**Supplemental table 1** The primers for *C5orf66* gene amplification and sequencing.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SNP** | **1st-PCR primer**  | **2nd-PCR primer** | **UEP-DIR** | **UEP-SEQ** |
| rs254563 | ACGTTGGATGGGAAGCTAGATGAGAGAAAG | ACGTTGGATGGTCTTGGAGAATCCTCATGC | F | TCCTCATGCACATTATTATATCAAAG |
| rs4976270 | ACGTTGGATGGAGGACAAGGCTCTGGATCA | ACGTTGGATGTGCAGCACAGCAGGCCTTTT | R | ccGCAGGCCTTTTGCTAAT |
| rs639933 | ACGTTGGATGCGGCACTCTCCAAAGCTTAT | ACGTTGGATGCTGTGTCACCACTGGTTATC | R | GAGCACCTGGACGTG |
| rs647161 | ACGTTGGATGAACCTCTACCTGGGCAAATG | ACGTTGGATGCTGAGGAGCCATGTGTGATG | F | ttgttAGCGTAGGGATCCTGGCAGAGT |

SNP: single nucleotide polymorphism; UEP-DIR: Unique extension primer-direction; UEP-SEQ: Unique extension primer-sequencing.

**Supplemental table 2** The SNPs of*C5orf66* associated with susceptibility of CRC in the subgroup tests (Gender and Smoking).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **SNP ID** | **Model** | **genotype** | **Gender** |  | **Smoking** |
| **OR (95% CI)** | ***p*** | **OR (95% CI)** | ***p*** |  | **OR (95% CI)** | ***p*** | **OR (95% CI)** | ***p*** |
| **Male** | **Female** |  | **Yes** | **No** |
| **rs254563** | Allele | A | 1.04(0.77-1.40) | 0.820  | 1.12(0.80-1.56) | 0.519  |  | 1.39(0.83-2.32) | 0.211  | 1.13(0.81-1.57) | 0.468  |
|  |  | G | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Genotype | AA | 0.76(0.34-1.70) | 0.505  | 0.90(0.29-2.75) | 0.853  |  | 0.96(0.22-4.18) | 0.957  | 1.07(0.39-2.98) | 0.892  |
|  |  | AG | 1.19(0.82-1.72) | 0.357  | 1.21(0.81-1.80) | 0.352  |  | 1.63(0.86-3.09) | 0.131  | 1.18(0.79-1.76) | 0.410  |
|  |  | GG | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Dominant | AA-AG | 1.12(0.79-1.59) | 0.533  | 1.18(0.80-1.74) | 0.402  |  | 1.54(0.84-2.81) | 0.165  | 1.17(0.80-1.72) | 0.421  |
|  |  | GG | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Recessive | AA | 0.72(0.33-1.60) | 0.424  | 0.84(0.28-2.56) | 0.762  |  | 0.83(0.19-3.60) | 0.808  | 1.02(0.37-2.82) | 0.968  |
|  |  | AG-GG | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Log-additive | - | 1.03(0.77-1.38) | 0.827  | 1.12(0.80-1.58) | 0.518  |  | 1.34(0.80-2.26) | 0.269  | 1.13(0.81-1.57) | 0.478  |
| **rs4976270** | Allele | C | 1.18(0.92-1.51) | 0.204  | 1.20(0.91-1.59) | 0.193  |  | 1.23(0.81-1.86) | 0.334  | 1.25(0.95-1.64) | 0.112  |
|  |  | T | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Genotype | CC | 1.63(0.89-2.99) | 0.110  | 1.77(0.91-3.42) | 0.092  |  | 3.36(0.90-12.56) | 0.071  | 1.63(0.85-3.12) | 0.143  |
|  |  | CT | 1.06(0.75-1.49) | 0.757  | 1.04(0.70-1.53) | 0.855  |  | 0.89(0.50-1.58) | 0.690  | 1.23(0.84-1.79) | 0.283  |
