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BRIEF REPORT

# Comparative genome-wide analysis of gastric adenocarcinomas with hyperplastic polyp components

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## Abstract

Gastric hyperplastic polyps are common and generally regarded as benign lesions, whereas gastric adenocarcinomas infrequently occur from gastric hyperplastic polyps. Although gastric hyperplastic polyps have received a lot of attention because of their association with malignant transformation, it remains unclear whether gastric hyperplastic polyps are neoplastic lesions that have sporadic genetic changes similar to colorectal hyperplastic polyps. We performed genome-wide analyses of two gastric adenocarcinomas with hyperplastic polyp components. The interface between "adenocarcinoma" and "hyperplastic polyp" components was fairly sharp, and the adenocarcinoma components had copy number alterations and *TP53* mutations, whereas the hyperplastic polyp components had only single nucleotide polymorphisms, which were also found in adenocarcinoma components. We did not detect any somatic changes in the hyperplastic polyp components, even in genome-wide analyses, which was in contrast to the adenocarcinoma components. However, due to the small number of cases examined herein, further genetic analyses of more cases are needed.

**Keywords** Hyperplastic polyp · Adenocarcinoma · Malignant transformation · Array comparative genomic hybridization · Targeted amplicon sequencing

## Introduction

Gastric hyperplastic polyps are generally regarded as benign lesions, but some studies suggested that gastric hyperplastic polyps progressed to adenocarcinomas, similar to colorectal hyperplastic polyps. The incidence of malignant transformation of gastric hyperplastic polyps is low (1.5–4.5%) [1].

The molecular genetics of colorectal hyperplastic polyps have gradually been revealed. Jass was the first to propose the hyperplastic polyp-carcinoma sequence [2]. Colorectal hyperplastic polyps are classified into three subtypes: microvesicular, goblet cell-rich, and mucin-poor subtypes [3]. Microvesicular subtypes have *BRAF* mutations (66.7–88.0%) and may progress to sessile serrated adenomas/polyps, whereas goblet cell-rich subtypes have *KRAS* mutations (8.0–72.7%) [4]. Therefore, colorectal hyperplastic polyps are regarded as neoplastic lesions that progress to adenocarcinomas [4].

Although gastric hyperplastic polyps have received a lot of attention because of their association with malignant transformation, they have not been investigated in as much detail as colorectal hyperplastic polyps. Previous studies suggested that

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gastric adenocarcinomas in hyperplastic polyps follow a multistep progression model, such as the hyperplasia-dysplasia-adenocarcinoma sequence [5, 6]. However, it currently remains unclear whether gastric hyperplastic polyps have sporadic genetic changes [7–11]. Furthermore, the number of studies that have performed genome-wide analyses is limited [7, 9–11].

A limited number of studies have investigated gastric hyperplastic polyps without dysplasia/adenocarcinoma using genetic analyses [7–9]. Salomao et al. did not detect genetic mutations in five gastric hyperplastic polyps [7]. A few sporadic mutations (KRAS mutations in 1/10 [8] and 1/21 cases [9], and a BRAF mutation in 1/21 cases [9]) were discovered in gastric hyperplastic polyps, with a lower incidence than in colorectal hyperplastic polyps, as described above [4].

We herein examined two gastric adenocarcinomas with hyperplastic polyp components that showed the interface between "adenocarcinoma" and "hyperplastic polyp" components was fairly sharp within the same lesion. To investigate the genetic relationship between these histologically distinct components, we performed genome-wide analyses separately for each component.

## Materials and methods

### Conventional histological analysis

We reviewed endoscopically resected gastric hyperplastic polyps that were archived between 2009 and 2016 at Shirakawa Clinic and Keiaido Hospital. All tissue samples were routinely formalin-fixed, paraffin-embedded, cut into 3-μm-thick sections, and stained by hematoxylin and eosin. We identified five cases of adenocarcinomas with hyperplastic polyp components. Among them, we selected two cases with a clear border between the adenocarcinoma and hyperplastic polyp components within the same lesion to ensure that the molecular analyses using genomic DNA extraction from the adenocarcinoma and hyperplastic polyp components were performed without contamination (for details, see "Results").

