Japanese genome-wide association study identifies a significant colorectal cancer susceptibility locus at chromosome 10p14

Yusuke Takahashi,1,2 Keishi Sugimachi,1,3 Ken Yamamoto,4 Atsushi Niida,5 Teppei Shimamura,5,6 Tetsuya Sato,7 Masahiko Watanabe,8 Junichi Tanaka,9 Shinee Kudo,9 Kenichi Sugihara,10 Kazuo Hase,11 Masato Kusunoki,12 Kazutaka Yamada,13 Yasuhiro Shimada,14 Yoshihiro Moriya,14 Yutaka Suzuki,15 Satoru Miyano,5 Masaki Mori2 and Koshi Mimori1

1Department of Surgery, Kyushu University Beppu Hospital, Beppu; 2Department of Gastroenterological Surgery, Osaka University, Suita; 3Department of Hepatobiliopancreatic surgery, National Kyushu Cancer Center, Fukuoka; 4Department of Medical Biochemistry, Kurume University, Kurume; 5Laboratory of DNA Information Analysis, Human Genome Center Institute of Medical Science, University of Tokyo, Tokyo; 6Division of System Biology, Graduate School of Medicine, Nagoya University, Nagoya, Japan; 7Division of Bioinformatics, Medical Institute of Bioregulation, Kyushu University, Fukuoka; 8Department of Surgery, Kitazato University, Sagamihara; 9Digestive Disease Center, Northern Yokohama Hospital, Showa University, Yokohama; 10Department of Surgery, Tokyo Medical and Dental University, Tokyo; 11Department of Surgery, National Defense University, Tokorozawa; 12Department of Surgery, Mie University, Tsu; 13Department of Surgery, Takano Hospital, Kumamoto; 14Department of Surgery and Digestive Tract Medicine, National Cancer Center, Tokyo; 15Department of Medical Genome Sciences, Graduate School of Frontier Sciences, University of Tokyo, Kashiwa, Japan

Key words
Colorectal cancer, genome-wide association study, Japanese, single nucleotide polymorphism, 10p14

Correspondence
Koshi Mimori, Department of Surgery, Kyushu University Beppu Hospital, 4546 Tsurumihara, Beppu 874-0838, Japan.
Tel: +81-977-27-1650, Fax: +81-977-27-1652; E-mail: kmimori@beppu.kyushu-u.ac.jp

and
Masaki Mori, Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Yamadaoka 2-2, Suita 565-0871, Japan.Tel: +81-6-6879-3251, Fax: +81-6-6879-3259; E-mail: mmori@gesurg.med.osaka-u.ac.jp

Yusuke Takahashi and Keishi Sugimachi are equally contributed to the manuscript.

Masaki Mori and Koshi Mimori are also equally contributed to the manuscript.

Funding Information
This work was supported in part by the following grants and foundations: CREST, Japan Science and Technology Agency (JST); Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research, grant numbers 15H0912, 15H05707, 21591644, 21791295, 21791297, 215921014 and 21679006; the Funding Program for Next Generation World-Leading Researchers (LS094); New Energy and Industrial Technology Development Organization (NEDO) Technological Development for Chromosome Generation World-Leading Researchers (h170227 and hp160219).

Received June 15, 2017; Revised August 19, 2017; Accepted August 29, 2017

Cancer Sci 108 (2017) 2239–2247
doi: 10.1111/cas.13391

Genome-wide association studies are a powerful tool for searching for disease susceptibility loci. Several studies identifying single nucleotide polymorphisms (SNP) connected intimately to the onset of colorectal cancer (CRC) have been published, but there are few reports of genome-wide association studies in Japan. To identify genetic variants that modify the risk of CRC oncogenesis, especially in the Japanese population, we performed a multi-stage genome-wide association study using a large number of samples: 1846 CRC cases and 2675 controls. We identified 4 SNP (rs7912831, rs4749812, rs7898455 and rs10905453) in chromosome region 10p14 associated with CRC; however, there are no coding or non-coding genes within this region of fairly extensive linkage disequilibrium (a 500-kb block) on 10p14. Our study revealed that the 10p14 locus is significantly correlated with susceptibility to CRC in the Japanese population, in accordance with the results of multiple studies in other races.

