

Supplementary Information

for

Impaired 8-hydroxyguanine repair activity of MUTYH variant p.Arg109Trp found in a Japanese patient with early-onset colorectal cancer

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The distribution of *supF* base substitution-type mutations in a *supF* forward mutation assay using the pMY189 plasmid containing 8-hydroxyguanine (8OHG) at position 159 of *supF* in H1299 human cell lines inducibly expressing MUTYH protein

Supplementary TABLE S1: Primers used for PCR amplification of the *MUTYH* gene.

Amplicon number	Forward sequence (5'->3')	Reverse sequence (5'->3')	Product size (bp)
1	gaagctcgggagctgaaac	cggcgacccgacggcgagac	160
2	tggcctcattgtgactgac	gcacctggcccttagtaagtc	280
3	aggctgctgtgtccaagac	gaggacactgctgacctgcc	444
4	ggcaggtcagcagtgctctc	ccctagctcctctaccacctg	542
5	ggtaggaaccaggagtcttg	agagctccttgcagacacc	406
6	ctgcaaaggagctctgcttc	cttagggccaacctagagag	463
7	taggttgcccctaaagccc	gccaacgctgtagttcctg	448
8	tggcaggactgtgggagttc	caacagagcgattctccgtc	488
9	tgggattataggcgtgagcc	acctatggactcaggcctgg	239
10	tccccaaactacaaggcctc	aacatagcgagacccccatctc	278

Supplementary TABLE S2: Primers used for PCR amplification of the *OGG1* gene.

Amplicon number	Forward sequence (5'->3')	Reverse sequence (5'->3')	Product size (bp)
1	cttaagggtcgtggccttg	gaggggacaggcttctcag	268
2	catgttgctcagactggaagatag	cttatgtccaagaaccctaacc	427
3	ctctaacggtgctgacttcatt	gaacagatcttgaaagctgatgg	367
4	cttgaagatgcctgatgcttg	ctttctccttctgctctgc	346
5	gggctataagcaagatgctgg	gagaagttctaccatcccagc	274
6	cacctctcctcagaccctac	tggggaatttctttgtccag	493
7	cttgtgcaggacagcaatctc	attcattccaagcaggc	473

Supplementary TABLE S3: Primers used for PCR-CTPP of the *MUTYH* c.55C>T and c.325C>T variants.

Variant	Primer name ^a	Primer sequence (5'→3') ^b
c.55C>T	F1	CTTTGCATGTCTCCAGGGCTG
c.55C>T	F2	GGCCATCATGAGGAAGCCAC
c.55C>T	R1	CACTTCCCACGGCTGCTCA
c.55C>T	R2	CCTTGGGCCACAACCTAGTTCC
c.325C>T	F1	AGCCTGTGCAGGGATGATT
c.325C>T	F2	CTAAGCTGGTACGACCAAGAGAAAC
c.325C>T	R1	TCTTCTCCATGGTAGGTCCCA
c.325C>T	R2	CATGACCTCTGAGACCCACAC

^aThis primer name is corresponded to that in Figure 1C,D.

^bNucleotide at the variant position is shown in bold.

Supplementary TABLE S4: *MUTYH* nucleotide variations in its coding region and splice-site region found in 34 Japanese patients with early-onset CRC

Case No.	c.55C>T (p.Arg19*)	c.325C>T (p.Arg109Trp)	c.934-2A>G	c.1014G>C (p.Gln338His)	c.1118C>T (p.Ala373Val)	c.1431G>C (p.Thr477Thr)
1	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
2	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
3	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
4	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
5	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
6	wt/wt	wt/wt	wt/wt	vt/vt	wt/wt	wt/wt
7	wt/wt	wt/wt	wt/vt	wt/vt	wt/wt	wt/wt
8	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
9	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
10	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/vt
11	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/vt
12	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
13	wt/wt	wt/wt	wt/wt	vt/vt	wt/wt	wt/wt
14	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
15	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
16	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
17	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
18	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
19	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
20	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
21	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
22	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
23	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
24	wt/wt	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt
25	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
26	wt/wt	wt/wt	wt/wt	vt/vt	wt/wt	wt/wt
27	wt/vt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
28	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
29	wt/wt	wt/wt	wt/wt	vt/vt	wt/wt	wt/wt
30	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt
31	wt/wt	wt/vt	wt/wt	wt/vt	wt/wt	wt/wt
32	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
33	wt/wt	wt/wt	wt/wt	wt/vt	wt/wt	wt/wt
34	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt	wt/wt

wt: wild type, vt: variant type.

Genotype containing variant type is shown in red font.

Supplementary TABLE S5: *OGGI* nucleotide variations in its coding region found in 34 Japanese patients with early-onset CRC.

Case No.	c.294G>A (p.Lys98Lys)	c.966C>T (p.Asp322Asp)	c.977C>G (p.Ser326Cys)
1	wt/wt	wt/wt	wt/vt
2	wt/wt	wt/wt	vt/vt
3	wt/wt	wt/wt	wt/wt
4	wt/wt	wt/wt	wt/vt
5	wt/wt	wt/wt	wt/vt
6	wt/wt	wt/wt	wt/vt
7	wt/wt	wt/wt	wt/vt
8	wt/wt	wt/wt	wt/wt
9	wt/wt	wt/wt	wt/wt
10	wt/wt	wt/wt	wt/wt
11	wt/vt	wt/wt	wt/wt
12	wt/wt	wt/wt	wt/wt
13	wt/wt	wt/wt	wt/vt
14	wt/wt	wt/wt	wt/vt
15	wt/wt	wt/wt	vt/vt
16	wt/wt	wt/wt	wt/vt
17	wt/wt	wt/wt	wt/wt
18	wt/wt	wt/wt	wt/wt
19	wt/wt	wt/wt	wt/vt
20	wt/wt	wt/wt	vt/vt
21	wt/wt	wt/wt	vt/vt
22	wt/wt	wt/wt	wt/vt
23	wt/wt	wt/wt	wt/wt
24	wt/wt	wt/wt	wt/vt
25	wt/wt	wt/wt	vt/vt
26	wt/wt	wt/wt	vt/vt
27	wt/wt	wt/wt	wt/vt
28	wt/wt	wt/vt	wt/vt
29	wt/wt	wt/wt	wt/wt
30	wt/wt	wt/wt	wt/vt
31	wt/wt	wt/wt	wt/vt
32	wt/wt	wt/wt	wt/wt
33	wt/wt	wt/wt	wt/vt
34	wt/wt	wt/wt	wt/vt

