APS PTR V, control peptide; KKW KMR RNP FWI KIQ RC – CGI RVV DNS PPG ALG PLL RRS APS PTR V. The KKW KMR RNP FWI KIQ RC was the shuttle tag sequence performing a receptor-independent cell entry. The chimeric peptide was synthesized and purified by using reverse-phase high performance liquid chromatography (HPLC) (Toray Research Center, Otsu, Japan). Peptide stocks were prepared in DMSO and stored in aliquots at -80°C.

### Statistical analysis

The statistical analysis was performed using an unpaired *t*-test, chi-square test, or Dunnett's test. JMP version 7.0.1 software (SAS Institute, Cary, NC) was used for the analyses. *P* values less than 0.05 were considered statistically significant.

## Results

### Identification of *CRKL* amplification in gastric cancer

To search for highly amplified genes in gastric adenocarcinoma, we adopted a genome-wide high-resolution SNP microarray approach in three cell lines of differentiated gastric adenocarcinoma: MKN7, MKN28, and MKN74. Genotype calls were obtained at more than 95% of the 262,264 SNP sites on the array, meaning that the SNP microarray analysis had been performed properly. The SNP microarray data were then used to determine the chromosomal copy number using the CNAG program (Figures 1A and 1B). Five highly amplified regions with a copy number of more than 6 (9p13, 17q12-q21, 19q12, 19q13, and 22q11) were identified, as shown in Table 1. These regions contained various kinds of genes, a total of 22 genes (Table 1). Among them, we decided to focus on the *CRKL* gene at chromosome 22q11.21, the product

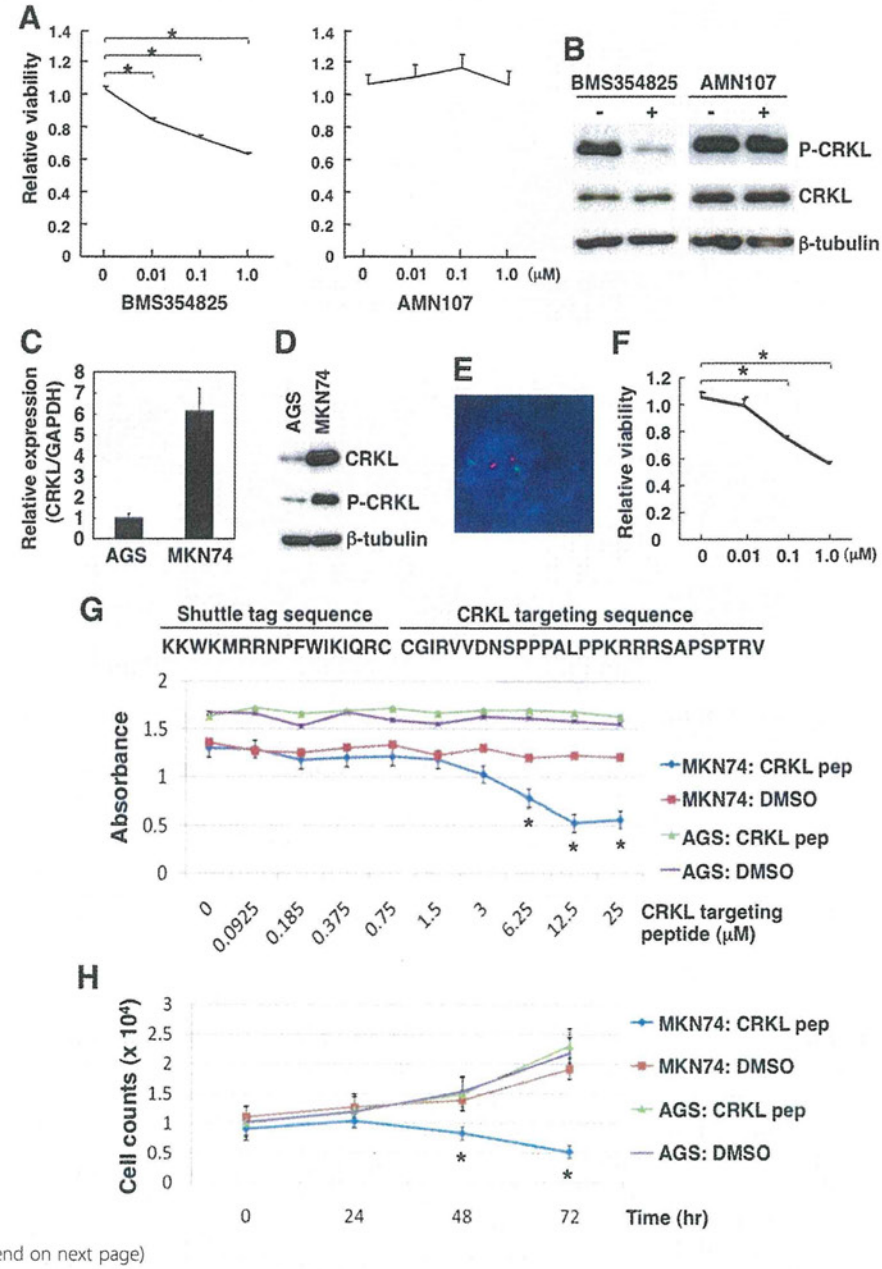


**Figure 4** (See legend on next page)

of which is an SH2 and SH3 domain-containing adaptor protein that shares homology with the CRK oncoprotein, because CRKL is a known substrate of BCR-ABL kinase in Philadelphia chromosome-positive leukemia [27,28] and its role in gastric cancer has not been previously analyzed. To confirm that *CRKL* gene amplification was detectable in the MKN74 cell line, we performed a FISH analysis using a probe specific for *CRKL*. As expected, an extreme increase in the *CRKL* copy number was detected in the MKN74 cells using a FISH analysis (Figure 1C). When the level of CRKL mRNA expression was examined in MKN74 cells using a real-time QRT-PCR analysis, the level was much higher than that in non-cancerous gastric tissue (Figure 1D). Moreover, a western blot analysis showed that the level of CRKL protein expression was higher in MKN74 cells than in non-cancerous gastric tissue (Figure 1E). These results suggested that the *CRKL* gene is highly amplified and that CRKL is overexpressed in a subset of gastric cancer cell lines.

(See figure on previous page)

**Figure 4 Responses of the MKN74 gastric cancer cell line with *CRKL* amplification to treatment with BMS354825 (a dual Src/BCR-ABL kinase inhibitor) and CRKL-targeting peptide. (A)** Viability of MKN74 cells treated with BMS354825 but not those treated with AMN107 (a highly selective BCR-ABL kinase inhibitor) is decreased. The cells were seeded in 96-well microplates at a density of $1 \times 10^4$ per well; after 24 h, the drug (0.01–1.0 μM) or 0.1% DMSO solution was added. Viability was examined in the MKN74 cells after 72 h of treatment at the indicated concentration using WST-8 reagent. The number of viable cells after treatment with each inhibitor was normalized to the number of viable cells without treatment, and the relative viability is shown in the graph. Values are the mean ± standard error. *P* values were calculated using the Dunnett's multiple comparison test, and * indicates a statistically significant decrease. **(B)** Effective inhibition of CRKL phosphorylation in MKN74 cells treated with BMS354825. Cells were treated with each inhibitor (DMSO only or 0.01 μM of drug) for 90 min, and the expression of CRKL protein was examined using a western blot analysis with anti-phospho CRKL polyclonal antibody (Y207; 1:1,000 dilution) or anti-CRKL monoclonal antibody (Y244; 1:500 dilution). The expression of β-tubulin protein was analyzed as an internal control. **(C)** Comparison of CRKL mRNA transcripts between AGS and MKN74 cells using real-time QRT-PCR analysis. The amounts of CRKL transcripts normalized to the amount of GAPDH transcripts are shown in the graph. **(D)** Comparison of expression of CRKL protein between AGS and MKN74 cells using a western blot analysis. The expression of CRKL was examined using the primary antibodies shown in (B). The expression of β-tubulin protein was analyzed as an internal control. **(E)** Detection of *CRKL* gene copy number in AGS cells using a FISH analysis. The *CRKL* signal is red, and the control signal for chromosome 22 is green. Nuclei are stained with DAPI. **(F)** Viability of AGS cells decreased after BMS354825 treatment. Viability was examined as described in (A). Values are the mean ± standard error. *P* values were calculated using a *t*-test, and * indicates a statistically significant decrease. **(G)** MKN74 cells with *CRKL* amplification and AGS cells without *CRKL* amplification were seeded in 96-well microplates at a density of $1 \times 10^4$ per well. 24 h after seeding, cells were treated with CRKL-targeting peptide (0.0925–25 μM) or 0.2% DMSO solution at the indicated concentration. The sequence of the CRKL-targeting peptide that was used is shown above the graph. After 72 h of incubation, viability was determined using an MTT assay. The results are presented as the mean ± standard deviation of three independent experiments. *P* values were calculated using a *t*-test, and * indicates a statistically significant difference between the cells treated with CRKL-targeting peptide and those treated with DMSO. **(H)** Cell proliferation of MKN74 and AGS cells treated with CRKL-targeting peptide (6.25 μM) or DMSO as measured by counting cells using a hemocytometer. Cells ($1 \times 10^4$) were seeded in 24-well plates and treated with CRKL-targeting peptide or DMSO. The cell counting was performed every 24 h for 3 days. Data are shown as the mean ± standard deviation of three independent experiments. *P* values were calculated using a *t*-test, and * indicates a statistically significant difference between the cells treated with CRKL-targeting peptide and those treated with DMSO.