© 2017 The Authors. Cancer Science published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
### Table 1. Summary of previously reported single nucleotide polymorphisms (SNP) associated with colorectal cancer

| SNP    | Chromosome | Gene  | Odds ratio | P-value | Population                      | First author | Journal         | Year | Reference number |
|--------|------------|-------|------------|---------|---------------------------------|--------------|-----------------|------|-----------------|
| rs4939627 | 18q21     | SMAD7 | 1.15       | 1.0 x 10^(-12) | Caucasian | Broderick P | Nat Genet | 2007 | 4               |
| rs6983267 | 8q24      | --    | 1.17       | 3 x 10^-11    | European | Tomlinson IP | Nat Genet | 2007 | 1               |
| rs4444423 | 14q22.2   | BMP4  | 1.11       | 8.1 x 10^-10  | European | Houlston RS | Nat Genet | 2008 | 6               |
| rs9929218 | 16q22.1   | CDH1  | 0.91       | 1.2 x 10^-8   | European | Houlston RS | Nat Genet | 2008 | 6               |
| rs10411210 | 19q13.1   | RHPN2 | 0.87       | 4.6 x 10^-9   | European | Houlston RS | Nat Genet | 2008 | 6               |
| rs961253  | 20p12.3   | --    | 1.12       | 2.0 x 10^-10  | European | Houlston RS | Nat Genet | 2008 | 6               |
| rs4779584 | 15q13     | CRAC1 | 1.26       | 4.4 x 10^-14  | Ashkenazi Jews and Europeans | Jaeger E | Cancer Science | 2008 | 7               |
| rs3802842 | 11q23     | --    | 1.1        | 5.8 x 10^-10  | European, Japanese, Israeli | Tenesa A | Nat Genet | 2008 | 5               |
| rs10795668 | 10p14     | --    | 1.12       | 2.5 x 10^-13  | European | Tomlinson IP | Nat Genet | 2008 | 2               |
| rs16892766 | 8q23.3    | EBF3H | 1.26       | 3.3 x 10^-18  | European | Tomlinson IP | Nat Genet | 2008 | 2               |
| rs7136702 | 12q13.13  | --    | 1.06       | 4.0 x 10^-8   | European | Houlston RS | Nat Genet | 2010 | 8               |
| rs6687758 | 1q41      | --    | 1.09       | 2.2 x 10^-9   | European | Houlston RS | Nat Genet | 2010 | 8               |
| rs6691170 | 1q41      | --    | 1.06       | 9.5 x 10^-10  | European | Houlston RS | Nat Genet | 2010 | 8               |
| rs4925386 | 20q13.33  | LAMAS | 0.93       | 1.8 x 10^-10  | European | Houlston RS | Nat Genet | 2010 | 8               |
| rs10936599 | 3q26      | MYNN  | 0.93       | 3.9 x 10^-8   | European | Houlston RS | Nat Genet | 2010 | 8               |
| rs7758229 | 6q26      | SLC22A3 | 1.28     | 7.9 x 10^-9   | Japanese and Korean | Cui R | Gut | 2011 | 9               |
| rs1957636 | 14q22     | BMP4  | 1.084      | 3.9 x 10^-10  | Caucasian | Tomlinson IP | Nat Genet | 2011 | 11              |
| rs11632715 | 15p      | GREM1 | 1.116      | 2.3 x 10^-10  | Caucasian | Tomlinson IP | Nat Genet | 2011 | 11              |
| rs16969681 | 15p      | GREM1 | 1.181      | 5.3 x 10^-8   | Caucasian | Tomlinson IP | Nat Genet | 2011 | 11              |
| rs4813802 | 20p12     | BMP2  | 1.093      | 4.6 x 10^-11  | Caucasian | Tomlinson IP | Nat Genet | 2011 | 11              |
| rs3824999 | 11q13.4   | POLD3 | 1.08       | 3.6 x 10^-10  | European and Japanese | Dunlop MG | Nat Genet | 2012 | 10              |
| rs1321311 | 6p21      | CDKN1A | 1.1       | 1.1 x 10^-10  | European and Japanese | Dunlop MG | Nat Genet | 2012 | 10              |
| rs5934683 | Xp22.2    | SHROOM2 | 1.07     | 7.3 x 10^-10  | European and Japanese | Dunlop MG | Nat Genet | 2012 | 10              |
