**Table 3. Selected lifestyle and medical characteristics of participants by sex and study area**

| | *n* | Study area | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | Chiba | Shizuoka | Okazaki | ACC | Takashima | Kyoto | Tokushima | Fukuoka | Saga | Amami |
| Men | | | | | | | | | | | | |
| Current smokers (%) | 2123 | 29.1 | 23.5 | 23.6 | 25.6 | 31.0 | 35.7 | 41.8 | 31.5 | 33.0 | 34.3 | 23.3 |
| Ex-smokers (%) | 2123 | 42.9 | 42.3 | 48.3 | 50.2 | 44.2 | 33.3 | 33.6 | 38.4 | 39.4 | 42.9 | 39.1 |
| Current drinkers (%)[a] | 2121 | 71.4 | 78.5 | 73.4 | 62.6 | 66.9 | 73.8 | 68.0 | 61.6 | 70.2 | 71.4 | 84.1 |
| Exercise ≥1/month (%) | 2109 | 82.9 | 87.1 | 88.7 | 81.0 | 83.5 | 60.1 | 81.0 | 94.5 | 89.9 | 85.3 | 75.9 |
| Body mass index ≥25.0 (%) | 2114 | 29.5 | 29.1 | 25.2 | 26.4 | 22.6 | 27.0 | 23.8 | 41.1 | 31.4 | 27.7 | 52.8 |
| History of hypertension (%) | 2052 | 25.2 | 28.2 | 14.7 | 29.5 | 24.0 | 28.7 | 9.9 | 21.9 | 44.4 | 28.6 | 32.1 |
| Systolic blood pressure (mm Hg) | 1698 | 130.1 ± 18.8 | NA | 121.9 ± 14.8 | 128.7 ± 15.2 | NA | 133.0 ± 19.5 | 122.8 ± 16.0 | 119.8 ± 17.6 | 144.0 ± 19.6 | 138.3 ± 19.3 | 132.0 ± 17.7 |
| Diastolic blood pressure (mm Hg) | 1698 | 80.8 ± 11.9 | NA | 76.5 ± 10.5 | 81.4 ± 9.1 | NA | 81.2 ± 11.2 | 74.3 ± 11.9 | 73.7 ± 12.8 | 88.0 ± 11.1 | 86.0 ± 12.8 | 81.9 ± 11.1 |
| Total cholesterol (mg/dl) | 1296 | 205.2 ± 32.3 | NA | 201.3 ± 29.3 | 205.9 ± 32.0 | NA | 208.3 ± 35.6 | —[b] | 209.0 ± 31.4 | 209.9 ± 31.5 | 203.6 ± 33.6 | 208.0 ± 35.7 |
| HDL-cholesterol (mg/dl) | 1378 | 58.9 ± 15.9 | NA | 58.3 ± 15.4 | 64.1 ± 17.5 | NA | 59.4 ± 17.0 | 61.4 ± 17.6 | 52.5 ± 11.6 | 54.5 ± 16.1 | 55.3 ± 14.2 | 57.7 ± 13.4 |
| Triglyceride (mg/dl) | 1377 | 135.9 ± 93.0 | NA | 122.8 ± 68.6 | 120.8 ± 86.5 | NA | 116.9 ± 60.0 | 127.9 ± 85.7 | 126.7 ± 64.2 | 181.5 ± 131.9 | 164.9 ± 93.6 | 165.7 ± 130.8 |
| Blood glucose (mg/dl) | 1175 | 102.1 ± 20.3 | NA | 101.1 ± 14.3 | 100.0 ± 17.0 | NA | 97.9 ± 16.3 | 98.7 ± 25.0 | 104.2 ± 26.4 | NA | NA | 110.3 ± 26.8 |
| HbA1c (%) | 1354 | 5.27 ± 0.74 | NA | 5.27 ± 0.56 | 5.36 ± 0.64 | NA | 5.24 ± 0.79 | NA | 5.17 ± 0.52 | 5.17 ± 0.82 | 5.28 ± 1.04 | 5.31 ± 0.42 |