### Ability of CRKL to control gastric cell proliferation

To explore the functional significance of *CRKL* amplification in gastric cancer, we attempted to examine the effect of overexpressed CRKL on gastric cell proliferation. For this purpose, we prepared MKN74 cells with distinct CRKL expression levels using the siRNA knockdown of CRKL expression. CRKL-specific siRNA transfection effectively decreased the level of CRKL protein expression in MKN74 cells by approximately 70% of the levels observed in negative control siRNA-transfected cells (Figure 2A). A cell proliferation assay showed that the number of CRKL siRNA-transfected MKN74 cells was significantly lower at 3 and 4 days after transfection than the number of negative control siRNA-transfected cells (Figure 2B), meaning that CRKL has the ability to upregulate cell proliferation.

**Table 1 Detection of chromosomal regions with a high copy number (more than 6) in the gastric cancer cell lines MKN7, MKN28, and MKN74 using a genome-wide SNP microarray analysis**

| Chromosomal regions[a] | Genes with a high copy number in the region |
|---|---|
| 9p13 | *PAX5* |
| 17q12-q21 | *FBXL20, MED1, PERLD1, ERBB2, IKZF3, ZPBP2* |
| 19q12 | *CCNE1* |
| 19q13 | *CD22* |
| 22q11 | *DGCR8, USP41, ZNF74, SCARF2, KLHL22, MED15, PI4KA, SERPIND1, SNAP29, CRKL, THAP7, P2RX6, LOC729526* |

[a] If more than four consecutive SNP probes with a copy number of more than six were detected in either of the three cell lines, the chromosomal region was regarded as being a "highly amplified region" and was listed in this table.

### Overexpression of CRKL protein in gastric cancer

Next, we investigated the expression status of CRKL protein in primary gastric cancer using an immunohistochemical analysis with anti-CRKL monoclonal antibody (Y243). CRKL was mainly observed in the cytoplasm, consistent with previous reports [29]. When we compared the level of CRKL expression between non-cancerous gastric foveolar epithelium ($n = 41$) and gastric cancer ($n = 360$), the level of CRKL expression in gastric cancer (mean ± standard deviation = 0.42 ± 0.63) was significantly higher than that in non-cancerous tissue (0.20 ± 0.26) ($P = 0.032$) (Figures 3A-3E). When an expression level of 1.00, which corresponds to a value 5-fold of the mean expression level in non-cancerous gastric foveolar epithelium, was used as a cutoff value for the expression status in gastric cancer (i.e., low expression group, 0–0.99; high expression group, 1.00–3.00), 88 (24.4%) of the 360 primary gastric cancers were included in the high expression group (Figure 3F). To examine whether CRKL overexpression is associated with *CRKL* amplification in gastric cancer, we performed a FISH analysis for the *CRKL* gene in the 360 primary gastric cancers and compared the prevalence of *CRKL* amplification between the low expression group and the high expression group. As

expected, the percentage of gastric cancer cells with *CRKL* amplification was significantly higher in the high expression group (9.1%; 8/88 cases) than in the low expression group (2.2%; 6/272 cases) ($P = 0.028$, chi-square test). This result suggests that *CRKL* amplification contributes to CRKL overexpression in primary gastric cancer. We further investigated whether the levels of CRKL expression is associated with clinicopathological features in primary gastric cancer patients, the high CRKL expression was observed significantly more often in male and differentiated-type gastric cancer (Table 2). These results suggested that CRKL protein is overexpressed partly due to *CRKL* amplification in a subset of primary gastric cancers and is associated with the gender and histopathology.

### Decrease in the viability of CRKL-expressing MKN74 cells treated with BMS354825

Finally, we tested the possibility of using CRKL as a therapeutic target in MKN74 cells with *CRKL* amplification. Since Philadelphia chromosome-positive leukemia expressing BCR-ABL is responsive to BMS354825 (a dual Src/BCR-ABL kinase inhibitor) and AMN107 (a highly selective BCR-ABL kinase inhibitor) [22,24], we checked the response of MKN74 cells to both inhibitors. Cell viability was significantly decreased in BMS354825-treated (0.01–1.0 μM) MKN74 cells, compared with cells treated with the solvent only, while it was not significantly decreased in AMN107-treated cells (Figure 4A). When the status of CRKL phosphorylation was examined in the MKN74 cells using western blot analysis with an anti-phospho CRKL antibody, CRKL phosphorylation was found to be inhibited more effectively by BMS354825 than by AMN107 (Figure 4B). These results suggested that BMS354825 has the potential to suppress the viability of MKN74 cells expressing CRKL, likely via the inhibition of CRKL phosphorylation.

To further characterize the role of CRKL in the BMS354825-induced suppression of MKN74 cell viability, we examined the effect of BMS354825 on gastric cancer cells without *CRKL* amplification. Since the AGS gastric cancer cell line had lower CRKL mRNA and CRKL protein expression levels than MKN74 cells (Figures 4C and D) and had a normal *CRKL* genomic copy number (Figure 4E), these cells were treated with BMS354825. Unexpectedly, the viability of the BMS354825-treated (0.1–1.0 μM) AGS cells decreased significantly (Figure 4F). Moreover, although the $IC_{50}$ value (inhibitory concentration producing a 50% response) for BMS354825 was slightly higher in AGS cells than in MKN74 cells, the values were not much different between AGS and MKN74 cells (data not shown). These results suggest that BMS354825 has the potential to suppress the viability of AGS cells, likely via a CRKL-independent pathway.

**Table 2 Association between CRKL expression and clinicopathological factors in 360 patients with primary gastric cancer**

| | | CRKL expression level | | |
|---|---|---|---|---|
| Factor | Patient | Low ($n = 272$) | High ($n = 88$) | *P* |
| Age | | | | |
| Year, mean ± SD[a] | 62.0 ± 11.2 | 61.7 ± 11.7 | 62.9 ± 11.4 | 0.3936[b] |
| (range) | (29–86) | (29–86) | (31–85) | |
| Gender | | | | |
| Male | 255 | 182 (66.9%) | 73 (83.0%) | 0.0028[c] |
| Female | 105 | 90 (33.1%) | 15 (17.0%) | |
| Histological type | | | | |
| Differentiated | 172 | 118 (43.4%) | 54 (61.4%) | 0.0033[c] |
| Undifferentiated | 188 | 154 (56.6%) | 34 (38.6%) | |
| pT stage | | | | |
| pT1 | 143 | 103 (37.9%) | 40 (45.5%) | 0.2082[c] |
| pT2-pT4 | 217 | 169 (62.1%) | 48 (54.5%) | |

[a] SD, standard deviation. [b] *t*-test. [c] Chi-square test.

### Decrease in the viability/proliferation of CRKL-expressing MKN74 cells treated with a CRKL-targeting peptide

We then planned to use a more specific inhibitor of CRKL and examined the response of MKN74 and AGS cells to a CRKL-targeting peptide [26]. Cell viability decreased significantly in MKN74 cells treated with the CRKL-targeting peptide (6.25–25 μM), compared with DMSO (solvent)-treated cells, but a similar decrease was not found in AGS gastric cancer cells without *CRKL* amplification (Figure 4G). When cell proliferation was compared after treatment with 6.25 μM of the CRKL-targeting peptide, the cell proliferation was significantly suppressed in MKN74 cells treated with the peptide, compared with DMSO-treated MKN74 cells, but no inhibition of cell proliferation was seen in the AGS cells (Figure 4H). Control peptide had no effect on the gastric cancer cell proliferation. These results suggested that the CRKL-targeting peptide has the potential to suppress the viability/proliferation of gastric cells exhibiting *CRKL* amplification, but not of gastric cells that do not exhibit *CRKL* amplification.