| rs4813802 | 20p12     | BMP2  | 1.18       | 7.3 x 10^-5   | Caucasian | Peters U | Hum Genet | 2012 | 12              |
| rs2853668 | 5p33.15   | TERT-CLPTM1L | 0.85 | 1.9 x 10^-4  | Caucasian | Peters U | Hum Genet | 2012 | 12              |
| rs10774214 | 12p13.32  | CCND2 | 1.17       | 3.06 x 10^-8  | East Asian and European | Jia WH | Nat Genet | 2013 | 13              |
| rs2432279 | 20p12.3   | HAQ1, PLCB1 | 1.14 | 6.6 x 10^-9  | East Asian and European | Jia WH | Nat Genet | 2013 | 13              |
| rs647161  | 5q31.1    | P1YX1 | 1.17       | 1.2 x 10^-10  | East Asian and European | Jia WH | Nat Genet | 2013 | 13              |
| rs3987    | 4q26      | NDST3 | 1.36       | 4.0 x 10^-8   | Spanish | Real LM | Plos One | 2014 | 14              |
| rs35509282 | 4q32.2    | RSTL5 | 1.53       | 8.2 x 10^-9   | Ashkenazi Jews and Europeans | Schmitt SL | Carcinogenesis | 2014 | 15              |
| rs12241008 | 10q25     | VTI1A | 1.19       | 1.4 x 10^-9   | European, African and Japanese | Wang H | Nat Commun | 2014 | 16              |
| rs1035209 | 10p24.2   | MRP2  | 1.13       | 4.54 x 10^-11 | East Asians in our European | Whiffin N | Hum Mol Genet | 2014 | 17              |
| rs3217810 | 12p13.32  | CCND2 | 1.19       | 2.16 x 10^-10 | East Asians in our European | Whiffin N | Hum Mol Genet | 2014 | 17              |
| rs10911251 | 1q25.3    | LAMC1 | 1.09       | 1.75 x 10^-8  | East Asians in our European | Whiffin N | Hum Mol Genet | 2014 | 17              |
| rs7229639 | 18q21.1   | SMAD7 | 1.22       | 2.39 x 10^-11 | East Asians | Zhang B | Int J Cancer | 2014 | 18              |
| rs7064017 | 10q22.3   | ZMIZ1-AS1 | 1.1     | 2.07 x 10^-8  | East Asians | Zhang B | Nat Genet | 2014 | 18              |
| rs11196172 | 10q25.2   | TCF7L2 | 1.14       | 1.04 x 10^-12 | East Asians | Zhang B | Nat Genet | 2014 | 18              |
| rs1535    | 11q12.2   | FADS2 | 1.15       | 8.21 x 10^-20 | East Asians | Zhang B | Nat Genet | 2014 | 18              |
| rs174537  | 11q12.2   | MYRF  | 1.16       | 9.2 x 10^-21  | East Asians | Zhang B | Nat Genet | 2014 | 18              |
| rs174550  | 11q12.2   | FADS1 | 1.15       | 1.58 x 10^-19 | East Asians | Zhang B | Nat Genet | 2014 | 18              |
| SNP       | Chromosome | Gene                | Odds ratio | P-value          | Population       | First author | Journal    | Year | Reference number |
|-----------|------------|---------------------|------------|------------------|------------------|--------------|------------|------|------------------|
| rs4246215 | 11q12.2    | FEN1                | 1.15       | 7.65 × 10⁻²⁰     | East Asians      | Zhang B      | Nat Genet  | 2014 | 18               |
| rs10849432| 12p13.31   | CD9                 | 1.14       | 5.81 × 10⁻¹⁰     | East Asians      | Zhang B      | Nat Genet  | 2014 | 18               |
| rs12603526| 17p13.3    | NXN                 | 1.1        | 3.42 × 10⁻⁸      | East Asians      | Zhang B      | Nat Genet  | 2014 | 18               |
| rs1800469 | 19q13.2    | TGFBI               | 1.09       | 1.17 × 10⁻⁸      | East Asians      | Zhang B      | Nat Genet  | 2014 | 18               |
| rs2241714 | 19q13.2    | B9D2                | 1.09       | 1.36 × 10⁻⁸      | East Asians      | Zhang B      | Nat Genet  | 2014 | 18               |
| rs10904849| 10p13      | CUBN                | 1.14       | 7.01 × 10⁻⁸      | East Asians      | Al-Tassan NA | Sci Rep    | 2015 | 19               |