### Immunohistochemical analysis

A panel of primary antibodies to the following antigens was used for immunohistochemistry: p53 (DO-7; 1:50; Leica Biosystems, Newcastle upon Tyne, UK) and HER2 (4B5; prediluted; Ventana Medical Systems, Inc., Tucson, AZ, USA). Cells were visualized using biotin-streptavidin immunoperoxidase kits (Histofine, Nichirei, Tokyo, Japan) and diaminobenzidine.

**Fig. 1** Loupe and microscopic images of case 1 (**a**–**d**) and case 2 (**e**–**h**). **a**, **e**. Loupe images. The black dotted lines indicate the interface between the adenocarcinoma and hyperplastic polyp components. DNA of each case was separately extracted from adenocarcinoma (red circle) and hyperplastic poly (blue circle) components. **b**, **f**. The black dotted lines indicate the interface between adenocarcinoma (right side) and hyperplastic polyp (left side) components. **c**, **g**. The adenocarcinoma components. The tumor cells had oval nuclei and eosinophilic columnar cytoplasm, and formed dilated, fused, and irregular glands. **d**, **h**. The hyperplastic components. The columnar epithelial cells without atypia formed dilated glands and papillary arrangements. Bar = 5 mm (**a**, **e**), 1 mm (**b**, **f**), and 200 μm (**c**, **d**, **g**, **h**).

### DNA extraction

DNA was extracted from paraffin-embedded sections separately from adenocarcinoma and hyperplastic polyp areas, as previously described [12].

### Array CGH

Array comparative genomic hybridization (CGH) analysis was performed using a 4 × 180 K CGH oligonucleotide microarray (Agilent Technologies, Santa Clara, CA, USA) as described previously [12, 13].

### Targeted amplicon sequencing

The mutation profiles of the primary tumors were determined by targeted amplicon sequencing using a next-generation sequencer as described previously [14]. The details of this method are described in Supplementary Methods.

### Direct DNA sequencing for *TP53* mutations

Genomic DNA extracted from formalin-fixed paraffin-embedded sections was amplified and sequenced using the primers described previously [15].