## Discussion

Through a genome-wide SNP microarray analysis performed in this study, the *CRKL* gene was identified as a highly amplified gene in gastric cancer. An increase in the copy number was confirmed in MKN74 gastric cancer cells with *CRKL* amplification using a FISH analysis, and a high CRKL expression level was also observed in these cells. The ability of CRKL to upregulate cell proliferation was shown in MKN74 cells by comparing the cell proliferation rate between CRKL siRNA-transfected cells and negative control siRNA-transfected cells. CRKL

protein was overexpressed in 24.4% of the primary gastric cancers, and its level in the gastric cancer was associated with the gender and histopathology. *CRKL* amplification was more frequently found in primary gastric cancers with high CRKL protein expression levels than in those with low CRKL expression levels. Finally, we showed that MKN74 cells with *CRKL* amplification were responsive to the kinase inhibitor BMS354825, likely via the inhibition of CRKL phosphorylation, and a CRKL-targeting peptide. Our current findings suggest that CRKL has an important role in the development of a subset of gastric cancers and has the potential to be a molecular therapy target for gastric cancer.

CRKL is an adaptor cell signaling protein that contains an SH2 domain and two tandem SH3 domains, both of which mediate protein-protein interactions [27,28,30]. CRKL is well known as a surrogate substrate of BCR-ABL kinase in chronic myeloid leukemia and acute lymphoblastic leukemia [11,27,28], and intensive studies of CRKL in Philadelphia chromosome-positive leukemia have been performed. However, only one paper by Kim *et al.* [31] has reported the CRKL status in gastric cancer. They revealed that the expression of CRKL mRNA in a cancer cell line was stimulated by proteins released by *Helicobacter pylori*, although the underlying mechanism was not resolved and the *CRKL* genomic copy number was not analyzed. Our genome-wide SNP microarray analysis successfully revealed, for the first time, that the *CRKL* gene is highly amplified in a subset of gastric cancers. We also showed that the CRKL protein can upregulate cell proliferation using the RNA-interference-mediated knockdown of CRKL in a gastric cancer cell line with *CRKL* amplification. Thus, CRKL overexpression arising from genomic amplification likely contributes to the aggressiveness of gastric cancer.

Recent progress in the development of molecular cancer therapy has revealed new molecular-targeting drugs, such as EGFR-targeting drug ZD1839 (Iressa) and HER2-targeting anti-HER2 monoclonal antibody trastuzumab (Herceptin), to be potent therapies for specific cancers [32-34]. In this study, BMS354825, a dual inhibitor for Src and BCR-ABL kinases, but not AMN107, a BCR-ABL specific inhibitor, showed an inhibitory effect on the survival of MKN74 cells with *CRKL* amplification. A decrease in CRKL phosphorylation through the inhibition of a currently unknown Src kinase seems to be one of the main mechanisms of BMS354825-mediated cytotoxicity in MKN74 cells. BMS354825 is currently being studied clinically in colorectal cancer, prostate cancer, breast cancer, lung cancer, and Philadelphia chromosome-positive leukemia [22,23,35]. Our results suggest that the CRKL protein may be a target of BMS354825-mediated therapy for a subset of gastric cancers. In our analyses, BMS354825 suppressed the viability of AGS cells without *CRKL* amplification as well as the viability of MKN74 cells with *CRKL* amplification, suggesting that a CRKL-independent pathway, which has been previously implicated [36], may also be involved in the BMS354825-mediated cytotoxicity seen in gastric cancers. We also presented the usefulness of a CRKL-targeting peptide for suppressing the proliferation of MKN74 cells with *CRKL* amplification. Our results should contribute to the establishment of CRKL-targeting therapy for a subset of gastric cancers in the future.

In the present study, a genome-wide, high-resolution SNP microarray analysis was successfully performed and five highly amplified chromosome regions containing 22 genes were identified in gastric cancers, as listed in Table 1. Although the *ERBB2* gene, a well-known oncogene that is often amplified in gastric cancer [4], was included in this list, the roles of the most of the genes in the Table have not been studied in gastric cancer. Further investigation of these roles is needed in the future.

## Conclusion

We conclude that CRKL protein is overexpressed in a subset of gastric cancers and is associated with *CRKL* amplification in gastric cancer. Furthermore, we conclude that CRKL protein has the ability to regulate gastric cell proliferation and has the potential to serve as a molecular therapy target for gastric cancer.

### Abbreviations

DAPI: 4',6-diamidino-2-phenylindole; DMSO: Dimethyl sulfoxide; FISH: Fluorescence *in situ* hybridization; QRT-PCR: Quantitative reverse-transcription-polymerase chain reaction; SNP: Single nucleotide polymorphism; siRNA: Small interfering RNA; TMA: Tissue microarray; SH2/SH3: Src homology 2 and 3.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

HN performed the experiments and wrote the paper draft. KS and SN interpreted the data and revised the paper. HT, HI, MS, KN, MG, SN,

and HY performed a part of the experiments. MM and HK provided tissue samples. SN performed a part of the experiments and was involved in the experimental design. HS conceived the research, designed the experiment, and revised the paper. All authors have read and approved the manuscript.

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Review Article Open Access

# Assessment Scales for Nicotine Addiction

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## Abstract

The genetics of nicotine addiction has been probed using combinations of genetic markers and questionnaire results regarding individual smoking behavior. Recently, the association of nicotine addiction with various candidate gene or gene polymorphisms has been proposed based on genome-wide association studies and candidate gene approaches, but the assessment of smoking behaviors including how severely each smoker is addicted, is typically performed using limited measures such as questionnaires. In this review, we present part of our recent data in which different scaling methods detected different genetic polymorphisms associated with different aspects of addicted smoking behaviors, as determined using questionnaire responses and genotyping data for 2500 Japanese elderly subjects. Several scaling methods have been developed to estimate nicotine addiction; here, we briefly review four scaling methods in addition to the Fagerström Tolerance Questionnaire (FTQ) and the Fagerström Test for Nicotine Dependence (FTND): The Tobacco Dependence Screener (TDS), the Wisconsin Inventory of Smoking Dependence Motives (WISDM), the Cigarette Dependence Scale (CDS), and the Nicotine Dependence Syndrome Scale (NDSS). The characteristics and powers of these scales are also discussed. These scales are used regionally; for example, the TDS is mainly used in Japan, while the NDSS and the WISDM are used in the US. Taking advantage of the characteristics of these scaling methods and comparing them with each other in various populations may be useful for elucidating the genetic and non-genetic nature of nicotine dependence.

**Keywords:** Nicotine addiction; Nicotine dependence

**Abbreviations**: FTQ: Fagerström Tolerance Questionnaire; FTND: Fagerström Test for Nicotine Dependence; TDS: Tobacco Dependence Screener; WISDM: Wisconsin Inventory of Smoking Dependence Motives; CDS: Cigarette Dependence Scale; NDSS: Nicotine Dependence Syndrome Scale

## Introduction

Cigarette smoking is still a major cause of many preventable diseases [1]. The pharmacologic effect of nicotine plays a crucial role in tobacco addiction [1,2]. When issues around tobacco use are considered, "nicotine addiction" can be regarded as a roadblock that needs to be overcome. The importance of nicotine in maintaining smoking and in cessation difficulty has been well acknowledged [3]. Numerous twin studies have investigated the contributions of genetic and environmental factors to nicotine addiction [4-6]. Recent advances made through the use of linkage and association approaches, especially genome-wide association (GWA) studies, have identified susceptibility genes for addiction including nicotine addiction [7].

Along with the progress of research regarding nicotine addiction, the measurement of nicotine addiction has been recognized as an important issue. Progress in tobacco research may depend on improved measurement [8]. How nicotine addiction is defined and measured may influence the results and interpretations of research or clinical outcomes.

From a historical perspective, the Fagerström Tolerance Questionnaire (FTQ) [9] and its shorter version, the Fagerström Test for Nicotine Dependence (FTND) [10], are the most notable scales and have been used in both clinical and research settings [7]. However, the Fagerström scales were intended as measures of physical tolerance per se [9]; therefore, they do not assess several important aspects of nicotine dependence, such as cravings, subjective compulsion to smoke, nicotine withdrawal, behavioral saliency, or behavioral automaticity, which are often regarded as core constructs for dependence [11].

Nicotine addiction can also be assessed using diagnostic criteria based on the Diagnostic and Statistical Manual (DSM) of the American Psychiatric Association [12,13] and the International Classification of Disease 10th revision (ICD-10) from the World Health Organization (WHO) [14]. The Tobacco Dependence Screener (TDS, reviewed later) [15] is a 10-item questionnaire for screening tobacco/nicotine dependence according to these criteria.

We previously reported the association between neuropeptide Y receptor 2 (*NPY2R*) polymorphism and smoking behavior of elderly Japanese [16], in which both the FTND and the TDS were used as assessment scales for nicotine addiction. Analyses of information about smoking behavior and genotyping data of rs4425326 and rs6857715 from about 2500 subjects including various smoking status revealed that male but not female ever-smokers (current and ex-smokers) having the rs4425326 TT genotype had significantly higher FTND scores ($P$= .003) and greater CPD (cigarettes smoked per day) than those with other genotypes. No association was found between the TDS and these polymorphisms. We also conducted association study between smoking behavior and the Neurexin 1 (*NRXN1*) gene polymorphisms, rs2193225 and rs6721498 using the same subjects [17]. In contrast, we have found that male ever-smokers with the rs2193225 GG type were more prevalent in the higher TDS score category ($P$= .056), but not in the higher FTND score category. These observations indicate that the traits detected by the scores of the two questionnaires are supposedly different, and how the genetic components control these traits in establishing individual nicotine dependence has not been elucidated.