| Women | | | | | | | | | | | | |
| Current smokers (%) | 2390 | 7.1 | 7.6 | 5.0 | 6.3 | 12.4 | 5.6 | 15.8 | 0.0 | 9.0 | 5.4 | 4.4 |
| Ex-smokers (%) | 2390 | 4.8 | 6.7 | 4.4 | 5.9 | 8.4 | 1.3 | 7.9 | 4.6 | 4.7 | 5.7 | 1.0 |
| Current drinkers (%)[a] | 2383 | 27.7 | 36.1 | 27.2 | 22.4 | 31.7 | 24.6 | 34.2 | 22.7 | 25.0 | 28.2 | 23.3 |
| Exercise ≥1/month (%) | 2381 | 76.0 | 82.8 | 81.8 | 83.9 | 70.9 | 55.6 | 44.7 | 81.8 | 88.3 | 83.4 | 73.1 |
| Body mass index ≥25.0 (%) | 2359 | 19.3 | 12.8 | 15.5 | 17.3 | 14.0 | 21.9 | 15.8 | 27.3 | 19.5 | 16.5 | 36.2 |
| History of hypertension (%) | 2289 | 17.9 | 10.4 | 15.8 | 19.3 | 12.9 | 18.5 | 7.9 | 18.2 | 37.9 | 17.6 | 22.0 |
| Systolic blood pressure (mm Hg) | 1732 | 126.5 ± 19.9 | NA | 115.7 ± 16.8 | 122.7 ± 15.6 | NA | 124.6 ± 19.5 | 120.4 ± 19.5 | 111.5 ± 11.7 | 137.5 ± 21.9 | 129.5 ± 19.5 | 126.8 ± 19.0 |
| Diastolic blood pressure (mm Hg) | 1732 | 76.4 ± 11.7 | NA | 70.3 ± 10.5 | 76.7 ± 9.5 | NA | 73.6 ± 11.4 | 71.8 ± 11.1 | 68.2 ± 10.3 | 84.0 ± 11.3 | 77.9 ± 12.2 | 76.0 ± 10.4 |
| Total cholesterol (mg/dl) | 1318 | 217.9 ± 34.6 | NA | 206.4 ± 31.9 | 213.5 ± 34.1 | NA | 222.6 ± 36.5 | NA | 210.0 ± 35.7 | 230.1 ± 31.4 | 220.3 ± 33.5 | 218.0 ± 33.7 |
| HDL-cholesterol (mg/dl) | 1347 | 68.0 ± 15.5 | NA | 72.3 ± 16.1 | 75.0 ± 16.9 | NA | 67.0 ± 15.0 | 69.3 ± 13.7 | 65.5 ± 12.0 | 66.1 ± 16.6 | 64.3 ± 14.1 | 63.4 ± 12.6 |
| Triglyceride (mg/dl) | 1347 | 103.4 ± 65.7 | NA | 80.0 ± 40.9 | 97.3 ± 60.9 | NA | 96.8 ± 54.9 | 68.6 ± 27.8 | 84.3 ± 41.2 | 128.0 ± 66.0 | 129.2 ± 83.4 | 109.4 ± 74.2 |
| Blood glucose (mg/dl) | 1072 | 95.1 ± 16.9 | NA | 91.9 ± 8.3 | 94.7 ± 13.7 | NA | 91.0 ± 10.2 | 89.6 ± 7.1 | 101.9 ± 31.9 | NA | NA | 101.8 ± 24.3 |
| HbA1c (%) | 1396 | 5.17 ± 0.57 | NA | 5.15 ± 0.35 | 5.32 ± 0.57 | NA | 5.11 ± 0.54 | NA | —[b] | 5.16 ± 0.65 | 5.10 ± 0.59 | —[b] |

Abbreviations: ACC, Aichi Cancer Center; HDL, high-density lipoprotein; NA, not available.
Plus-minus values are means ± SDs.
[a]Individuals who drank alcoholic beverages ≥1 day/week.
[b]Data available for fewer than 20 participants.