| rs17836917| 17q12      | MYO1D, CCL8, CCL13  | 0.75       | 4.55 × 10⁻⁸      | Han Chinese      | Jiang K      | Oncotarget | 2015 | 20               |
| rs12522693| 5q23.3     | HINT1               | 1.31       | 2.08 × 10⁻⁸      | Han Chinese      | Jiang K      | Oncotarget | 2015 | 20               |
| rs17094983| 14q23.1    | –                   | 0.87       | 2.5 × 10⁻¹⁰      | African          | Lemire M     | Hum Genet  | 2015 | 21               |
| rs16941835| 16q24.1    | RPI1-SBA18          | 1.15       | 5.06 × 10⁻⁸      | African          | Lemire M     | Hum Genet  | 2015 | 21               |
| rs72647484| 1q36.2     | CDC42, WNT4         | 1.21       | 1.21 × 10⁻⁸      | African          | Lemire M     | Hum Genet  | 2015 | 21               |
| rs1119016410| 10p24.1 | SLC25A28, ENTPD7, COX15, CUTC, ABC2 | 1.09 | 4.0 × 10⁻⁸ | European and Asian | Schumacher FR | Nat Commun | 2015 | 22               |
| rs318450412| 12q24.12  | SH2B3               | 1.09       | 1.7 × 10⁻⁸       | European and Asian | Schumacher FR | Nat Commun | 2015 | 22               |
| rs7320812012| 12q24.22 | NOS1                | 1.16       | 2.8 × 10⁻⁸       | European and Asian | Schumacher FR | Nat Commun | 2015 | 22               |
| rs606682520| 20q13.13  | PREX1               | 1.09       | 4.4 × 10⁻⁹       | European and Asian | Schumacher FR | Nat Commun | 2015 | 22               |
| rs8124813 | 3p14.1     | LRRG1               | 1.09       | 2.0 × 10⁻⁸       | European and Asian | Schumacher FR | Nat Commun | 2015 | 22               |
| rs35360328 | 3p22.1     | CTNNB1              | 1.14       | 3.1 × 10⁻⁹       | European and Asian | Schumacher FR | Nat Commun | 2015 | 22               |
| rs4711689 | 6p21.1     | TFB1                | 1.11       | 3.9 × 10⁻⁸       | East Asian       | Zeng C       | Gastroenterology | 2016 | 23               |
| rs2450115 | 8q23.3     | EIF3H               | 1.12       | 1.2 × 10⁻¹²      | East Asian       | Zeng C       | Gastroenterology | 2016 | 23               |
| rs6469656 | 8q23.3     | EIF3H               | 1.11       | 2.0 × 10⁻¹¹      | East Asian       | Zeng C       | Gastroenterology | 2016 | 23               |
| rs4919687 | 10q24.3    | CYP17A1             | 1.14       | 7.8 × 10⁻¹²      | East Asian       | Zeng C       | Gastroenterology | 2016 | 23               |
| rs11064437| 12p13.3    | SP582               | 1.12       | 4.5 × 10⁻¹¹      | East Asian       | Zeng C       | Gastroenterology | 2016 | 23               |
of the disease. For the last ten years, several genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNP) intimately connected to the onset of CRC. Tomlinson et al. identified rs6983267 at 8q24.21 as the SNP most strongly connected to the onset of CRC.\(^{1,2}\) Zanke et al.\(^{3}\) investigated 100k SNP in 7480 cases of CRC with double screening among different races and discovered SNP at 8q24 as well as one at 9q24. Brodelick et al.\(^{4}\) report the SNP rs4938827 at 18q21 located within the gene SMAD7 from among 550k SNP in 7473 cases of CRC. Tenesa et al.\(^{5}\) report the SNP rs3802842 at 11q23, rs7014346 at 8q24 and rs4938827 at 18q21. Table 1 shows the data derived from previous GWAS on CRC.\(^{1,2,4,23}\) which reveal that risky genetic polymorphisms are different among various populations. For example, in the 8q23–24 region, rs6983267 is a risk factor for CRC among Caucasians, Asians and Africans, rs7014346 and rs10505477 are risky only among Caucasians, and rs16892766 is a risk factor for those with Caucasian and African ancestry.\(^{24}\)