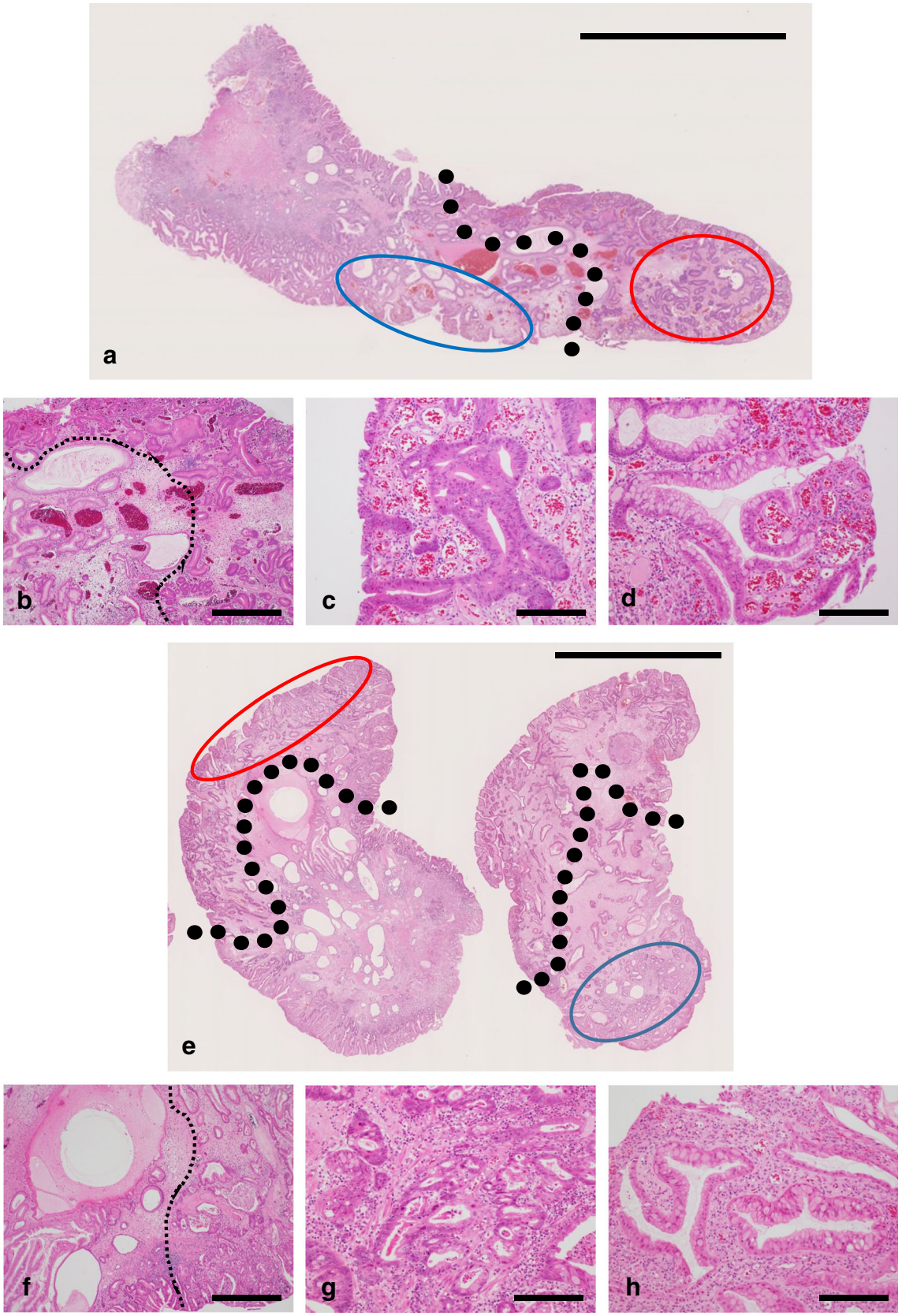
## Results

### Histological and immunohistochemical features

Cases 1 and 2 were a 67- and a 84-year-old, respectively, and both cases were women. The polyp lesion of case 1 was 11 × 5 × 5 mm in size on the greater curvature of the lower body (Supplementary Fig. 1a–c). The polyp lesion of case 2 was 17 × 15 × 13 mm in size on the greater curvature of the antrum (Supplementary Fig. 1d).

Representative histological features are shown in Fig. 1 (Case 1: Fig. 1a-d, Case 2: Fig. 1e-h).

The lesions in each case had two distinct morphologies by histology, which were clearly independent of each other (Fig. 1a, b, e, f). We did not detect any areas of




dysplasia/adenoma in either case. Both cases were negative for *Helicobacter pylori*.

The first lesion was a well to moderately differentiated tubular adenocarcinoma component (Fig. 1c, g). The tumor