**Table 4. Genotype distributions and allele frequencies of 108 selected genetic polymorphisms (107 SNPs and 1 insertion/deletion polymorphism)**

| Gene | Polymorphism | rs number | Genotype[a] | | | *n*[a] | | | | Frequency (proportion) | | | | | | *P* for HWE | MAF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Observed | | | Expected[b] | | | | |
| | | | AA | Aa | aa | AA | Aa | aa | XX | AA | Aa | aa | AA | Aa | aa | | |
| *ABCA1* | C-565T | rs2422493 | CC | CT | TT | 1634 | 2137 | 746 | 2 | 0.362 | 0.473 | 0.165 | 0.358 | 0.481 | 0.161 | 0.29 | 0.402 |
| *ABCA1* | G-191C | rs1800976 | GG | GC | CC | 1628 | 2144 | 746 | 1 | 0.360 | 0.475 | 0.165 | 0.357 | 0.481 | 0.162 | 0.37 | 0.402 |
| *ABCA1* | G-17C | rs2740483 | GG | GC | CC | 2211 | 1898 | 409 | 1 | 0.489 | 0.420 | 0.091 | 0.489 | 0.420 | 0.090 | 0.94 | 0.301 |
| *ABCA1* | Val825Ile (G/A) | rs2066715 | GG | GA | AA | 1877 | 2064 | 577 | 1 | 0.415 | 0.457 | 0.128 | 0.415 | 0.459 | 0.127 | 0.80 | 0.356 |
| *ABCA1* | Val771Met (G/A) | rs2066718 | GG | GA | AA | 3945 | 550 | 23 | 1 | 0.873 | 0.122 | 0.005 | 0.872 | 0.123 | 0.004 | 0.40 | 0.066 |
| *ABCA1* | Arg1587Lys (G/A) | rs2230808 | GG | GA | AA | 1673 | 2154 | 689 | 3 | 0.370 | 0.477 | 0.153 | 0.371 | 0.476 | 0.153 | 0.95 | 0.391 |
| *ABCC11* | Arg180Gly (T/C) | rs17822931 | TT | TC | CC | 3368 | 1051 | 95 | 5 | 0.746 | 0.233 | 0.021 | 0.744 | 0.237 | 0.019 | 0.23 | 0.137 |
| *ACE* | Insertion/Deletion (I/D) | rs1799752 | I/I | I/D | D/D | 1854 | 2021 | 634 | 10 | 0.411 | 0.448 | 0.141 | 0.404 | 0.463 | 0.133 | 0.029 | 0.365 |
| *ADD1* | Trp460Gly (T/G) | rs4961 | TT | TG | GG | 1399 | 2163 | 956 | 1 | 0.310 | 0.479 | 0.212 | 0.301 | 0.495 | 0.203 | 0.026 | 0.451 |
| *ADH1B* | His47Arg (A/G) | rs1229984 | AA | AG | GG | 2607 | 1659 | 250 | 3 | 0.577 | 0.367 | 0.055 | 0.579 | 0.364 | 0.057 | 0.54 | 0.239 |
| *ADH1C* | Arg272Gln (C/T) | rs1693482 | CC | CT | TT | 3882 | 591 | 43 | 3 | 0.860 | 0.131 | 0.010 | 0.856 | 0.139 | 0.006 | 0.0005 | 0.075 |
| *ADIPOQ* | C-11377G | rs266729 | CC | CG | GG | 2553 | 1663 | 301 | 2 | 0.565 | 0.368 | 0.067 | 0.561 | 0.376 | 0.063 | 0.18 | 0.251 |
| *ADIPOQ* | Gly15Gly (T/G) | rs2241766 | TT | TG | GG | 2314 | 1789 | 415 | 1 | 0.512 | 0.396 | 0.092 | 0.504 | 0.412 | 0.084 | 0.011 | 0.290 |
| *ADIPOQ* | G276T | rs1501299 | GG | GT | TT | 2498 | 1674 | 346 | 1 | 0.553 | 0.371 | 0.077 | 0.545 | 0.387 | 0.069 | 0.006 | 0.262 |
| *ADIPOQ* | IVS-3971A/G | rs822396 | AA | AG | GG | 3984 | 517 | 16 | 2 | 0.882 | 0.114 | 0.004 | 0.882 | 0.114 | 0.004 | 1.00 | 0.061 |