As for the Japanese population, several SNP-based GWAS have been performed for CRC. Matsuo et al.\(^{25}\) performed a case-control study using 481 cases and 962 controls and report an association between CRC and rs6983267 at 8q24. Furthermore, Cui et al.\(^{9}\) performed GWAS using 1583 Japanese CRC cases and 1898 controls and replication analyses using a total of 4809 CRC cases and 2973 controls, including 225 Korean subjects with distal colon cancer and 377 controls. They found an association between CRC and a known carcinogenic SNP at 8q24 and an association between distal CRC and rs7758229 in intron 5 of SLC22A3 at 6q26.\(^{26}\) Zhang et al.\(^{26}\) performed a case-control study and reported that microsomal glutathione S-transferase 1 (MGST1) gene polymorphisms had an association with CRC risk (OR = 1.682, \(P = 0.004\)) among the Han Chinese. However, the molecular biological mechanisms by which these SNP are involved in colorectal carcinogenesis remain unclear.

Using a case-control study on 1511 CRC cases and 2098 controls, we previously reported that the risk allele of rs6983267 in 8q24 is associated with CRC, especially in diabetes mellitus patients.\(^{27}\) However, these SNPs, except for those in 8q24, have not been defined as the regulating polymorphisms of colorectal carcinogenesis beyond racial differences. To find the responsible host genetic factors, we designed an SNP-based GWAS to identify SNP associated with susceptibility to morbidity from CRC in a pure Japanese population.\(^{28}\) We performed a multistage genome-wide association study in Japanese individuals, with a total of

---

**Fig. 1.** The complete design of the genome-wide association study. In phase 1, 577 patients with colorectal cancer (CRC) and 571 controls were genotyped for 500 568 single nucleotide polymorphisms (SNP) with Affymetrix 500K chip sets. Two additional rounds of screening using the Illumina GoldenGate Assay (1536 SNP for phase 2.2) and TaqMan Assay (21 SNP for phase 3) were performed to identify significant SNP.

**Fig. 2.** (a) Manhattan plots from the phase 1 genome-wide association results. \(P\)-values (–log10 \(P\), \(y\)-axis) are plotted against their respective chromosomal positions (\(x\)-axis). Each chromosome is depicted in alternating blue and red. (b) Log quantile-quantile \(P\)-values between the expected (theoretical) \(P\)-value and the observed \(P\)-value. The genomic inflation factor (based on median \(\chi^2\)) is 1.10. If we set the odds ratio of the CRC-related genotype as 1.4 and the allelic frequency in the control as 0.2, it will be located at the 36th quantile by the \(P\)-value distribution. If we set the odds ratio of the CRC-related genotype as 1.4 and the allelic frequency in the control as 0.4, it will be located at the 350th. If we set the odds ratio of the CRC-related genotype as 1.2 and the allelic frequency in the control as 0.2, it will be located in greater than the 1000th, and the genotype will be difficult to determine.

© 2017 The Authors. Cancer Science published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association.
Table 2. Fast tracked second screening (phase 2.1) of single nucleotide polymorphisms (SNP) related to colorectal cancer