wt: wild type, vt: variant type.

Genotype containing variant type is shown in red font.

LEGENDS FOR SUPPLEMENTARY FIGURES

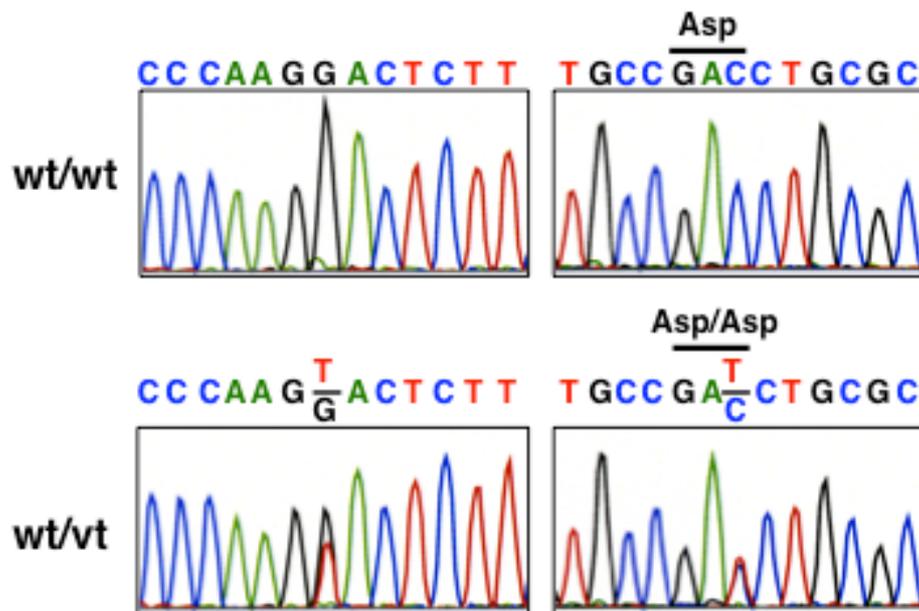
Supplementary FIGURE S1: Identification of novel *OGGI* variants of c.949-89G>T and c.966C>T (p.Asp322Asp) in Japanese patients with early-onset CRC by sequencing analysis. Sequencing electropherograms show a G>T and C>T nucleotide variation at c.949-89 and c.966 position, respectively (lower panels). wt: wild type, vt: variant type. These *OGGI* nucleotide variations have not been reported to date, meaning they are novel *OGGI* variants.

Supplementary FIGURE S2: The distribution of *supF* base substitution-type mutations in a *supF* forward mutation assay using the pMY189 plasmid containing 8-hydroxyguanine (8OHG) at position 159 of *supF* in H1299 human cell lines inducibly expressing MUTYH protein. Empty vector-transposed cells and cells inducibly expressing WT type 2 MUTYH, type 2 p.Arg81Trp MUTYH variant, or p.Asp208Asn negative control in the presence of cumate were analyzed.

Supplementary Figure S1

c.949-89G>T

c.966C>T(p.Asp322Asp)



Supplementary Figure S2

Empty vector-transposed cells

```
100      110      120      130      140      150      160      170      180
|        |        |        |        |        |        |        |
5'-GGTGGGGTTC CCGAGCGGCCAAAGGGAGCAGACTCTAAATCTGCCGTCATCGACTTCGAAGGTTTCGAATCCTTCCCCCACCACC-3'
                                     C
                                     A
                                     T
                                     TTT
                                     TTTT
                                     TTTTTT
                                     TTTTTTTT
```

WT MUTYH-transposed cells

```
100      110      120      130      140      150      160      170      180
|        |        |        |        |        |        |        |
5'-GGTGGGGTTC CCGAGCGGCCAAAGGGAGCAGACTCTAAATCTGCCGTCATCGACTTCGAAGGTTTCGAATCCTTCCCCCACCACC-3'
          C          T          C          T          T          T
          C          T          A          T          T          T
          TTT
          TTT
```

p.R81W MUTYH-transposed cells

```
100      110      120      130      140      150      160      170      180
|        |        |        |        |        |        |        |
5'-GGTGGGGTTC CCGAGCGGCCAAAGGGAGCAGACTCTAAATCTGCCGTCATCGACTTCGAAGGTTTCGAATCCTTCCCCCACCACC-3'
          C
          T
          TTT
          TTTT
          TTTTTT
          TTTTTTTT
```

p.D208N MUTYH-transposed cells

```
100      110      120      130      140      150      160      170      180
|        |        |        |        |        |        |        |
5'-GGTGGGGTTC CCGAGCGGCCAAAGGGAGCAGACTCTAAATCTGCCGTCATCGACTTCGAAGGTTTCGAATCCTTCCCCCACCACC-3'
          T
          T
          TTT
          TTTT
          TTTTTT
          TTTTTTTT
```