**Supplementary Table 1** **Demographic characteristics of luminal A patients and controls with peripheral blood leukocyte DNA**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristicsa** | **Luminal A cases**  | **Controls** | ***P*-value** |
| **No. (%)** | **No. (%)** |
|  | 17 | 439 |  |
| **Age (year)** |  |  | **0.020** |
| <40 | 0(0.00) | 50(11.49) |  |
| 40-49 | 3(17.65) | 149(34.25) |  |
| 50-59 | 11(64.71) | 131(30.11) |  |
| ≥60 | 3(17.64) | 105(24.15) |  |
| Median (CV%) | 53.50 (11.94%) | 50.00 (19.33%) |  |
| **Marital status** |  |  | 0.06 |
|  Single | 3(17.65) | 26(6.00) |  |
| Married | 14(82.35) | 407(94.00) |  |
| **Education level** |  |  | 0.62 |
|  Primary and below | 5(29.41) | 126(29.1) |  |
|  Junior high school | 5(29.41) | 140(32.33) |  |
|  Senior middle school | 4(23.53) | 130(30.02) |  |
|  College and above | 3(17.65) | 37(8.55) |  |
| **Occupation** |  |  | **0.040** |
|  Mental worker | 8(50.00) | 86(23.76) |  |
|  Manual worker | 8(50.00) | 241(66.57) |  |
|  Mixed | 0(0.00) | 35(9.67) |  |
| **Family history of cancer** |  | 0.10 |
|  Yes | 5(31.25) | 60(15.75) |  |
|  No | 11(68.75) | 321(84.25) |  |
| **BMI (kg/m2)** |  |  | 0.68 |
| ≤18.5 | 0(0.00) | 16(3.70) |  |
|  18.5-23.0 | 7(43.75) | 162(37.41) |  |
|  ≥23.0 | 9(56.25) | 255(58.89) |  |
| Median (CV%) | 23.85 (10.86%) | 23.80 (15.79%) |  |

**Supplementary Table 2 Association of *CDH4* methylation in peripheral blood leukocyte DNA and luminal A breast cancer risk**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Methylation status** | **Luminal A cases No. (%)** | **Controls No. (%)** | **ORcrude (95%CI)** | ***P*-value** | **ORadj (95%CI)** | ***P*-value** |
|  |
| ***CDH4*** |  |  |  |  |  |  |
| Hypomethylated | 10 (58.82) | 324 (73.80) | 1.00  |  | 1.00  |  |  |
| Hypermethylated | 7 (41.18) | 115 (26.20) | 1.95 (0.72-5.24) | 0.19 | 2.36(0.82-6.78) | 0.11 |  |

**Supplementary Table 3** **Demographic characteristics of luminal B patients and controls with peripheral blood leukocyte DNA**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristicsa** | **Luminal B cases** | **Controls** | ***P*-value** |
| **No. (%)** | **No. (%)** |
|  | 146 | 439 |  |
| **Age (year)** |  |  | 0.59 |
| <40 | 12(8.22) | 50(11.49) |  |
| 40-49 | 56(38.36) | 149(34.25) |  |
| 50-59 | 46(31.51) | 131(30.11) |  |
| ≥60 | 32(21.91) | 105(24.15) |  |
| Median (CV%) | 51.00 (18.60%) | 50.00 (19.33%) |  |
| **Marital status** |  |  | **0.046** |
|  Single | 16(10.96) | 26(6.00) |  |
| Married | 130(89.04) | 407(94.00) |  |
| **Education level** |  |  | 0.09 |
|  Primary and below | 38(26.03) | 126(29.10) |  |
|  Junior high school | 47(32.19) | 140(32.33) |  |
|  Senior middle school | 38(26.03) | 130(30.02) |  |
|  College and above | 23(15.75) | 37(8.55) |  |
| **Occupation** |  |  | **0.001** |
|  Mental worker | 30(22.90) | 86(23.76) |  |
|  Manual worker | 71(54.20) | 241(66.57) |  |
|  Mixed | 30(22.90) | 35(9.67) |  |
| **Family history of cancer** |  | **<0.001** |
|  Yes | 43(29.86) | 60(15.75) |  |
|  No | 101(70.14) | 321(84.25) |  |
| **BMI (kg/m2)** |  |  | 0.79 |
| ≤18.5 | 4(2.74) | 16(3.70) |  |
|  18.5-23.0 | 58(39.73) | 162(37.41) |  |
|  ≥23.0 | 84(57.53) | 255(58.89) |  |
| Median (CV%) | 23.44 (27.33%) | 23.80 (15.79%) |  |