| Chromosome | SNP          | Phase 1 | Phase 2.1 | First PCR | Second PCR | Total screening |
|------------|--------------|---------|-----------|-----------|------------|-----------------|
| 1          | rs325914     | 4.86E-05| 8.08E-01  |           |            |                 |
| 1          | rs1510310    | 1.24E-04| 5.82E-01  |           |            |                 |
| 1          | rs1442459    | 4.32E-05| 8.54E-01  |           |            |                 |
| 1          | rs6692131    | 1.24E-04| 2.75E-01  |           |            |                 |
| 1          | rs7586098    | 2.25E-04| 4.17E-01  |           |            |                 |
| 2          | rs10178331   | 2.63E-05| 7.25E-01  |           |            |                 |
| 2          | rs1036069    | 9.97E-06| 7.52E-01  |           |            |                 |
| 2          | rs12105972   | 2.13E-04| 5.61E-01  |           |            |                 |
| 2          | rs13000465   | 5.16E-05| 5.09E-02  | 5.43E-01  |            |                 |
| 2          | rs12624259   | 2.00E-05| 3.16E-01  |           |            |                 |
| 3          | rs3771021    | 2.84E-04| 6.82E-01  |           |            |                 |
| 3          | rs7597875    | 1.88E-04| 3.59E-02  | 8.13E-01  |            |                 |
| 3          | rs2881606    | 8.55E-05| 4.95E-01  |           |            |                 |
| 3          | rs6794054    | 1.45E-04| 8.50E-01  |           |            |                 |
| 3          | rs9023024    | 1.63E-04| 9.13E-01  |           |            |                 |
| 4          | rs12491172   | 6.77E-05| 3.80E-01  |           |            |                 |
| 4          | rs1436656    | 1.26E-04| 2.34E-01  |           |            |                 |
| 4          | rs1725389    | 1.13E-04| 8.91E-01  |           |            |                 |
| 5          | rs4512014    | 1.82E-04| 2.90E-01  |           |            |                 |
| 5          | rs13112145   | 6.74E-05| 3.20E-01  |           |            |                 |
| 5          | rs250222     | 2.15E-04| 7.35E-01  |           |            |                 |
| 6          | rs6595624    | 2.65E-05| 1.69E-01  |           |            |                 |
| 6          | rs2864246    | 1.73E-04| 1.62E-01  |           |            |                 |
| 6          | rs900402     | 1.70E-04| 5.04E-01  |           |            |                 |
| 6          | rs2523865    | 8.50E-05| 1.67E-01  |           |            |                 |
| 7          | rs220687     | 1.45E-04| 1.71E-00  |           |            |                 |
| 7          | rs2040432    | 3.83E-05| 4.80E-01  |           |            |                 |
| 8          | rs10242940   | 1.38E-04| 6.46E-01  |           |            |                 |
| 9          | rs6975897    | 9.73E-05| 2.84E-01  |           |            |                 |
| 10         | rs3107548    | 8.50E-05| 9.40E-01  |           |            |                 |
| 10         | rs7041802    | 8.59E-05| 2.09E-01  |           |            |                 |
| 10         | rs11251410   | 7.79E-05| 1.81E-01  |           |            |                 |
| 11         | rs7912831    | 6.57E-05| 7.10E-02  | 1.25E-04  | 2.42E-05  | 9.31E-08        |
| 11         | rs11239278   | 2.14E-04| 5.73E-01  |           |            |                 |
| 11         | rs5030317    | 8.75E-05| 7.18E-01  |           |            |                 |
| 11         | rs11032820   | 2.57E-04| 4.90E-01  |           |            |                 |
| 12         | rs7124825    | 8.12E-05| 9.79E-01  |           |            |                 |
| 13         | rs22576154   | 1.59E-04| 6.54E-01  |           |            |                 |
| 13         | rs11068349   | 1.79E-04| 3.16E-01  |           |            |                 |
| 13         | rs7327880    | 2.75E-06| 8.87E-01  |           |            |                 |
| 13         | rs8002855    | 5.18E-05| 4.41E-01  |           |            |                 |
| 13         | rs9544316    | 7.94E-06| 8.24E-01  |           |            |                 |
| 13         | rs1329338    | 1.80E-04| 4.27E-01  |           |            |                 |
| 13         | rs2803215    | 3.57E-05| 7.87E-01  |           |            |                 |
| 13         | rs9577345    | 1.12E-04| 1.51E-01  |           |            |                 |
| 14         | rs6573776    | 1.72E-04| 8.25E-01  |           |            |                 |
| 14         | rs234588     | 2.14E-04| 2.17E-02  | 2.33E-01  |            |                 |
| 15         | rs539901     | 5.63E-05| 1.72E-01  |           |            |                 |
| 16         | rs11860241   | 3.63E-05| 1.44E-01  |           |            |                 |
| 16         | rs150073     | 1.43E-04| 7.16E-01  |           |            |                 |
| 16         | rs8047051    | 2.17E-04| 1.79E-01  |           |            |                 |
| 16         | rs1110560    | 1.61E-04| 6.57E-01  |           |            |                 |
| 17         | rs7214294    | 1.04E-04| 2.65E-01  |           |            |                 |
| 18         | rs9303936    | 1.56E-04| 2.23E-01  |           |            |                 |
| 18         | rs9575443    | 1.19E-04| 5.47E-01  |           |            |                 |
| 18         | rs11082969   | 1.84E-04| 8.56E-01  |           |            |                 |
| 18         | rs2879526    | 1.13E-04| 2.52E-01  |           |            |                 |
| 19         | rs12690781   | 9.21E-05| 9.59E-01  |           |            |                 |
| 19         | rs326444     | 7.44E-05| 4.48E-01  |           |            |                 |
Table 2 (Continued)

| Chromosome | SNP   | Phase 1       | Phase 2.1      | First PCR | Second PCR | Total screening |
|------------|-------|---------------|----------------|-----------|------------|-----------------|
| 20         | rs736232 | 4.30E–06       | 6.78E–01       |           |            |                 |
| 21         | rs2825545 | 1.93E–04       | 7.94E–01       |           |            |                 |
| 22         | rs4822015 | 7.76E–05       | 4.01E–01       |           |            |                 |

Materials and Methods

**Study samples.** We collected peripheral blood samples from nine collaborating institutes and hospitals for this project investigating the genetic risk factors of CRC cases. Newly diagnosed CRC cases were identified in eight hospitals (Kyushu University Beppu Hospital [Beppu, Japan], Kitazato University [Kanagawa, Japan], National Cancer Center Hospital [Tokyo, Japan], Northern Yokohama Hospital Showa University [Kanagawa, Japan], National Defense Medical College Hospital [Saitama, Japan], Tokyo Medical and Dental University Hospital [Tokyo, Japan], Mie University Hospital [Mie, Japan] and Takano Hospital [Kumamoto, Japan]) from 2000 to 2007. Controls without a prior history of CRC at the time of enrollment were also recruited from those hospitals. All controls were enrolled after having a colonoscopy to ensure that they had no disease. All participants provided documented informed consent. The study protocol was reviewed and approved by each institute. We included 1846 cases and 2675 controls into the GWAS study. The average age of CRC patients was 61.9±15.0 years. Age and gender details for each phase are shown in Table S1. All cases and controls were of East Asian ancestry and from Japan.