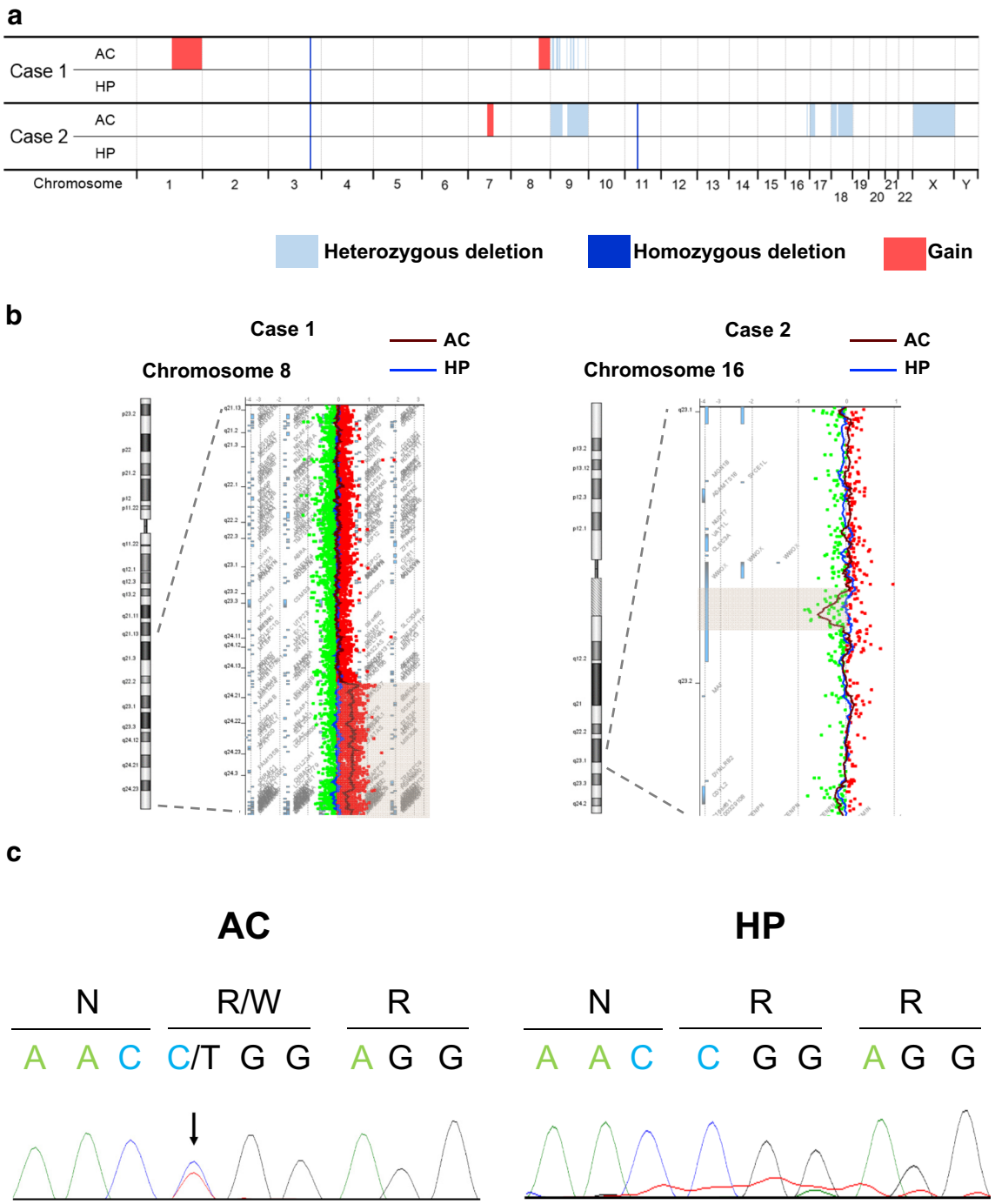



**Fig. 2** Results of the genetic analysis. **a** Copy number changes detected by array comparative genomic hybridization (CGH) performed separately for the adenocarcinoma and hyperplastic polyp components are visualized. **b** Representative images of array CGH. A gain in 8q24.13-q24.3 and a heterozygous deletion in 16q23.1 were observed only in the adenocarcinoma components of case 1 (left) and case 2 (right), respectively. **c** A *TP53* Arg248Trp mutation was detected only in the adenocarcinoma component (arrow) of case 2 by direct sequencing, and not in the hyperplastic polyp component. AC, adenocarcinoma component; HP, hyperplastic polyp component

cells had oval nuclei and eosinophilic columnar cytoplasm and formed dilated, fused, and irregular glands. The nuclear arrangement was either mono- or multilayered. Immunohistochemically, the tumor cells of case 1 were focally and weakly positive for p53, while those of case 2 were diffusely and strongly positive (Supplementary Fig. 2a, b). HER2 overexpression was not observed in either case (data not shown).