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Some assessment scales have been developed with the goal of capturing diverse aspects of nicotine dependence. These scaling methods are being improved in each successive version. In the present article, we briefly review four relatively new scales and discuss their characteristics and powers (Table 1).

| Assessment scales | Number and gender of subjects | Race of subject | Age of subjects, years old | Reliability (Internal consistency), α | Indexes correlated with the scale | References associated with the scale |
|---|---|---|---|---|---|---|
| TDS [15] | Sample1: 58 males<br>Sample2: 115 males<br>: 33 females<br>Sample3: 194 males | Japanese | 27.6 (±11.1)[a]<br>43.1 (±15.6)[a]<br>33.0 (±12.4)[a]<br>NM | .81<br>.76<br>.77<br>.77 | Years of smoking, CPD<br>Years of smoking, CPD, CO levels<br>None<br>Age, Years of smoking CPD | Studies on varenicline treatment [19,20]<br>Studies developing new tools for assessing nicotine dependence [21,22] |
| WISDM-68 [22] | 303 males, 454 females, 18 not identified | 638 White<br>83 African-American<br>54 others | ≥18 | Total WISDM-68: .98- .99<br>Subscales: .84- .96 | Total WISDM-68: TDS, CO, CPD,<br>Some subscales: End of treatment relapse | Replication study [26,27]<br>Studies on PDM and SDM [28-30]<br>Brief WISDM [31] |
| CDS-12, CDS-5 [33] | Preliminary survey: mail 384, internet 145 (Gender: NM)<br>Main survey: 3009 47% males | NM | 18-70<br>32 (range 12-74) | CDS-12: .90<br>CDS-5: .84 | Cotinine, CPD, Urge to smoke, Switching from daily to occasional smoking | Replication studies on predictivity [34,35] |
| NDSS [24] | Study 1: 317 57% females<br>Study 2 : 802 57% females<br>Study 3: 91 59% males | NM<br>66% White 31% Black 3% others<br>81% White | 44.2 (±10.3)[a]<br>39.2 (±10.6)[a]<br>34.5 (±9.0) [a] (range 20-55) | 30-item version, NDSS-T: .84<br>Factors: .55- .76 | 30-item version: CPD, Difficulty abstaining, Past severity of withdrawal | Studies comparing some scales [26,38] |

Abbreviation: TDS= Tobacco Dependence Screener, WISDM= Wisconsin Inventory of Smoking Dependence Motives, CDS= Cigarette Dependence Scale, NDSS= Nicotine Dependence Syndrome Scale, NM= Not mentioned, NDSS-T=Total NDSS, CPD= Cigarettes smoked per day, CO= Carbon monoxide, PDM= primary dependence motives, SDM= secondary dependence motives

a. Mean (± standard deviation)

**Table1:** Summary of assessment scales for nicotine addiction.

## Assessment Scales

**Tobacco Dependence Screener (TDS):** The Tobacco Dependence Screener (TDS) [15] is a 10-item questionnaire for screening tobacco/nicotine dependence according to the International Classification of Diseases 10th revision (ICD-10) [14], the Diagnostic and Statistical Manual of Mental Disorders third edition revised [12] and fourth edition [13] (DSM-III-R and DSM-IV).

The questions were derived from the tobacco use section of the World Health Organization's Composite International Diagnostic Interview (WHO-CIDI), version 1.1 [18], which was designed to assess 11 dependence symptoms of ICD-10 tobacco dependence, and the DSM-III-R. The questions in a later version of the CIDI are also adapted to the DSM-IV. After combining the second and third symptoms, the TDS was developed based on these 10 symptoms. The symptoms were as follows: (1) smoking more than he/she intended, (2) a desire to quit smoking and unsuccessful efforts to quit smoking, (3) craving for tobacco, (4) withdrawal symptoms, (5) smoking to avoid withdrawal symptoms, (6) smoking despite a serious illness, (7) smoking despite health problems, (8) smoking despite mental problems, (9) feeling dependent on tobacco, and (10) giving up important activities for smoking. For each question regarding each symptom, a dichotomous response category ("yes" or "no") was possible. The number of "yes" responses was counted as the scale score.

Three samples of Japanese smokers (n=400, in total) were used to assess the reliability and validity of this scale. The Cronbach alpha coefficients of internal reliability for the TDS were .76 or greater for all the samples. The TDS was found to have a better screening performance than the Fagerström Tolerance Questionnaire (FTQ) for any of the three diagnostic criteria (ICD-10, DSM-III-R, and DSM-IV) based on Receiver Operating Characteristic (ROC) analyses.

The TDS score was significantly and positively correlated with the number of cigarettes smoked per day, the years of smoking, the severity of the three diagnoses and breath carbon monoxide levels. Additionally, the TDS score was significantly lower among those who had quit smoking, compared with those who had not.

This simplified scale showed an acceptable reliability, construct and predictive validity, and screening performance based on psychiatric diagnosis criteria.

In 2006, the Japanese National Health Insurance program began to cover smoking cessation treatment for patients who met the criteria for nicotine dependence; one of these criteria was a score of 5 or greater on the TDS. Therefore, the TDS is widely used in Japanese clinical settings. In some research on varenicline treatment, the TDS has also been used as a measure for assessing nicotine dependence [19,20].

The TDS has also been used as a smoking index in studies developing

new tools for assessing nicotine dependence [21,22]. A lower TDS score among subjects who quit smoking, compared with those who did not, has been replicated in a study reporting the predictability of the FTQ and the TDS among inpatients with coronary heart attacks [23].

The TDS is assumed to be a reliable, concise, and useful measure based on the DSM and ICD-10 assessments per se. However, the DSM assessment of dependence has been pointed out to be a dichotomous diagnostic decision conveniently classifying people as "dependent" or "not dependent" [7,24], despite its underlying multidimensionality [25]. Dichotomous measures are useful for case-finding and epidemiological studies, but may present difficulties in some research settings when the nature of nicotine dependence is considered.

**Wisconsin inventory of smoking dependence motives (WISDM-68):** The Wisconsin Inventory of Smoking Dependence Motives (WISDM-68) is a multidimensional measure of dependence based on theoretically grounded motives for drug use [22]. The measure has 68 items consisting of 13 domains that identify separate motives for tobacco use. The number of items related to each motive ranges from 4 to 7. Each item is answered using a 7-point Likert scale ranging from 1 - "Not true of me at all" to 7 - "Extremely true of me". The authors attempted to define and measure dependence based on 'motivations', which were intended to illuminate mechanisms underlying the compulsive use of tobacco/nicotine using a large sample of 775 smokers.

The 13 domains (subscales) are listed below:

1. Affiliative Attachment
2. Automaticity
3. Behavioral Choice/Melioration/Alternative Reinforcement
4. Cognitive Enhancement
5. Craving
6. Cue Exposure/Associative Processes
7. Loss of Control
8. Negative Reinforcement
9. Positive Reinforcement
10. Social and Environmental Goads
11. Taste and Sensory Properties
12. Tolerance
13. Weight Control

The internal consistency coefficient of each subscale was greater than .90, with the exception of the Cue Exposure/Associative Processes subscale, for which the reliability coefficient was .88. The WISDM-68 subscales had fair-to-excellent internal consistencies (range = .73 - .95) for all six of the groups examined: men, women, daily, non-daily, white, and non-white smokers. This result means that the WISDM-68 subscales are appropriate for various populations.

Confirmatory factor analysis models were used to examine the hypothesis that the WISDM-68 is multidimensional. The results indicated that dependence measured by the WISDM-68 was not a unitary factor, but a diverse collection of distinct motives for drug use. However, strong inter-correlations among some of the subscales were seen, which does not mean that the 13-factor model was the best-fitting model possible.

Concerning measurement validation, concurrent validity and preliminary predictive validity were investigated. For concurrent validity, three indices were assessed: heaviness of smoking measured via self-report of smoking rate and via alveolar carbon monoxide (CO) levels, and the DSM-IV criteria for tobacco dependence as assessed using the Tobacco Dependence Screener (TDS). All the subscales were correlated with smoking heaviness (cigarettes per day $r$= .23- .76; CO $r$= .15- .70) and the TDS ($r$= .31-73). The total WISDM-68 was also correlated with the smoking indices (cigarettes per day $r$= .63; CO $r$= .55; TDS $r$= .72). Regression analyses revealed that the Tolerance subscale best predicted the CO level, but the Craving, Cue Exposure/Associative Processes, and Tolerance subscales were the best predictors of DSM-IV dependence.