|  |  | TT | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Dominant | CC-CT | 1.14(0.82-1.58) | 0.448  | 1.14(0.79-1.65) | 0.486  |  | 1.06(0.61-1.84) | 0.841  | 1.29(0.90-1.85) | 0.162  |
|  |  | TT | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Recessive | CC | 1.59(0.89-2.83) | 0.115  | 1.73(0.92-3.27) | 0.089  |  | 3.56(0.98-12.9) | 0.053  | 1.47(0.79-2.75) | 0.227  |
|  |  | CT-TT | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Log-additive | - | 1.18(0.92-1.53) | 0.194  | 1.21(0.91-1.60) | 0.189  |  | 1.27(0.82-1.96) | 0.293  | 1.26(0.95-1.66) | 0.106  |
| **rs639933** | Allele | C | 1.20(0.94-1.54) | 0.145  | 1.18(0.89-1.55) | 0.248  |  | 1.23(0.81-1.86) | 0.334  | 1.27(0.96-1.66) | 0.091  |
|  |  | A | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Genotype | CC | 1.61(0.89-2.92) | 0.117  | 1.73(0.89-3.36) | 0.104  |  | 2.46(0.75-8.08) | 0.138  | 1.72(0.90-3.29) | 0.103  |
|  |  | CA | 1.13(0.80-1.60) | 0.478  | 1.00(0.68-1.47) | 0.993  |  | 1.01(0.57-1.79) | 0.968  | 1.22(0.83-1.78) | 0.306  |
|  |  | AA | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Dominant | CC-CA | 1.20(0.86-1.68) | 0.273  | 1.10(0.76-1.59) | 0.612  |  | 1.15(0.66-1.99) | 0.625  | 1.30(0.90-1.86) | 0.157  |
|  |  | AA | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Recessive | CC | 1.51(0.86-2.67) | 0.155  | 1.74(0.92-3.27) | 0.088  |  | 2.45(0.77-7.77) | 0.130  | 1.56(0.84-2.90) | 0.163  |
|  |  | CA-AA | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Log-additive | - | 1.21(0.94-1.57) | 0.137  | 1.18(0.89-1.57) | 0.241  |  | 1.27(0.82-1.97) | 0.288  | 1.27(0.97-1.68) | 0.086  |
| **rs647161** | Allele | A | 1.14(0.88-1.47) | 0.328  | 1.15(0.87-1.52) | 0.311  |  | 1.26(0.82-1.94) | 0.289  | 1.27(0.96-1.67) | 0.097  |
|  |  | C | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Genotype | AA | 1.11(0.59-2.10) | 0.742  | 1.54(0.79-2.98) | 0.203  |  | 1.82(0.53-6.31) | 0.344  | 1.53(0.78-3.01) | 0.215  |
|  |  | AC | 1.26(0.89-1.78) | 0.189  | 1.05(0.71-1.54) | 0.806  |  | 1.32(0.74-2.34) | 0.343  | 1.31(0.90-1.91) | 0.156  |
|  |  | CC | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Dominant | AA-AC | 1.24(0.89-1.72) | 0.208  | 1.12(0.78-1.62) | 0.545  |  | 1.37(0.79-2.39) | 0.263  | 1.35(0.94-1.93) | 0.104  |
|  |  | CC | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Recessive | AA | 1.00(0.54-1.85) | 0.998  | 1.50(0.80-2.82) | 0.209  |  | 1.60(0.48-5.39) | 0.447  | 1.35(0.70-2.58) | 0.370  |
|  |  | AC-CC | 1.00 |  | 1.00 |  |  | 1.00 |  | 1.00 |  |
|  | Log-additive | - | 1.14(0.88-1.48) | 0.319  | 1.16(0.88-1.54) | 0.299  |  | 1.33(0.84-2.12) | 0.223  | 1.27(0.96-1.68) | 0.098  |

SNP: Single nucleotide polymorphisms; OR: Odds ratio; CI: Confidence interval; *p* < 0.05 indicates statistical significance; “-” indicates Log-additive model.