| *ADIPOR1* | G-8503A | rs6666089 | GG | GA | AA | 4234 | 272 | 7 | 6 | 0.938 | 0.060 | 0.002 | 0.938 | 0.061 | 0.001 | 0.22 | 0.032 |
| *ADIPOR1* | C5843T | rs1342387 | CC | CT | TT | 1230 | 2206 | 1073 | 10 | 0.273 | 0.489 | 0.238 | 0.268 | 0.499 | 0.233 | 0.17 | 0.483 |
| *ADIPOR1* | C10224G | rs7539542 | CC | CG | GG | 2788 | 1505 | 225 | 1 | 0.617 | 0.333 | 0.050 | 0.614 | 0.339 | 0.047 | 0.24 | 0.216 |
| *ADRB2* | Gln27Glu (C/G) | rs1042714 | CC | CG | GG | 3943 | 551 | 20 | 5 | 0.874 | 0.122 | 0.004 | 0.873 | 0.122 | 0.004 | 0.81 | 0.065 |
| *ADRB3* | Trp64Arg (T/C) | rs4994 | TT | TC | CC | 2932 | 1397 | 182 | 8 | 0.650 | 0.310 | 0.040 | 0.648 | 0.314 | 0.038 | 0.34 | 0.195 |
| *AGT* | Thr174Met (C/T) | rs4762 | CC | CT | TT | 3615 | 848 | 52 | 4 | 0.801 | 0.188 | 0.012 | 0.800 | 0.189 | 0.011 | 0.75 | 0.105 |
| *AGT* | Thr235Met (C/T) | rs699 | CC | CT | TT | 2989 | 1372 | 157 | 1 | 0.662 | 0.304 | 0.035 | 0.662 | 0.304 | 0.035 | 1.00 | 0.187 |
| *AGTR1(ATR1)* | A1166C (at 3'UTR) | rs5186 | AA | AC | CC | 3777 | 695 | 45 | 2 | 0.836 | 0.154 | 0.010 | 0.834 | 0.159 | 0.008 | 0.048 | 0.087 |
| *ALDH2* | Glu487Lys (G/A) | rs671 | GG | GA | AA | 2544 | 1649 | 321 | 5 | 0.564 | 0.365 | 0.071 | 0.557 | 0.379 | 0.064 | 0.018 | 0.254 |
| *APOA1* | Ala61Thr (C/T) | rs12718465 | CC | CT | TT | 4036 | 463 | 18 | 2 | 0.894 | 0.103 | 0.004 | 0.893 | 0.104 | 0.003 | 0.25 | 0.055 |
| *APOA1* | Arg184Pro (G/C) | rs5078 | GG | GC | CC | 4512 | 0 | 0 | 7 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.00 | 0.000 |
| *APOA5* | T-1131C | rs662799 | TT | TC | CC | 1955 | 2001 | 556 | 7 | 0.433 | 0.443 | 0.123 | 0.429 | 0.452 | 0.119 | 0.21 | 0.345 |
| *APOA5* | Gly185Cys (G553T) | rs2075291 | GG | GT | TT | 3920 | 559 | 32 | 8 | 0.869 | 0.124 | 0.007 | 0.867 | 0.129 | 0.005 | 0.020 | 0.069 |
| *APOE* | T-203G | rs405509 | TT | TG | GG | 2213 | 1847 | 451 | 8 | 0.491 | 0.409 | 0.100 | 0.483 | 0.424 | 0.093 | 0.025 | 0.305 |
| *APOE* | Arg158Cys (C/T) (at exon2) | rs7412 | CC | CT | TT | 4140 | 369 | 9 | 1 | 0.916 | 0.082 | 0.002 | 0.916 | 0.082 | 0.002 | 0.72 | 0.043 |
| *APOE* | Cys112Arg (T/C) (at exon4) | rs429358 | TT | TC | CC | 3669 | 782 | 67 | 1 | 0.812 | 0.173 | 0.015 | 0.808 | 0.182 | 0.010 | 0.001 | 0.101 |
| *ARNTL(BMAL1)* | A/G | rs7950226 | AA | AG | GG | 1638 | 2102 | 773 | 6 | 0.363 | 0.466 | 0.171 | 0.355 | 0.482 | 0.163 | 0.028 | 0.404 |
| *ART4(DOK1)* | Leu208Leu (G/A) | rs3088189 | GG | GA | AA | 3595 | 875 | 47 | 2 | 0.796 | 0.194 | 0.010 | 0.797 | 0.192 | 0.012 | 0.48 | 0.107 |