**Supplementary Table 4** **Association of *CDH4* methylation in peripheral blood leukocyte DNA and luminal B breast cancer risk**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Methylation status** | **Luminal B cases No. (%)** | **Controls No. (%)** | **ORcrude (95%CI)** | ***P*-value** | **ORadj (95%CI)** | ***P*-value** |
|  |
| ***CDH4*** |  |  |  |  |  |  |
| Hypomethylated | 76 (52.05) | 324 (73.80) | 1.00  |  | 1.00  |  |  |
| Hypermethylated | 70 (47.95) | 115 (26.20) | 2.57 (1.74-3.78) | <0.001  | 2.93(1.89-4.56) | <0.001  |  |

**Supplementary Table 5. Univariate and multivariate analyses for the associations between dietary factors and lifestyle and breast cancer risk**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Environmental factors** | **Cases** **No. (%)** | **Controls No. (%)** | **Univeriate analysis** |  | **Multivariate analysis** |
| **ORadj (95%CI)** | ***P*-value** |  | **ORadj (95%CI)** | ***P*-value** |
| **Cereal (g/week)** |  |  |  |  |  |  |  |
| <50 | 173 (55.45) | 210 (48.28) | 1.00  |  |  |  |  |
| ≥50 | 139 (44.55) | 225 (51.72) | 0.72 (0.51-1.01) | 0.057 |  |  |  |
| **Vegetables (g/day)** |  |  |  |  |  |  |
| <500 | 220 (59.46) | 196 (45.37) | 1.00  |  |  | 1.00  |  |
| ≥500 | 150 (40.54) | 236 (54.63) | 0.61 (0.44-0.85) | **0.003** |  | 0.59 (0.41-0.84) | **0.003** |
| **Allium vegetables (times/week)** |  |  |  |  |  |
| ≤3 | 268 (70.53) | 199 (45.33) | 1.00  |  |  | 1.00  |  |
| >3 | 112 (29.47) | 240 (54.67) | 0.40 (0.29-0.56) | **0.000** |  | 0.37 (0.26-0.53) | **<0.001**  |
| **Poultry (times/month)** |  |  |  |  |  |  |
| No | 345(90.79) | 388(88.79) | 1.00  |  |  |  |  |
| Yes | 35(9.21) | 49(11.21) | 0.85 (0.51-1.43) | 0.54 |  |  |  |
| **Fish (times/month)** |  |  |  |  |  |  |
| <3 | 345(92.25) | 377(86.27) | 1.00  |  |  | 1.00  |  |
| ≥3 | 29(7.75) | 60(13.73) | 0.39 (0.23-0.66) | **0.001** |  | 0.45 (0.25-0.81) | **0.007** |
| **Milk (times/week)** |  |  |  |  |  |  |
| <1 | 328(87.00) | 338(77.52) | 1.00  |  |  | 1.00  |  |
| ≥1 | 49(13.00) | 98(22.48) | 0.44 (0.28-0.69) | **<0.001** |  | 0.57 (0.35-0.93) | **0.024** |
| **Overnight fooda (times/week)** |  |  |  |  |  |  |
| 0 | 66 (17.46) | 113 (26.04) | 1.00  |  |  | 1.00  |  |
| 1-3 | 149 (39.42) | 195 (44.93) | 1.18 (0.76-1.84) | 0.45 |  | 1.23 (0.76-1.98) | 0.402 |