Hierarchical logistic regression using data from a cessation study (N=238) indicated that Automaticity, Cognitive Enhancement, Negative Reinforcement and Social/Environmental Goads predicted relapse, but the total WISDM-68 score did not significantly predict relapse.

The internal consistency and validity of this scale were replicated by the group of investigators and by another one [26,27]. Interestingly, in a later study, the group of investigators has suggested that just four subscales (automaticity, craving, loss of control, and tolerance) had represented the core features of dependence [28-30]. These subscales were dubbed the "primary dependence motives (PDM)" and the remaining scales were labeled as the "secondary dependence motives (SDM)". The findings suggest that the PDM captures the more fundamental dependence-related variance and that the PDM score may reflect the emergence of clinical features especially characteristic of advanced or problematic tobacco use [30].

This measurement was followed by the Brief Wisconsin Inventory of Smoking Dependence Motives (Brief WISDM) [31]. Research using data from three independent samples aimed to shorten the WISDM by selecting subscales and reducing the sets of items. Thirty-one items were dropped, and the Behavioral Choice-Melioration subscale was dropped from the WISDM; the Negative and Positive Reinforcement subscales were then consolidated. The new WISDM short-form, comprised of 37 items and 11 subscales, was found to have a comparable internal consistency, long-term stability, concurrent validity, predictive validity and model fit with the original WISDM. The 37-item Brief WISDM is useful when the assessment burden is a consideration.

The psychometric properties and construct validity of these full and brief versions of the Wisconsin Inventory of Smoking Dependence were assessed using an internet-based sample of treatment-seeking Hungarian smokers [32]. The WISDM-37 had sufficient psychometric properties and good construct validity, compared with the WISDM-68.

In conclusion, the WISDM scales showed appropriate psychometric characteristics for the measurement of a wide variety of smoking motives and were related to some indices with regard to nicotine dependence or smoking behavior. However, most of the studies using the WISDM scales were conducted in the US or in Caucasian populations. For further confirmation of the reliability and validity, attempts to use these scales among various populations are needed.

**Cigarette dependence scale – 12 (CDS-12), cigarette dependence scale – 5 (CDS-5):** The Cigarette Dependence Scale [33] was developed by asking smokers via mail and through a web site to assess signs indicating a dependence on cigarettes. There are two types of scale, the CDS-12 and the CDS-5, each of which is rated using a 5 point scale. The CDS-12 is a 12-item instrument covering the main components of the DSM-IV and ICD-10 and some of the FTND. The CDS-5 is a 5-item version of the CDS-12. These items were designed to index the

dependence outcomes, such as "Please rate your addiction to cigarettes on a scale of 0 to 100" and "On average, how many cigarettes do you smoke per day?"

Internal consistency was assessed using internal consistency coefficients (Cronbach α). The internal consistency coefficients were .90(α) for CDS-12 and .84(α) for CDS-5. These coefficients were larger than the recommended level (.70). Furthermore, they were higher than that of the FTND (α= .66). Thus, a high internal consistency for both the CDS-12 and the CDS-5 was demonstrated.

To assess reliability, the intraclass coefficient correlation (ICC) for each item and scale was tested at baseline and at 15-31 days after the baseline survey. The ICC for all the items was .60 or higher, and the ICC for CDS-12 and CDS-5 was .83 or higher. The ICC for CDS-12 and CDS-5 was significantly higher than that of the FTND ($P < .001$).

The construct validity was tested to assess the "dependence in daily smokers and occasional smokers", "a 0-100 rating of the strength of the urge to smoke during the last attempt to quit", "the number of cigarette smoked per day", etc. Higher scores for the dependence items and scales were obtained for the daily smokers than for the occasional smokers. All the items and scales had a strong association with the 0-100 rating of the strength of the urge to smoke during the last attempt to quit.

A recent study [34] reported that CDS-12 predicted smoking cessation after 8 days and 6 weeks. They assessed the dependence rating, withdrawal intensity, and self-efficacy rating. The higher CDS-12 score at baseline predicted smoking abstinence after 6 days and 8 weeks, although the CDS-5 and FTND did not predict smoking cessation. A higher CDS-12 also predicted a higher withdrawal rating and a lower self-efficacy rating after 8 days, but the FTND did not yield similar predictions. These results suggest that the CDS-12 has a predictive validity. In a more recent study, the predictive validity of five cigarette dependence questionnaires (CDS-12, CDS-5, FTND, Heaviness of Smoking Index, and the Nicotine Dependence Syndrome Scale, reviewed later) was investigated. The results of this study indicated that the CDS-12 was the best predictor (OR: 3.98 per SD unit) of smoking abstinence at an 8-day follow-up [35].

In addition, the cotinine level was measured in saliva from volunteers. All the items and scales were associated with the level of cotinine. The CDS-5 was more strongly associated with the cotinine level ($R^2 = 0.21$) than the CDS-12 ($R^2 = 0.17$). The association between the CDS-5 and the cotinine level was equivalent to the association of the FTND with the cotinine level. However, the CDS-12 was less strongly associated with the cotinine level.

In conclusion, the recently developed CDS is considered to reflect the DSM-IV and the ICD-10 and FTND. The CDS has a high reliability, but its predictive validity was only recently demonstrated. This scale is expected to be used in current clinical practice because of its high reliability and validity.

**Nicotine dependence syndrome scale (NDSS):** The Nicotine Dependence Syndrome Scale (NDSS) [24] is a multidimensional questionnaire based on Edwards's syndromal conceptualization of dependence [36,37]. After three successive studies, a 19-item self-report scale consisting of five factors was developed using mainly participants in smoking cessation studies.

The essential elements of syndrome that Edwards proposed, which were the theoretical foundation of this scale, were as follows: a narrowing in the repertoire of drug use behavior, an increased salience of drug-seeking behavior, an increased tolerance to the drug, repeated withdrawal symptoms, repeated relief or avoidance of withdrawal symptoms by further drug use, subjective awareness of a compulsion to use the drug, and rapid reinforcement of the syndrome after relapse. Starting with these concepts applied to nicotine dependence, a 23- item questionnaire was first developed. After psychometric analysis, seven items were added in the next step, and the psychometric properties were also investigated. Finally, a factor analysis extracted five factors: drive (craving and withdrawal, and subjective compulsion to smoke), priority (preference for smoking over other reinforcers), tolerance (reduced sensitivity to the effects of smoking), continuity (regularity of smoking rate), and stereotypy (invariance of smoking), leading to a 19-item questionnaire.

The internal consistency of the omnibus summary score, the NDSS-T (for total) showed good values for both the first 23-item version and the second 30-item version (α= .86, and .84, respectively). Each factor also indicated relatively acceptable reliability coefficients for both version (.55- .83) with the exception of stereotypy in the 23-item version (α= .49), which was improved in the 30-item revision (α=.70). The test-retest reliability for the NDSS-T and each factor using the 30-item version showed an adequate level (.71- .83).

Concerning the validation analysis, more data was obtained from the first 23-item version than from the 30-item version. The NDSS-T and factor scores showed strong associations with dependent-relevant measures such as cigarettes smoked per day (CPD), difficulty abstaining, and past severity of withdrawal on both the 23-item and the 30-item version. The 23-item NDSS-T and certain factor scores were correlated with some scales reflecting smoking motives or occasions. They also predicted subsequent real-world experience such as urges when smoking, withdrawal symptoms (e.g. urge, restlessness) in acute abstinence, and outcome of cessation. These relations were maintained even when the FTQ scores were controlled and similar results were observed when the CPD was controlled. This finding supports the idea that the NDSS has an incremental utility and validity.

The results of a simultaneous regression analysis in the 30-item version showed an incremental utility of multiple scales. For smoking rate (CPD) and difficulty abstaining, all five subscales indicated independent predictive utility, while for severity of withdrawal, all the subscales except continuity showed independent utility.

Differences in the dependence measures between two ethnicities (White and African American) were examined. The NDSS-T did not show a significant difference, but the FTQ did, and significant ethnic group differences were seen in the subscales (e.g. African American showed significant lower scores for drive and continuity but higher scores for stereotypy).

In conclusion, the NDSS showed evidence of being a valid measure of dependence, though the reliability of some subscale was relatively low. The NDSS samples the multidimensional components of dependence and represents a broad conceptual framework for nicotine dependence. Several improvements are possible and needed; for example, scale revisions especially for stereotypy and continuity, incorporating FTQ scales content into the NDSS, assessing the relationship between the NDSS and DSM-based measures.

## Conclusions

We have briefly reviewed the relatively new scales that are being used to assess nicotine addiction. In an effort to expand the breadth of the theories and mechanisms underlying nicotine addiction, multidimensional scales have been developed. The references which report the process of developing these measures also support that

nicotine dependence is a heterogeneous construct. Continuous efforts to capture different aspects of nicotine dependence are needed.