The other lesion was a hyperplastic polyp component (Fig. 1d, h). In this lesion, the columnar epithelial cells without atypia formed dilated glands and papillary arrangements. Immunohistochemically, the columnar

**Table 1** Variants detected by targeted amplicon sequencing

| | Gene | Chr | Position* | Ref | Variant | Genotype | Frequency | Read depth | Altered read depth | Protein | dbSNP build 138 ref. number | COSMIC ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case 1 | *ERBB4* | 2 | 212812097 | T | C | hom | 100 | 849 | 849 | Intronic | rs839541 | |
| | *PIK3CA* | 3 | 178917005 | A | G | het | 49.1 | 442 | 217 | Intronic | rs3729674 | |
| | *FGFR3* | 4 | 1807894 | G | A | hom | 100 | 1831 | 1831 | Synonymous | rs7688609 | |
| | *PDGFRA* | 4 | 55141055 | A | G | hom | 100 | 1693 | 1693 | Synonymous | rs1873778 | |
| | *KDR* | 4 | 55980239 | C | T | het | 48.7 | 1181 | 575 | Intronic | rs7692791 | |
| | *APC* | 5 | 112175770 | G | A | hom | 100 | 1939 | 1939 | Synonymous | rs41115 | |
| | *HMGXB3/CSF1R* | 5 | 149433596 | TG | GA | het | 30.8 | 1806 | 557 | Intronic | rs35308019 | |
| | *EGFR* | 7 | 55249063 | G | A | hom | 100 | 1257 | 1257 | Synonymous | rs1050171 | |
| | *MET* | 7 | 116339672 | C | T | het | 52.5 | 2000 | 1051 | Synonymous | rs35775721 | |
| | *MET* | 7 | 116340262 | A | G | het | 51.8 | 951 | 493 | p.Asn375Ser | rs33917957 | COSM710 |
| | *FLT3* | 13 | 28610183 | A | G | hom | 100 | 1999 | 1999 | Intronic | rs2491231 | |
| | *TP53* | 17 | 7579472 | G | C | het | 52.6 | 1934 | 1018 | p.Pro72Arg | rs1042522rs1042522 | |
| Case 2 | *ERBB4* | 2 | 212812097 | T | C | het | 58.9 | 531 | 313 | Intronic | rs839541rs839541 | |
| | *FGFR3* | 4 | 1807894 | G | A | hom | 100 | 1111 | 1111 | Synonymous | rs7688609rs7688609 | |
| | *PDGFRA* | 4 | 55141055 | A | G | hom | 100 | 1346 | 1346 | Synonymous | rs1873778 rs1873778 | |
| | *KDR* | 4 | 55962546 | – | G | het | 55.1 | 1994 | 1098 | Intronic | rs3214870 | |
| | *KDR* | 4 | 55972974 | T | A | het | 51.7 | 1998 | 1033 | p.Gln472His | rs1870377 | |
| | *APC* | 5 | 112175770 | G | A | hom | 100 | 1994 | 1994 | Intronic | rs41115 | |
| | *RET* | 10 | 43613843 | G | T | hom | 100 | 1998 | 1998 | Synonymous | rs1800861 | |
| | *HRAS* | 11 | 534242 | A | G | hom | 100 | 927 | 927 | Synonymous | rs12628 | COSM249860 |
| | *FLT3* | 13 | 28610183 | A | G | het | 52.2 | 2000 | 1044 | Intronic | rs2491231 | |
| | *TP53* | *17* | *7577539* | *G* | *A* | *het* | *29.4* | *1993* | *586* | *p.Arg248Trp* | | *COSM10656* |
| | *TP53* | 17 | 7579472 | G | C | het | 70.8 | 1977 | 1399 | p.Pro72Arg | rs1042522 | |
| | *STK11* | 19 | 1220321 | T | C | hom | 100 | 1990 | 1990 | Intronic | rs2075606 | |

Variants detected only in the adenocarcinoma component are shown in italics. Genome mapping based on genome build hg19

*Chr* chromosome, *het* heterozygous, *hom* homozygous, *ref* reference

epithelial cells were mostly negative for p53 (Supplementary Fig. 2a, b).