**Supplementary table 3** The FPRP and statistical power values of all the positive results in this study.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| SNP ID | Model | Genotype | OR (95%CI) | *p* | Statistical Power a (%) | Prior probability |
| 0.25 | 0.1 | 0.01 |
| **Overall analysis** |
| rs4976270 | Genotype | CC/TT | 1.69(1.08-2.64) | 0.021 | 77.0 | 0.076 b | 0.198 b | 0.731 |
|  | Recessive | CC/CT-TT | 1.65(1.08-2.53) | 0.021 | 81.1 | 0.074 b | 0.194 b | 0.726 |
| rs639933 | Genotype | CC/AA | 1.67(1.07-2.59) | 0.024 | 79.0 | 0.077 b | 0.200 | 0.734 |
|  | Recessive | CC/CA-AA | 1.61(1.05-2.46) | 0.028 | 84.2 | 0.090 b | 0.228 | 0.765 |
| **Subgroup analysis: age (age > 60)** |
| rs4976270 | Allele | C/T | 1.37(1.06-1.77) | 0.016  | 99.8 | 0.046 b | 0.126 b | 0.614 |
|  | Genotype | CC/TT | 2.36(1.27-4.37) | 0.007  | 29.9 | 0.059 b | 0.159 b | 0.676 |
|  | Recessive | CC/CT-TT | 2.25(1.24-4.07) | 0.008  | 34.8 | 0.059 b | 0.159 b | 0.675 |
|  | Log-additive | - | 1.35(1.05-1.76) | 0.022 | 99.8 | 0.074 b | 0.193 b | 0.725 |
| rs639933 | Allele | C/A | 1.34(1 .03-1.73) | 0.027  | 99.9 | 0.069 b | 0.182 b | 0.710 |
|  | Genotype | CC/AA | 2.00(1.09-3.67) | 0.025  | 50.0 | 0.131 b | 0.312 | 0.833 |
|  | Recessive | CC/CA-AA | 1.85(1.04-3.31) | 0.038  | 60.4 | 0.160 b | 0.363 | 0.862 |
|  | Log-additive | - | 1.32(1.02-1.71) | 0.036  | 99.9 | 0.096 b | 0.243 | 0.779 |
| rs647161 | Allele | A/C | 1.38(1.06-1.79) | 0.016  | 99.7 | 0.044 b | 0.121 b | 0.602 |
|  | Dominant | AA-AC/CC | 1.48(1.05-2.07) | 0.025  | 96.1 | 0.064 b | 0.171 b | 0.694 |
| **Subgroup analysis: gender (BMI ≤ 24)** |
| rs4976270 | Allele | C/T | 1.48(1.08-2.02) | 0.013  | 97.1 | 0.040 b | 0.111 b | 0.579 |
|  | Genotype | CC/TT | 2.32(1.06-5.11) | 0.036  | 35.6 | 0.236 | 0.481 | 0.911 |
|  | Dominant | CC-CT/TT | 1.57(1.06-2.34) | 0.025  | 88.3 | 0.083 b | 0.214 | 0.750 |
|  | Log-additive | - | 1.49(1.09-2.04) | 0.013  | 96.7 | 0.038 b | 0.107 b | 0.568 |