These scales are used regionally; therefore, taking advantage of the characteristics of these scaling methods and comparing them among various populations may be important for elucidating the genetic and non-genetic nature of nicotine dependence.

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problem solving. Predictably, this strategy provides distinct findings in different populations, highlighting the need of individualized medicine. Thus, the statement "significant controversy regarding ICS still exists" should read that there is no real controversy. Inhaled corticosteroids can be effective in suppressing bronchial inflammation with associated reduction of decline in lung function in particular subgroups of COPD (5). Therefore, it is not surprising that inflammatory phenotype-driven therapy has been extremely successful in COPD (6); that is, targeting treatment on one specific phenotype (eosinophilia) can improve disease outcome above guideline-based treatment, with effect sizes exceeding those by any novel drug in this disease. This has and can only become apparent by examining consistency of treatment effects along multiscale abnormalities of the disease.

Accordingly, the "current controversies" are the best fuel for existing perspectives in COPD. Subgroups of patients with unexpected phenotypes (e.g., those with eosinophilia [6], *see above*) have been captured as being responsive to available therapy. It is our task to give them the current perspective they deserve.

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## Reply

*From the Authors:*

We thank Drs. Postma and Sterk for their interest in our article (1). We really do not have anything to argue against their views since some of us (Drs. Sterk and Agustí) have recently coauthored an article exactly along these lines (2). We entirely concur on the importance of understanding the complexity of chronic obstructive pulmonary disease (3) and that systems biomedicine offers a very interesting research strategy to this end (4). We hope that other investigators in the field share this view, so more effective therapeutic alternatives can be offered to specific groups (phenotypes) of patients with chronic obstructive pulmonary disease.

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## Endobronchial Metastasis from Gastrinoma of the Pancreas

*To the Editor:*

Gastrinoma is a functional endocrine tumor of the digestive tract and pancreas that is known to cause Zollinger-Ellison syndrome. The main metastatic sites are regional lymph nodes, liver, and bones, and the lungs may also be involved (1). Here we describe a rare case of endobronchial metastasis from gastrinoma of the pancreas.

A 73-year-old male was referred to our hospital for detailed examination of prolonged cough. He had suffered from recurrent peptic ulcer during his fourth decade and was diagnosed as having pancreatic gastrinoma with liver metastasis. He had been carefully observed, receiving chronic proton pump inhibitor therapy, and had undergone resection of the right lobe of the liver to reduce the tumor mass 6 years previously, with postoperative chemotherapy of gemcitabine and uracil-tegafur. The maximal serum gastrin level was 4,300 pg/ml. He had never smoked. Computed tomography scan of the chest showed a nodule, 2.0 cm × 2.0 cm in size, in the right lower lobe and multiple micronodules in both lungs (Figure 1A). Bronchoscopic examination revealed total occlusion of the right basal bronchus by a polypoid mass (Figure 1B). A biopsy specimen from the endobronchial tumor showed the findings of neuroendocrine carcinoma (Figure 1C), immunohistochemically positive for gastrin (Figure 1D). The patient was diagnosed as having endobronchial metastasis from gastrinoma. The patient's cough disappeared with antitussive agents.

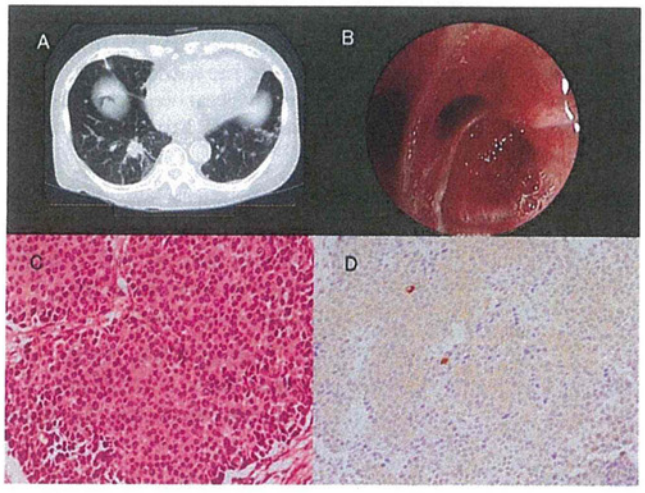


*Figure 1.* (*A*) Computed tomography of the chest shows a nodule, 2.0 cm × 2.0 cm in size, in the right lower lobe and multiple micronodules in both lungs. (*B*) Bronchoscopic findings show total obstruction of the right basal bronchus by a polypoid tumor. (*C*) Biopsy specimen from the endobronchial tumor shows findings of neuroendocrine carcinoma (hematoxylin and eosin staining, ×200). (*D*) Immunohistochemical staining for gastrin is positive.

Endobronchial metastasis is defined as nonpulmonary neoplasms that metastasize to the proximal central or subsegmental bronchus, in a bronchoscopically visible range, and is frequently associated with primary tumors of the kidney, colon/rectum, breast, and others (2). To the best of our knowledge, there have been no previous reports of endobronchial metastasis from gastrinoma. This is the first reported case of endobronchial metastasis from gastrinoma. Therefore, the present case reminds physicians to consider endobronchial metastasis from extrathoracic endocrine neoplasms.

Therapy for gastrinoma includes surgery for localized disease, debulking surgery for metastatic disease, and chemotherapy. More than sixty percent of gastrinomas are malignant; 5-year survival for patients with gastrinoma with liver metastases is between 40% and 75%, and it is almost 100% when no liver metastases are present (3). In this patient, tumor progression has been slow, and he remains almost asymptomatic after debulking surgery for liver metastases and chemotherapy.

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## Influence of Body Mass Index on Effects of a Shared Asthma Treatment Decision-Making Intervention

Asthma is a significant public health problem, with approximately 24.6 million Americans reporting current asthma in 2009 (1). Obesity prevalence has continuously increased over the last 30 years, reaching 33.8% among U.S. adults in 2007–2008 (2). A meta-analysis of prospective studies showed a dose-dependent relationship between increasing body mass index (BMI) and the risk of incident asthma (3). Understanding the influence of BMI and obesity on effectiveness of interventions to improve asthma control will help clinicians better care for obese patients with asthma.

The Better Outcomes of Asthma Treatment study, a randomized controlled trial in 612 adults with poorly controlled asthma, found that a shared treatment decision-making (SDM) intervention improved controller medication adherence and clinical outcomes (4). Given the increased attention to the links between obesity and asthma, we conducted *post hoc* analyses to investigate whether baseline BMI modified the SDM intervention effects on asthma medication acquisition outcomes (fill/refill adherence and regimen strength) and clinical outcomes in the follow-up year. We hypothesized that obese patients would have benefitted less from the SDM intervention than did overweight or normal weight patients. Some of the results reported here were previously reported in the form of an abstract (5).

Standard BMI categories were defined: normal (18.5–24.9 kg/m$^2$, n = 132) and underweight (<18.5 kg/m$^2$, n = 7) combined, overweight (25–29.9 kg/m$^2$, n = 185), and obese (≥30 kg/m$^2$, n = 286). Using comprehensive pharmacy dispensing records, we computed continuous medication acquisition (CMA) indices (6–8) to measure fill/refill adherence for controller medications (inhaled corticosteroids, leukotriene modifiers, and others) and for long-acting β agonists (LABA) during the 12 months before and after randomization of individual participants. Controller and LABA medication regimen strength was measured by cumulative beclomethasone canister-equivalents (C-E) and salmeterol diskus-equivalents (D-E), respectively, using a standardized weighting methodology (9). Clinical

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CASE REPORT

# Hepatocellular adenoma associated with familial adenomatous polyposis coli

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## Abstract

Hepatocellular adenoma (HCA) is a benign liver tumor that most frequently occurs in young women using oral contraceptives. We report a rare case of HCA in a 29 years old female with familial adenomatous polyposis (FAP). The first proband was her sister, who underwent a total colectomy and was genetically diagnosed as FAP. A tumor, 3.0 cm in diameter, was detected in the right lobe of the liver during a screening study for FAP. A colonoscopy and gastroendoscopy revealed numerous adenomatous polyps without carcinoma. The patient underwent a total colectomy and ileo-anal anastomosis and hepatic posterior sectoriectomy. The pathological findings of the liver tumor were compatible with HCA. The resected specimen of the colon revealed multiple colonic adenomatous polyps. Examination of genetic alteration revealed a germ-line mutation of the adenomatous polyposis coli (*APC*) gene. Inactivation of the second *APC* allele was not found. Other genetic alterations in the *hepatocyte nuclear factor 1 alpha* and *β-catenin* gene, which are reported to be associated with HCA, were not detected. Although FAP is reported to be complicated with various neoplasias in extracolic organs, only six cases of HCA associated with FAP, including the present case, have been reported. Additional reports will establish the precise mechanisms of HCA development in FAP patients.