**Extraction of genomic DNA and PCR of markers.** Genomic DNA was extracted from samples using conventional methodologies and quantified using PicoGreen (Invitrogen, Carlsbad, CA, USA). PCR was done using GeneAmp PCR System 9700 (Applied Biosystems, Carlsbad, CA, USA). Genotyping was done using the ABI 3100 Genetic Analyzer (Applied Biosystems) and analyses and assignment of marker alleles were done with the GENOTYPER programs (Applied Biosystems). Information on SNP was obtained from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/index.html).

**Genotyping.** In phase 1, the genotyping of the 577 CRC cases and 571 controls was carried out using the Affymetrix GeneChip Human Mapping 500K Array Set. In phase 1, we genotyped 500 568 tagSNP in 577 individuals with CRC and 571 controls using the Bayesian robust linear model with Mahalanobis (BRLMM) algorithm (http://media.affymetrix.com/support/technical/whitepapers/brlmm_whitepaper.pdf). Samples with a genotype call rate <0.94 for either the NspI or StyI GeneChip SNP were removed from analysis. To detect duplicate samples, relatives and DNA-contaminated samples, pairwise IBD estimation was carried out. After applying the strict quality control criteria described above, genotype

1846 cases and 2675 controls, to identify disease susceptibility SNP.

**Results**

An overview of the current study design is shown in Figure 1. The genome-wide association study was carried out using the Affymetrix GeneChip Human Mapping 500K Array Set. In phase 1, we genotyped 500 568 tagSNP in 577 individuals with CRC and 571 controls using the Bayesian robust linear model with Mahalanobis (BRLMM) algorithm (http://media.affymetrix.com/support/technical/whitepapers/brlmm_whitepaper.pdf). Samples with a genotype call rate <0.94 for either the NspI or StyI GeneChip SNP were removed from analysis. To detect duplicate samples, relatives and DNA-contaminated samples, pairwise IBD estimation was carried out. After applying the strict quality control criteria described above, genotype

**Fig. 3.** Linkage disequilibrium (LD) structure at 10p14. The polymorphic site rs7912831 is depicted in an LD block of 500 kb where there are coding and non-coding genes, such as non-coding RNA and micro RNA. The SNP rs10795668, which has been previously reported by Tomlinson et al., is located close to rs7912831. Data were analyzed using Haploview software (v3.2).
Table 3. Final Findings of genome-wide association studies in Japanese colorectal cancer cases

| Phase 1 | Phase 2.2 | Phase 3 | Combined |
|---------|-----------|---------|----------|
| SNP     | Odds ratio (95% CI) | Risk allele frequency | | |
| Chromosome | Position | | | |
| 10p14    | rs13000465 | 1.27 (1.16–1.39) | 0.61 | 0.53 | 0.0045 | 0.0032 | 0.0027 | 0.00082 | 0.25 | 0.20 | 0.00000043 |
| 10p14    | rs7597875 | 1.27 (1.17–1.39) | 0.61 | 0.53 | 0.0046 | 0.0032 | 0.0027 | 0.00082 | 0.25 | 0.20 | 0.00000043 |
| 10p14    | rs7912831 | 1.27 (1.17–1.39) | 0.61 | 0.53 | 0.0046 | 0.0032 | 0.0027 | 0.00082 | 0.25 | 0.20 | 0.00000043 |
| 10p14    | rs234588 | 1.27 (1.17–1.39) | 0.61 | 0.53 | 0.0046 | 0.0032 | 0.0027 | 0.00082 | 0.25 | 0.20 | 0.00000043 |

SNP, single nucleotide polymorphisms.

Discussion

In this study, we identified 4 SNP that are significantly associated with morbidity from CRC at the 10p14 locus. These novel SNP are close to the variants on 10p14 described by Tomlinson et al. These independent whole genome-wide association studies both found loci on 10p14 to be commonly associated with CRC. We consider this 10p14 locus to be a significant region of CRC susceptibility because it was identified in multiple studies in more than one race, including European and Japanese populations. Recently, a significant interaction between an SNP at 10p14 (rs4143094) and processed meat consumption was also reported in CRC patients. We performed data mining for genes that exist in this susceptibility locus in order to investigate the mechanism by which these SNP are connected to the causes of CRC. No genes, including noncoding RNA, were found in the susceptibility locus identified in the current study using the online human genome database (UCSC Genome Bioinformatics [http://genome.ucsc.edu/]) (Fig. S1). The genes nearest to our
susceptibility locus at 10p14 are GATA3 and CUGBP2. However, no significant correlations between our SNP and the expression of these genes were found by the combined SNP and CDNA expression arrays. We also sought to find new transcripts related to colorectal carcinogenesis at the 10p14 locus. First, we performed a RACE assay for each region where the SNP exist using total RNA extracted from CRC cell lines (Fig. S2), but we could not find any transcripts around all 4 SNPs. Second, we found a sequence homologous to mmu-mir-1981, which is a mouse micro RNA, just beside rs10905453. However, no transcript was found with northern bloting (Fig. S3). Moreover, we also did not find new protein-coding or RNA genes in the susceptibility region using mRNA whole-transcriptome analysis of 25 CRC cancer tissues. We could not find any functional genes around carcinogenic SNP in 10p14 in this study. Few reports have demonstrated the mechanism by which an SNP regulates the expression of genes or noncoding RNA to promote carcinogenesis and the development of CRC, but further study is warranted.