| rs639933 | Allele | C/A | 1.48(1.09-2.02) | 0.012 | 97.1 | 0.040 b | 0.111 | 0.579 |
|  | Genotype | CC/AA | 2.41(1.10-5.28) | 0.029  | 32.1 | 0.207 | 0.440 | 0.896 |
|  | Dominant | CC-CA/AA | 1.57(1.05-2.33) | 0.026  | 88.5 | 0.078 b | 0.203 | 0.738 |
|  | Log-additive | - | 1.50(1.09-2.05) | 0.012  | 96.4 | 0.033 b | 0.093 b | 0.529 |
| rs647161 | Allele | A/C | 1.45(1.06-1.99) | 0.020 | 97.7 | 0.062 b | 0.165 b | 0.685 |
|  | Genotype | AA/CC | 2.52(1.05-6.05) | 0.039  | 30.3 | 0.277 | 0.535 | 0.927 |
|  | Dominant | AA-AC/CC | 1.56(1.05-2.33) | 0.028  | 88.8 | 0.092 b | 0.232 | 0.769 |
|  | Log-additive | - | 1.51(1.09-2.10) | 0.013  | 95.3 | 0.043 b | 0.119 b | 0.598 |
| **Subgroup analysis: gender (Drinking: yes)** |
| rs254563 | Dominant | AA-AG/GG | 2.03(1.03-4.00) | 0.041  | 48.3 | 0.202 | 0.432 | 0.893 |
| rs4976270 | Genotype | CC/TT | 5.45(1.25-23.72) | 0.024  | 9.1 | 0.441 | 0.703 | 0.963 |
|  | Recessive | CC/CT-TT | 5.35(1.28-22.41) | 0.022  | 8.9 | 0.423 | 0.687 | 0.960 |
| rs639933 | Allele | C/A | 4.91(1.11-21.63) | 0.036  | 11.8 | 0.475 | 0.731 | 0.968 |
|  | Recessive | CC/CA-AA | 4.88(1.15-20.77) | 0.032  | 11.4 | 0.457 | 0.717 | 0.965 |
| **Subgroup analysis: gender (Adenocarcinoma)** |
| rs4976270 | Allele | C/T | 1.24(1.01-1.52) | 0.039  | 100.0 | 0.103 b | 0.257 | 0.792 |
|  | Genotype | CC/TT | 1.78(1.10-2.89) | 0.019 | 68.1 | 0.080 b | 0.207 | 0.741 |
|  | Recessive | CC/CT-TT | 1.67(1.05-2.65) | 0.029  | 77.8 | 0.102 b | 0.254 | 0.790 |
|  | Log-additive | - | 1.25(1.02-1.55) | 0.035  | 100.0 | 0.112 b | 0.274 | 0.806 |
| rs639933 | Allele | C/A | 1.25(1.02-1.53) | 0.035 | 100.0 | 0.084 b | 0.215 | 0.751 |
|  | Genotype | CC/AA | 1.82(1.13-2.93) | 0.014 | 65.1 | 0.059 b | 0.159 b | 0.676 |
|  | Recessive | CC/CA-AA | 1.72(1.09-2.71) | 0.020  | 74.2 | 0.073 b | 0.190 b | 0.721 |
|  | Log-additive | - | 1.26(1.02-1.55) | 0.031 | 100.0 | 0.079 b | 0.206 | 0.740 |