| >3 | 163 (43.12) | 126 (29.03) | 2.08 (1.32-3.28) | **0.002** |  | 2.38 (1.44-3.92) | **<0.001** |
| **Beans (times/week)** |  |  |  |  |  |  |
| ≤3 | 300 (80.86) | 324 (74.31) | 1.00  |  |  | 1.00  |  |
| >3 | 71 (19.14) | 112 (25.69) | 0.52 (0.35-0.78) | **0.002** |  | 0.74 (0.47-1.17) | 0.20 |
| **Pork (g/week)** |  |  |  |  |  |  |
| 0 | 47(12.37) | 84(19.18) | 1.00  |  |  | 1.00  |  |
| 0-250 | 166(43.68) | 175(39.95) | 1.76 (1.08-2.88) | **0.024** |  | 1.47 (0.86-2.50) | 0.16 |
| ≥250 | 167(43.95) | 179(40.87) | 1.93 (1.19-3.13) | **0.008** |  | 1.92 (1.14-3.24) | **0.015** |
| **Eggs (No./week)** |  |  |  |  |  |  |  |
| ≤3 | 162 (42.74) | 199 (45.33) | 1.00  |  |  |  |  |
| >3 | 217 (57.26) | 240 (54.67) | 0.85 (0.61-1.18) | 0.34 |  |  |  |
| **Pickles (times/week)** |  |  |  |  |  |  |
| <2 | 234 (61.58) | 249 (56.85) | 1.00  |  |  |  |  |
| ≥2 | 146 (38.42) | 189 (43.15) | 0.87 (0.62-1.22) | 0.43 |  |  |  |
| **Carbonated drinksb** |  |  |  |  |  |  |
| No | 364 (96.55) | 412 (94.06) | 1.00  |  |  |  |  |
| Yes | 13 (3.45) | 26 (5.94) | 0.54 (0.24-1.18) | 0.12 |  |  |  |
| **Juiceb** |  |  |  |  |  |  |  |
| No | 349 (92.82) | 403 (92.01) | 1.00  |  |  |  |  |
| Yes | 27 (7.18) | 35 (8.39) | 0.80 (0.44-1.46) | 0.46 |  |  |  |
| **Teac** |  |  |  |  |  |  |  |
| No | 319 (84.39) | 372 (85.71) | 1.00  |  |  |  |  |
| Yes | 59 (15.61) | 62 (14.29) | 0.93 (0.58-1.50) | 0.77 |  |  |  |
| **Canned fruit (times/month)** |  |  |  |  |  |  |
| <1 | 313 (83.02) | 380 (86.76) | 1.00  |  |  |  |  |
| ≥1 | 64 (16.98) | 58 (13.24) | 1.20 (0.77-1.87) | 0.42 |  |  |  |
| **Canned meat (times/month)** |  |  |  |  |  |  |
| <1 | 355 (93.67) | 406 (93.76) | 1.00  |  |  |  |  |
| ≥1 | 24 (6.33) | 27 (6.24) | 0.75 (0.38-1.46) | 0.39 |  |  |  |
| **Physical activitye (times/month)** |  |  |  |  |  |
| <1 | 243 (64.46) | 240 (55.3) | 1.00  |  |  | 1.000  |  |
| ≥1 | 134 (35.54) | 194 (44.7) | 0.53 (0.38-0.74) | **0.000** |  | 0.57 (0.39-0.83) | **0.003** |
| **Smoked** |  |  |  |  |  |  |  |
| No | 328 (86.54) | 387 (88.36) | 1.00  |  |  |  |  |
| Yes | 51 (13.46) | 51 (11.64) | 1.38 (0.83-2.31) | 0.22 |  |  |  |
| **Alcohol (times/month)** |  |  |  |  |  |  |
| <1 | 325 (86.67) | 388 (88.79) | 1.00  |  |  |  |  |
| ≥1 | 50 (13.33) | 49 (11.21) | 1.11 (0.69-1.81) | 0.67 |  |  |  |