**Key words:** Hepatic adenoma; Familial adenomatous polyposis coli; Extrahepatic manifestation; Adenomatous polyposis coli gene; Hepatocyte nuclear factor 1 alpha

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## INTRODUCTION

Hepatocellular adenoma (HCA) is a benign liver tumor that usually arises in women who are over 30 years old and have used oral contraceptives for over 5 years[1]. Other risk factors associated with HCA have been described, including glycogen-storage diseases, androgens, anabolic steroids, diabetes mellitus, some drugs and pregnancy[2-5].

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease caused by a mutation in the adenomatous polyposis coli (*APC*) gene. FAP is characterized by the early onset of multiple colorectal adenomatous polyps, with an inevitable progression to carcinoma if left untreated. Additionally, FAP is known to be associated with extracolic neoplasms in various other organs; adenomas and carcinomas of the upper gastrointestinal tract, desmoid tumors and thyroid carcinomas[6]. Due to familial screening and prophylactic colectomies, the prognosis of FAP patients has improved[6-8]. Thus, extracolic tumors have become more important causes of mortality[7]. Duodenal or periampullary cancer and desmoids are the two main causes of mortality after a total colectomy[9]. Other rare extracolic manifestations include cancers of the thyroid, liver, bile ducts and central nervous system[6,7,9]. HCA is rare for FAP-associated extracolic neoplasms[10].

Herein, we report a rare case of HCA concomitant with FAP. She had no history of oral contraceptive use or other risk factors for HCA. We summarize previous case reports[5,10-14] and consider HCA arising in FAP patients.

## CASE REPORT

A 29 years old Japanese woman was called for familial surveillance of FAP because her 27 years old sister had undergone a total colectomy due to the diagnosis of ascending colon cancer arising from FAP, already confirmed by gene analysis. Her 46 years old father died of gastric cancer but FAP was uncertain. Her son had suffered from hepatoblastoma which had been resected when he was 18 mo old. Her preoperative clinical laboratory tests, including liver function, were normal. Serologically, serum hepatitis B and hepatitis C virus markers were negative. Serum levels of alpha-fetoprotein and des-γ-carboxy prothrombin were also within normal ranges. Preoperative computed tomography (CT) showed a tumor in the posterior sector of the right lobe, measuring 28 mm in diameter. The tumor showed a slight inhomogeneous low density area on the unenhanced scan when compared with the surrounding liver parenchyma (Figure 1). The tumor was well enhanced in the early phase after the contrast medium injection. The tumor became indistinguishable in the late phase. Although the tumor was not detectable on T1-weighted magnetic resonance imaging (MRI), it was detected as a mild hyper-intense tumor in the posterior sector on T2-weighted MRI (Figure 1). The tumor was indiscernible in the arterial phase, but became a hypo-intense area in the hepato-biliary phase after gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid enhancement on T1-weighted MRI. No obvious

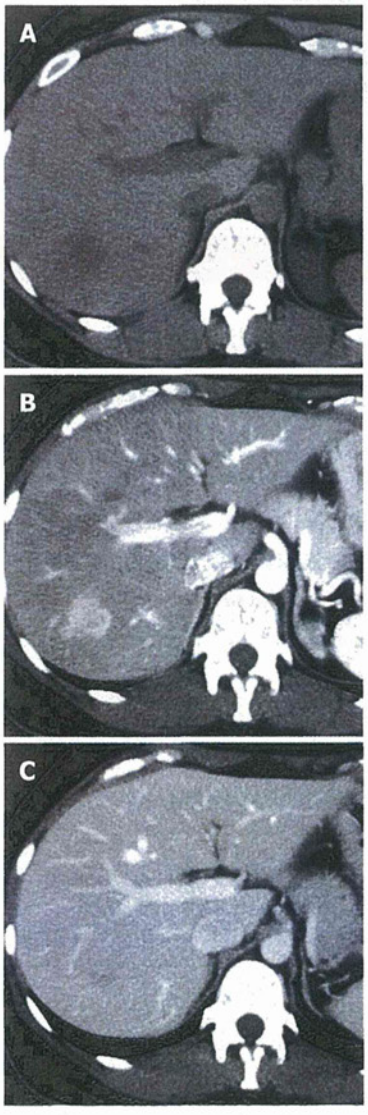


**Figure 1 Preoperative computed tomography scan revealed a 28 mm tumor in the posterior sector of the right hepatic lobe.** A: Plain computed tomography showed a tumor as a slight low density area; B: The tumor was inhomogeneously enhanced with a ragged border during the early phase; C: The tumor was indistinguishable in the late phase.

capsular formation or visible central scars were observed (Figure 2). Hepatic arteriography showed a tumor stain without any abnormalities in vascular structure or angioplany. A total colonoscopy revealed numerous polyps of various sizes throughout the colon and rectum but no obvious colorectal carcinoma was found. Gastroendoscopy also found thick polyps without carcinoma.

On the basis of clinical features[15] and previous literature[10-13], we performed a total colectomy and ileoanal anastomosis and hepatic posterior sectoriectomy on December 2008. Macroscopically, numerous polyps of various sizes, including one lateral spreading tumor in the ascending colon, were found in the mucosal surface of the resected colon specimen. In the cut surface of the resected liver specimen, the tumor grossly showed a faint yellow tumor without hemorrhage or necrosis. The

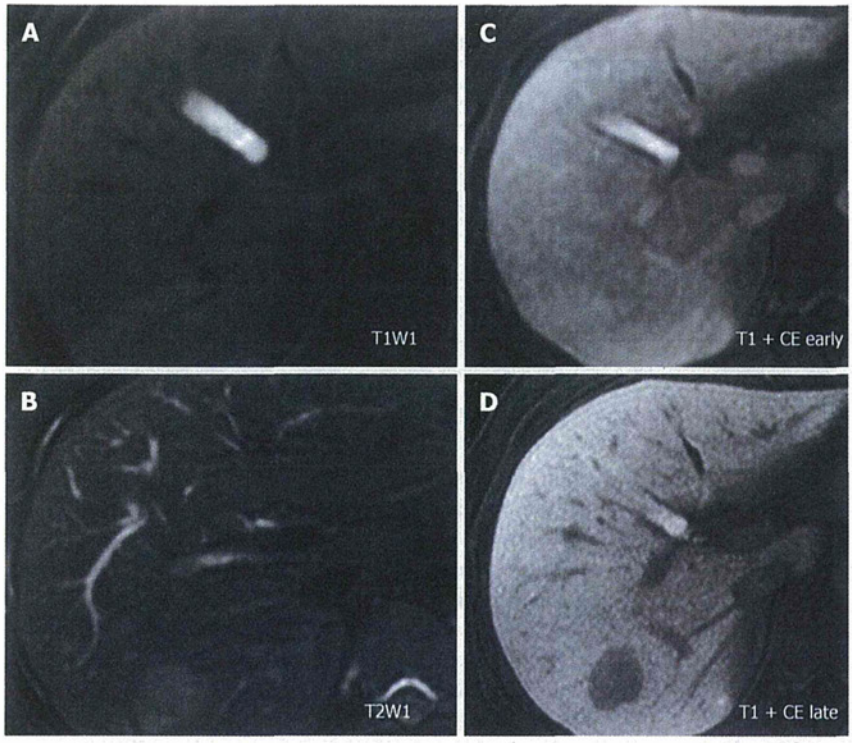


**Figure 2 Magnetic resonance imaging of the tumor.** A: The tumor showed an iso-intensity with the surrounding liver parenchyma on T1-weighted imaging; B: The tumor was visualized as a heterogeneous hyper-intense mass on T2-weighted imaging; C and D: After gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid enhancement, the tumor was discernible in the arterial phase and was clearly detected as a hypo-intense lesion in the hepato-biliary phase on T1-weighted imaging. CE: Contrast enhanced.