FPRP: false-positive report probability.

a Statistical power a was calculated using the number of observations in the subgroup and the OR and p values in this table.

b The level of false-positive report probability threshold was set at 0.2, and noteworthy findings are presented.

**Supplemental table 4** Clinical characteristics of patients based on the genotypes of selected SNPs.

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics** | **rs254563** |  | **rs4976270** |
| **AA** | **AG** | **GG** | ***p*** |  | **TT** | **CT** | **CC** | ***p*** |
| CEA (U/ml) | 20.40 ± 13.36 | 18.39 ± 12.6 | 16.74 ± 10.30 | 0.219 |  | 17.66 ± 12.52 | 16.68 ± 9.58 | 18.82 ± 11.25 | 0.419 |
| SF (ng/ml) | 91.49 ± 5.23 | 89.29 ± 9.65 | 89.09 ± 6.54 | 0.489 |  | 88.84 ± 6.54 | 89.30 ± 8.88 | 90.39 ± 6.07 | 0.380 |
| TNF (fmol/ml) | 0.88 ± 0.05 | 0.90 ± 0.08 | 0.89 ± 0.05 | 0.332 |  | 0.89 ± 0.06 | 0.89 ± 0.07 | 0.90 ± 0.07 | 0.797 |
| CA50 (U/ml) | 5.61 ± 8.42 | 6.18 ± 10.22 | 6.27 ± 11.91 | 0.975 |  | 5.33 ± 8.11 | 7.26 ± 14.46 | 5.69 ± 6.97 | 0.191 |
| CA19-9 (U/ml) | 77.67 ± 192.56 | 44.91 ± 79.98 | 49.17 ± 107.57 | 0.502 |  | 43.42 ± 86.52 | 52.27 ± 115.44 | 55.35 ± 114.25 | 0.600 |
| CA242 (U/ml) | 19.80 ± 27.84 | 15.94 ± 30.37 | 15.64 ± 28.43 | 0.871 |  | 16.15 ± 30.02 | 14.99 ± 27.80 | 18.07 ± 29.63 | 0.780 |
| AFP (ng/ml) | 9.78 ± 4.01 | 10.04 ± 4.36 | 10.05 ± 4.86 | 0.977 |  | 10.33 ± 5.08 | 9.96 ± 4.46 | 9.23 ± 3.73 | 0.270 |
| HB (g/L) | 106.33 ± 20.6 | 112.91 ± 36.73 | 108.29 ± 26.74 | 0.702 |  | 108.91 ± 24.91 | 108.27 ± 34.29 | 121.67 ± 25.74 | 0.355 |
| Leukocytes (g/L) | 4.05 ± 1.03 | 6.65 ± 4.06 | 6.54 ± 4.92 | 0.551 |  | 6.74 ± 5.58 | 6.72 ± 4.00 | 4.78 ± 1.85 | 0.278 |
| PLT (L) | 164.50 ± 67.18 | 238.26 ± 107.46 | 212.43 ± 108.41 | 0.369 |  | 206.04 ± 117.9 | 236.17 ± 101.59 | 195.08 ± 89.24 | 0.252 |
| **Characteristics** | **rs639933** |  | **rs647161** |
| **AA** | **AC** | **CC** | ***p*** |  | **AA** | **AC** | **CC** | ***p*** |
| CEA (U/ml) | 17.66 ± 12.52 | 16.68 ± 9.58 | 18.82 ± 11.25 | 0.246 |  | 19.01 ± 11.85 | 16.59 ± 9.68 | 17.81 ± 12.35 | 0.344 |
| SF (ng/ml) | 88.84 ± 6.54 | 89.30 ± 8.88 | 90.39 ± 6.07 | 0.319 |  | 89.51 ± 6.05 | 89.59 ± 8.8 | 88.80 ± 6.59 | 0.534 |
| TNF (fmol/ml) | 0.89 ± 0.06 | 0.89 ± 0.07 | 0.90 ± 0.07 | 0.777 |  | 0.89 ± 0.06 | 0.9 ± 0.07 | 0.89 ± 0.06 | 0.241 |
| CA50 (U/ml) | 5.33 ± 8.11 | 7.26 ± 14.46 | 5.69 ± 6.97 | 0.137 |  | 6.58 ± 7.55 | 7.26 ± 14.62 | 5.12 ± 7.40 | 0.132 |
| CA19-9 (U/ml) | 43.42 ± 86.52 | 52.27 ± 115.44 | 55.35 ± 114.25 | 0.505 |  | 64.04 ± 127.83 | 53.71 ± 124.39 | 40.72 ± 69.44 | 0.252 |
| CA242 (U/ml) | 16.15 ± 30.02 | 14.99 ± 27.80 | 18.07 ± 29.63 | 0.791 |  | 14.1 ± 21.74 | 14.73 ± 26.4 | 17.30 ± 32.39 | 0.618 |
| AFP (ng/ml) | 10.33 ± 5.08 | 9.96 ± 4.46 | 9.23 ± 3.73 | 0.311 |  | 9.58 ± 4.19 | 9.83 ± 4.40 | 10.34 ± 5.02 | 0.405 |
| HB (g/L) | 108.91 ± 24.91 | 108.27 ± 34.29 | 121.67 ± 25.74 | 0.366 |  | 120.00 ± 29.50 | 106.05 ± 26.20 | 112.13 ± 33.77 | 0.305 |
| Leukocytes (g/L) | 6.74 ± 5.58 | 6.72 ± 4.00 | 4.78 ± 1.85 | 0.349 |  | 4.77 ± 1.88 | 6.68 ± 3.93 | 6.68 ± 5.43 | 0.361 |
| PLT (L) | 206.04 ± 117.9 | 236.17 ± 101.59 | 195.08 ± 89.24 | 0.060 |  | 160.75 ± 62.27 | 242.64 ± 98.91 | 205.29 ± 116.90 | 0.051 |

CEA: carcinoembryonic antigen; CA-50: carbohydrate antigen 50; CA-19 9: carbohydrate antigen 19 9; CA 242: carbohydrate antigen 242; SF: serum ferritin; TNF: tumor necrosis factor; AFP: alpha-fetoprotein; HB: hemoglobin; PLT: platelet.