### Genetic analysis

The results of array CGH revealed that most copy number changes were observed only in the adenocarcinoma components: a gain of whole chromosome arm 1q (case 1), a gain in 8q24.13-q24.3 (case 1), multiple small heterozygous deletions in chromosome 9 (case 1), a gain in 7q21.11 (case 2), a heterozygous deletion of whole chromosome 9 (case 2), a heterozygous deletion in 16q23.1 (case 2), a heterozygous deletion of whole chromosome arm 17p (case 2), a heterozygous deletion of whole chromosome 18 (case 2), and a heterozygous deletion of whole chromosome X (case 2) (Fig. 2a, b). Small homozygous deletions in 3q26.1 (case 1, 2) and 11q12.1 (case 2) found in both the adenocarcinoma and hyperplastic polyp components were in regions of known benign copy number variants (polymorphisms) reported in the Database of Genomic Variants (DGV) (http://dgv.tcag.ca/dgv/app/home) (Fig. 2a).

Targeted amplicon sequencing revealed 12 and 11 shared variants in both components of cases 1 and 2, respectively, and all these variants were reported in dbSNP build 138 (Table 1); together with their allelic fractions, they were considered to be germline. In addition, a *TP53* mutation in hotspot codon 248 (Arg248Trp) was observed only in the adenocarcinoma component of case 2 and was confirmed by Sanger sequencing (Fig. 2c).

## Discussion

Genetic analyses of gastric hyperplastic polyps with dysplasia/adenocarcinoma were performed in previous studies [7–11]. Salomao et al. revealed *TP53*, *RB1*, and *PIK3CA* mutations in the gastric hyperplastic polyps with dysplasia by targeted next-generation sequencing [7]. However, they did not obtain adequate non-dysplastic areas in the gastric hyperplastic polyps with dysplasia for sequencing analysis. Weiss et al. detected copy number alterations in three gastric hyperplastic polyps with dysplasia using microarray CGH [11]. However, only "dysplasia" components were examined in three gastric hyperplastic polyps with dysplasia, and the copy number alterations of the "hyperplastic polyp" components were not investigated in detail.

A limited number of studies examined hyperplastic polyp components in gastric hyperplastic polyps with dysplasia/adenocarcinoma [8–10]. Dijkhuizen et al. detected the *Kras* mutation in the areas of hyperplasia, dysplasia, and adenocarcinoma in one gastric hyperplastic polyp with dysplasia/adenocarcinoma [8]. Saab et al. analyzed the "hyperplastic areas" and the "dysplastic areas" separately in four gastric hyperplastic polyps with dysplasia [9]. They described one case of a hyperplastic area with a *CTNNB1* mutation and one with an *APC* mutation. In another study, Anjiki et al. found a gain of 8q in the "adenocarcinoma" component of the gastric adenocarcinoma with a hyperplastic polyp component using array CGH [10]. They also investigated the "hyperplastic polyp" component within the same lesion; however, the area did not have any copy number alterations.

The present study is one of the few studies using genome-wide analyses and showed that only the "adenocarcinoma" components had possible somatic changes of copy number alterations and a *TP53* mutation (Fig. 2; Table 1). The "hyperplastic polyp" components showed only single nucleotide polymorphisms and copy number changes, which may be benign copy number variants (polymorphisms); therefore, we did not detect any possible somatic changes in the "hyperplastic polyp" components in contrast to the "adenocarcinoma" components, even using genome-wide analyses. However, pathogenic mutations may have been overlooked because of the limited targeted sequencing panel.

In conclusion, we separately analyzed histologically different components in two representative cases of gastric adenocarcinomas with hyperplastic polyp components; however, possible sporadic genetic changes could not be found in the "hyperplastic polyp" components, even using genome-wide analyses, in contrast to the "adenocarcinoma" components. Therefore, from a genetic point of view, we were unable to prove the gastric hyperplastic polyp-adenocarcinoma sequence as in colorectal hyperplastic polyps. Since the number of cases examined in the present study was limited, further genetic analyses of more cases are needed.

### Compliance with ethical standards

The present study was approved by the Gunma University Ethical Committee.

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Contributions** All the authors have contributed significantly to the content of the manuscript.

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