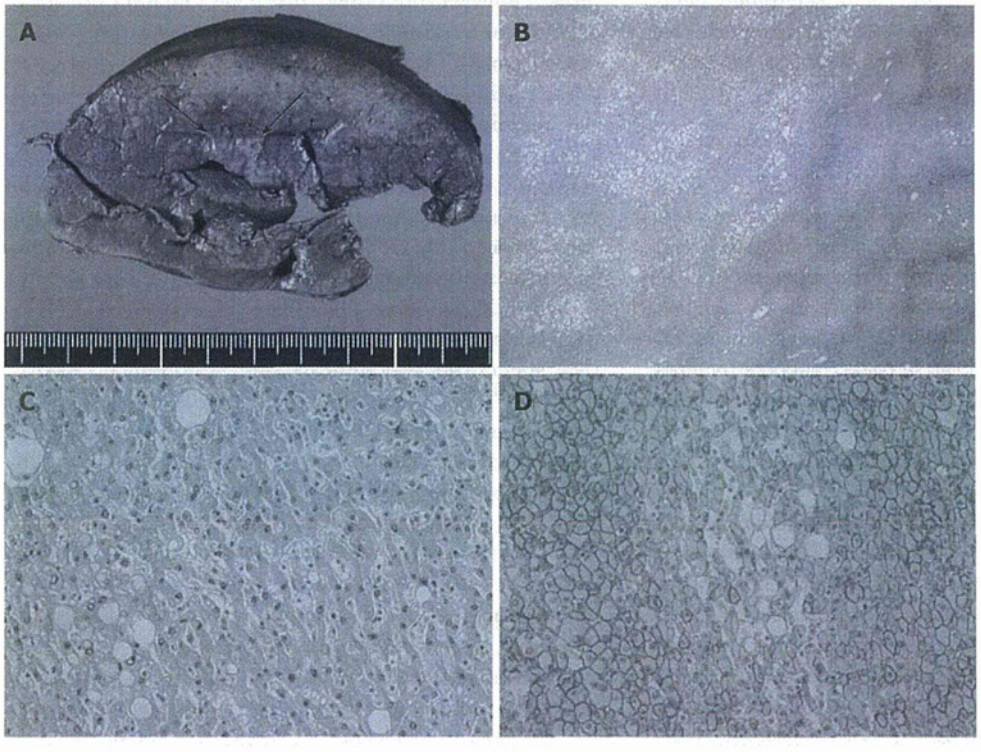


**Figure 3 Pathological findings of the liver tumor.** A: Cut surface of formalin-fixed liver specimen. The tumor was unencapsulated and its border was ill-defined (arrow); B, C: Microscopically, the tumor consisted of low-grade atypical hepatocytes, without cellular mitosis or changes in cellular density and structures. Fatty deposition in the tumor cells was ubiquitously remarkable. Neither biliary structures nor portal triads were present within the tumor (hematoxylin-eosin stain, original magnification ×40 in B and ×200 in C); D: β-catenin was immunohistochemically detected on the cytomembrane. Neither aberrant nuclear nor cytoplasmic accumulations were found (original magnification ×100).

tumor showed an ill-defined border and was unencapsulated (Figure 3A). The surrounding liver tissue seemed to be normal parenchyma.

Histologically, multiple colorectal polyps were adenomas with mild to moderate cellular atypia. A lateral spreading tumor was a tubular adenoma with severe atypia. The liver tumor consisted of low-grade atypical hepatocytes, without cellular mitosis or changes in cellular density and structures. Fatty deposition in the tumor cells was remarkable in some parts. No biliary structures or portal triads were present within the tumor (Figure 3B and C). There was no underlying hepatitis, fibrosis or cirrhosis in the adjacent liver parenchyma. These pathological findings were compatible for hepatic adenoma. To clarify the pathogen-

**Table 1 Reported cases of primary hepatocellular adenoma associated with familial adenomatous polyposis**

| Case | Age/gender | Location | No. of tumors | Size (cm) | Treatment | Oral contraception | Steroid use | Mutated codon in the *APC* gene | Disorder in the somatic gene of HCA |
|---|---|---|---|---|---|---|---|---|---|
| Bala *et al*[11] | 2/F | Right lobe | Solitary | 10 | Resection | (-) | (-) | 1451 | Loss of wild-type allele of *APC*, mutation of *p53* |
| Nakao *et al*[13] | 20/F | Left lobe | Multiple | 5.5 | Observation | (-) | (+) | ND | ND |
| Bläker *et al*[10] | 27/F | ND | ND | ND | ND | ND | ND | 1156 | 1516 |
| Jeannot *et al*[12] | 37/F | Right lobe | Solitary | 7 | Resection | (+) | (-) | 1062 | Mutation of *HNF1α* |
| Okamura *et al*[5] | 27/M | Left lobe | Solitary | 8.5 | Resection | (-) | (-) | ND | ND |
| [1]Toiyama *et al*[14] | 25/M | Left lobe | Solitary | 5.5 | Resection | (-) | (-) | ND | Mutation of *HNF1α* |
| This case | 29/F | Right lobe | Solitary | 3 | Resection | (-) | (-) | 499 | (-) |

[1]Case 6 is hepatocellular carcinoma within hepatocellular adenoma (HCA) in a familial adenomatous polyposis patient. *APC*: Adenomatous polyposis coli; ND: Not described; *HNF1α*: Hepatic nuclear factor 1 alpha; F: Female; M: Male.

esis of this patient, genetic alterations of the germ-line and somatic genes were examined[16]. Sequencing of the germline *APC* gene revealed a transition from ACG to ATG at codon 499 in exon 11. No loss of the *APC* gene in HCA cells was demonstrated by fluorescence in situ hybridization (data not shown). No additional somatic mutation of the *APC* gene was found in the HCA. Moreover, a mutation of the hepatocyte nuclear factor 1 alpha (*HNF1* gene, which is reported to be related to HCA[12]) was not detected.

The postoperative course was uneventful without any complications, and the patient was discharged twenty days after the operation. Follow-up CT scans revealed no signs of recurrence and other abdominal extracolic lesions 3 years after surgery.

## DISCUSSION

HCA is usually found in healthy young women, especially those who use oral contraceptives for a long time. More than 750 HCA cases have been reported since the first report, showing a possible etiological association between HCA and contraceptives[17]. Glycogen-storage diseases, androgens, anabolic steroids, diabetes mellitus, some drugs and pregnancy have been reported as other causal factors for HCA[2-5]. However, the present patient did not have any known exogenous or endogenous pathogenic factors, except for FAP.

Patients with FAP can develop extracolonic lesions such as desmoid tumors, adenomas and carcinomas of the upper gastrointestinal tract[6]. An increased risk of hepatic tumors, mainly hepatoblastoma and hepatocellular carcinoma[18-20], has also been shown in FAP patients. Hepatoblastomas develop in young patients with FAP at least 100 times more frequently than in the general population[18]. Kurahashi *et al*[19] reported a biallelic mutation in the *APC* gene in hepatoblastoma developed in a FAP patient showing a germline mutation in *APC*. In fact, this patient's son had hepatoblastoma at the age of 18 mo but the precise genetic information of hepatoblastoma has not been obtained.

Reported cases of HCAs arising in FAP patients are extremely rare. According to our literature review, only seven cases, including our case, have been reported (Table 1)[5,10-14]. Five of these patients were female and two were male. Among them, one patient used oral contraceptives[12] and another had a medical history of androgenic steroid use for the treatment of anaplastic anemia[13]. HCA containing HCC in a male FAP patient was recently presented[14].

The germ-line mutation of the *APC* gene was examined in four cases, including our patient[10-12]. Bala *et al*[11] suggested that inherited mutations in the *APC* gene between codon 1444 and 1578 significantly increase the risk of developing extraintestinal tumors, including liver tumors. However, the other *APC* gene mutation occurred at different codons in 3 cases[10,12], including the present case (Table 1). Biallelic inactivation of the *APC* gene was described in two cases[10,11] (Table 1). In the first case, loss of the wild-type *APC* allele, which caused hemizygosity of the inherited mutation, was demonstrated[11]. A somatic 4-bp insertion was detected at codon 1516 in another case[10]. These findings suggest that the relationship between the *APC* gene anomaly and HCA is more complicated than initially expected.

Recently, genotype/phenotype classifications of HCA have drawn attention as a noticeable phenomenon from the aspects of pathogenesis and pathological tumorigenesis[21-23]. In their reports, HCAs are classified into four categories: (1) HCAs with mutations of the *HNF1* gene (H-HCA, 35%-40%); (2) HCAs with mutations of the *β-catenin* gene (β-HCA, 10%-15%); (3) inflammatory HCAs with mutation of the *IL6ST* gene (I-HCA, 40%-50%); and (4) HCAs without markers (unclassified HCA, less than 5%-10%). Our patient showed no symptoms or signs of an inflammatory syndrome. Additionally, the HCA in the present case morphologically lacked the typical characteristics of I-HCA, such as inflammatory infiltrates, sinusoidal dilatation and numerous thick arteries[21-23]. The *β-catenin* gene was supposed to be normal[24,25] because *β-catenin* was immunohistochemically detected only around the cytomembrane, without aberrant nuclear and cytoplasmic staining distributed in random and heterogeneous patterns (Figure 3D). Thus, the tumor is not β-HCA. In our case, histopathological characteristics of the liver tumor were closely compatible for H-HCA (Figure 3B and C) since H-HCAs are pathologically characterized by marked lipid deposition in tumor cells without

cytological abnormalities or inflammatory infiltrates[21-23]. However, no *HNF1* gene mutation was identified (data not shown). Although this tumor may be categorized as an unclassified HCA, further investigation of tumorigenesis is necessary[26].

In conclusion, we reported here a rare case of HCA arising in a female FAP patient. Because of its rarity, the pathogenesis of HCAs in patients with FAP remains undefined. More cases should be examined to establish the genetic alterations associated with benign hepatic tumorigenesis in FAP patients. Results may shed light on a breakthrough for hepatocellular carcinogenesis[25].

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