ORadj: adjusted for marital status, education level, occupation, family history of cancer and history of hormone therapy.

aOvernight food, the cooked vegetables, eggs or meat which were left overnight, whether in the fridge or outside of the fridge.

bCarbonated drinks and bJuice**,** Yes means drinking at least 1cup/week for more than 3 months

cTea, Yes means drinking at least 2 times/ week for more than 3 months

dSmoke, Yes means smoking at least one per day for more than 6 weeks

ePhysical activity, Yes/No means exercise ≥ 70 minutes/< 70 minutes of moderate-intensity aerobic physical activity; or ≥ 30 minutes/< 30 minutes of vigorous-intensity aerobic physical activity; such as running, swimming, participating in ball games, etc.

**Supplementary Table 6. Correlation between clinicopathological characteristics and *CDH4* methylation in breast peripheral blood leukocyte DNA and tumor tissue DNA.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Character of clinical pathology** | **Peripheral blood leukocyte DNAa** | ***P-*value** | **Tumor tissue DNAb** | ***P-*value** |
| **Hypomethylated No. (%)** | **Hypermethylated No. (%)** | **Hypomethylated No. (%)** | **Hypermethylated No. (%)** |
| **Locationc** |  |  | 0.35  |  |  | 0.26  |
| Unilateral  | 169 (97.69) | 139 (95.86) |  | 163 (97.02) | 164 (98.80) |  |
| Both | 4 (2.31) | 6 (4.14) |  | 5 (3.98) | 2 (1.20) |  |
| **TNM Stage** |  |  | 0.50  |  |  | 0.95  |
| I | 55 (31.79) | 54 (37.24) |  | 40 (23.95) | 41 (24.55) |  |
| II | 102 (58.96) | 76 (52.41) |  | 85 (50.9) | 82 (49.1) |  |
| III-IV | 16 (9.25) | 15 (10.34) |  | 42 (25.15) | 44 (26.35) |  |
| **Tumour invasion** |  | 0.90  |  |  | 0.39  |
| T0, Tis | 1 (0.58) | 1 (0.69) |  | 2 (1.19) | 4 (2.45) |  |
| T1, T2, T3, T4 | 172 (99.42) | 144 (99.31) |  | 166 (98.81) | 159 (97.55) |  |
| **Lymphnodes involved** |  | 0.55  |  |  | 0.12  |
| N0 | 78 (45.88) | 69 (49.29) |  | 81 (48.80) | 93 (57.41) |  |
| N1, N2, N3 | 92 (54.12) | 71 (50.71) |  | 85 (51.20) | 69 (42.59) |  |
| **Metastasis status** |  | 0.23  |  |  | 0.60  |
| M0 | 167 (97.66) | 138 (95.17) |  | 161 (95.83) | 158 (94.61) |  |
| M1 | 4 (2.34) | 7 (4.83) |  | 7 (4.17) | 9 (5.39) |  |
| **ER status** |  |  | 0.92  |  |  | 0.42  |
| Negative | 48 (27.75) | 41 (28.28) |  | 55 (32.93) | 62 (37.13) |  |
| Positive | 125 (72.25) | 104 (71.72) |  | 112 (67.07) | 105 (62.87) |  |
| **PR status** |  |  | 0.74  |  |  | 0.32  |
| Negative | 64 (36.99) | 51 (35.17) |  | 76 (45.51) | 85 (50.9) |  |
| Positive | 109 (63.01) | 94 (64.83) |  | 91 (54.49) | 82 (49.1) |  |
| **HER2 expression** |  | 0.91  |  |  | 0.35  |
| Negative | 10 (8.55) | 9 (8.11) |  | 60 (35.93) | 52 (31.14) |  |
| Positive | 107 (91.45) | 102 (91.89) |  | 107 (64.07) | 115 (68.86) |  |
| **Molecular subtyped** |  | 0.45  |  |  | 0.26  |
| Luminal A | 10 (8.55) | 7 (6.31) |  | 17 (10.24) | 22 (13.41) |  |
| Luminal B | 76 (64.96) | 70 (63.06) |  | 44 (26.51) | 29 (17.68) |  |
| HER-2 enriched | 31 (26.50) | 32 (23.83) |  | 71 (42.77) | 76 (46.34) |  |
| Basal-like | 0 (0.00) | 2 (1.80) |  | 34 (20.48) | 37 (22.56) |  |
| **P53**  |  |  | 0.26  |  |  | 0.26  |
| Negative | 22 (30.99) | 22 (40.74) |  | 113 (68.07) | 123 (73.65) |  |
| Positive | 49 (69.01) | 32 (59.26) |  | 53 (31.93) | 44 (26.35) |  |
| **Histological typee** |  | 0.68  |  |  | 0.55  |
| Noninvasive | 10 (5.92) | 10 (7.09) |  | 39 (23.49) | 43 (26.38) |  |
| Invasive | 159 (94.08) | 131 (92.91) |  | 127 (76.51) | 120 (73.62) |  |
| **Ki-67 proliferation marker** |  | 0.45 |  |  | 0.53 |
| ≤5 | 42(24.71) | 42(29.37) |  | 45(27.27) | 35(21.88) |  |
| 5-30 | 102(60) | 85(59.44) |  | 94(56.97) | 98(61.25) |  |
| >30 | 26(15.29) | 16(11.19) |  | 26(15.76) | 27(16.88) |  |

aPeripheral blood leukocyte DNA, the methylation values were tested from peripheral blood leukocyte DNA of fasting peripheral venous blood. And the methylation cut-off value (0.778) was selected according to the maximum Youden index.

bTumor tissue DNA, the methylation values were tested from tumor tissue specimen, and its cut-off value was 55.5%, selected according to the 50% percentile.

cLocation, “left or right” means that breast cancer occurred on unilateral breast (left or right). “Both” means that breast cancer occurred on bilateral breast.

dSubtypes were classified by immunohistochemical surrogates as basal-like (ER-, PR-, HER-2−, triple-negative), luminal A (ER and/or PR+, HER-2−), luminal B (ER and/or PR+, HER-2+), or HER-2 enriched (ER and PR−, HER-2+).

eHistological type, noninvasive cancer means ductal carcinoma in situ (DCIS), invasive cancer includes invasive ductal carcinoma and invasive lobular carcinoma.

**Figure Legends**

**Supplementary Figure 1. Quantitative methylation-specific PCR (qMSP) of *CDH4* methylation for serial standards and samples** (A) Standard curve.The R2 value is 0.99, means the standard curve fits well. (B) Amplification plot. (C) Melt curve. The melting peak of serial dilutions of plasmid DNA and samples.