The mechanisms of CRC carcinogenesis caused by carcinogenic SNP, including the most common SNP rs6983267 at 8q24, are not well known because these SNPs do not exist in coding regions. Tuupanen et al. reported that the risk allele of rs6983267 is associated with microsatellite-stable cancer, and proposed that the underlying germ line genetic defect in 8q24 was a target in the somatic evolution of CRC. Interestingly, they also report that the risk allele G shows a copy number increase during CRC development and that rs6983267 affected a binding site for the Wnt-regulated transcription factor TCF4/LEF1, which leads to the enhancement of MYC transcription in vitro and in vivo. The abundant expression of MYC contributes to carcinogenesis and progression of CRC.

We consider that further analysis of such loci will enable us to understand the unknown mechanisms of colorectal carcinogenesis, including discovering new genes or noncoding RNA. For example, we reported that an SNP in miR-146a targeting EGFR and IRAK1 is associated with the prognosis of gastric cancer patients. As the underlying basis of the association identified at 10p14 is presently unclear, there are no clues to explain how this region is involved in morbidity from CRC. Our data reveal that 10p14 is a colorectal cancer susceptible region for more than one racial subgroup, but further studies are warranted to find the mechanistic relationship between 10p14 and colorectal carcinogenesis.

In conclusion, using a multistage GWAS in Japanese individuals, we identified a significant genome-wide level of association for 4 SNP on chromosome 10p14 associated with the onset of CRC.

Acknowledgements

We thank Ms Oda, Ms Kasagi and Ms Kawano for their technical assistance.

Disclosure Statement

The authors have no conflicts of interest to declare.
28 Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet 2007; 39: 906–13.

29 Qin Q, Liu L, Zhong R et al. The genetic variant on chromosome 10p14 is associated with risk of colorectal cancer: results from a case-control study and a meta-analysis. PLoS One 2013; 8: e64310.

30 Hong Y, Wu G, Li W, Liu D, He K. A comprehensive meta-analysis of genetic associations between five key SNPs and colorectal cancer risk. Oncotarget 2016; 7: 73945–59.

31 Figueiredo JC, Hsu L, Hutter CM et al. Genome-wide diet-gene interaction analyses for risk of colorectal cancer. PLoS Genet 2014; 10: e1004228.

32 Tuupanen S, Niittymaki I, Nousiainen K et al. Allelic imbalance at rs6983267 suggests selection of the risk allele in somatic colorectal tumor evolution. Cancer Res 2008; 68: 14–7.

33 Tuupanen S, Turunen M, Lehtonen R et al. The common colorectal cancer predisposition SNP rs6983267 at chromosome 8q24 confers potential to enhanced Wnt signaling. Nat Genet 2009; 41: 885–90.

34 Takatsuno Y, Mimori K, Yamamoto K et al. The rs6983267 SNP Is Associated with MYC Transcription Efficiency, Which Promotes Progression and Worsens Prognosis of Colorectal Cancer. Ann Surg Oncol 2013; 20: 1395–402.

35 Rochlitz CF, Herrmann R, de Kant E. Overexpression and amplification of c-myc during progression of human colorectal cancer. Oncology 1996; 53: 448-54.

36 Sugimachi K, Niida A, Yamamoto K et al. Allelic imbalance at an 8q24 oncogenic SNP is involved in activating MYC in human colorectal cancer. Ann Surg Oncol 2014; 21(Suppl 4): S515–21.

37 Kogo R, Mimori K, Tanaka F, Komune S, Mori M. Clinical significance of miR-146a in gastric cancer cases. Clin Cancer Res 2011; 17: 4277–84.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Demographic data of patients and controls in this study.

Fig. S1. Data mining for genes which exist in oncogenic susceptibility locus using online database.

Fig. S2. Four primer sets, sandwiching 4 single nucleotide polymorphisms at 10p14 were designed for qRT-PCR assay.

Fig. S3. Exploration of transcripts in 10p14 region with northern blotting.