| *ART4(DOK1)* | Asp265Asn (G/A) | rs11276 | GG | GA | AA | 3597 | 872 | 48 | 2 | 0.796 | 0.193 | 0.011 | 0.797 | 0.191 | 0.011 | 0.59 | 0.107 |
| *BHMT* | Arg239Gln (G742A) | rs3733890 | GG | GA | AA | 2765 | 1511 | 241 | 2 | 0.612 | 0.335 | 0.053 | 0.607 | 0.344 | 0.049 | 0.069 | 0.221 |
| *CBS* | Tyr233Tyr (C699T) | rs234706 | CC | CT | TT | 4302 | 212 | 2 | 3 | 0.953 | 0.047 | 0.000 | 0.953 | 0.047 | 0.001 | 1.00 | 0.024 |
| *CD14* | T-260C/T-159C | rs2569190 | TT | TC | CC | 1304 | 2256 | 958 | 1 | 0.289 | 0.499 | 0.212 | 0.290 | 0.497 | 0.213 | 0.79 | 0.462 |
| *CDKAL1* | G/C | rs7754840 | GG | GC | CC | 1581 | 2195 | 740 | 3 | 0.350 | 0.486 | 0.164 | 0.352 | 0.483 | 0.166 | 0.64 | 0.407 |
| *CDKN2A/B* | T/C | rs10811661 | TT | TC | CC | 1379 | 2205 | 934 | 1 | 0.305 | 0.488 | 0.207 | 0.302 | 0.495 | 0.203 | 0.34 | 0.451 |
| *CETP* | A-629C | rs1800775 | AA | AC | CC | 1384 | 2242 | 891 | 2 | 0.306 | 0.496 | 0.197 | 0.308 | 0.494 | 0.198 | 0.76 | 0.445 |
| *CETP* | Ile405Val (A/G) | rs5882 | AA | AG | GG | 1236 | 2116 | 1166 | 1 | 0.274 | 0.468 | 0.258 | 0.258 | 0.500 | 0.242 | <0.0001 | 0.492 |
| *CETP* | TaqIB (C/T) | rs708272 | CC | CT | TT | 1603 | 2174 | 741 | 1 | 0.355 | 0.481 | 0.164 | 0.354 | 0.482 | 0.164 | 0.93 | 0.405 |

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| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *CETP* | G/T | rs3764261 | GG | GT | TT | 2707 | 1512 | 298 | 2 | 0.599 | 0.335 | 0.066 | 0.588 | 0.358 | 0.054 | <0.0001 | 0.233 |
| *CHRNB2* | G-42A | rs2072658 | GG | GA | AA | 2837 | 1518 | 161 | 3 | 0.628 | 0.336 | 0.036 | 0.634 | 0.324 | 0.042 | 0.015 | 0.204 |
| *CHRNB2* | C/T (at 3'UTR) | rs2072660 | CC | CT | TT | 2601 | 1641 | 275 | 2 | 0.576 | 0.363 | 0.061 | 0.574 | 0.367 | 0.059 | 0.44 | 0.243 |
| *COMT* | Val158Met (G/A) | rs4680 | GG | GA | AA | 2006 | 1983 | 528 | 2 | 0.444 | 0.439 | 0.117 | 0.440 | 0.446 | 0.113 | 0.27 | 0.336 |
| *CYP1A1* | Ile462Val (A/G) | rs1048943 | AA | AG | GG | 2630 | 1620 | 268 | 1 | 0.582 | 0.359 | 0.059 | 0.580 | 0.363 | 0.057 | 0.39 | 0.239 |
| *CYP1A2* | A734C | rs762551 | AA | AC | CC | 1878 | 2043 | 592 | 6 | 0.416 | 0.453 | 0.131 | 0.413 | 0.459 | 0.128 | 0.33 | 0.358 |
| *CYP11B2* | T-344C | rs1799998 | TT | TC | CC | 2031 | 1976 | 507 | 5 | 0.450 | 0.438 | 0.112 | 0.447 | 0.443 | 0.110 | 0.42 | 0.331 |
| *CYP17A1* | T-34C | rs743572 | TT | TC | CC | 1281 | 2257 | 977 | 4 | 0.284 | 0.500 | 0.216 | 0.285 | 0.498 | 0.217 | 0.79 | 0.466 |
| *ESR1* | IVS1-351A/G (*Xba* I) | rs11155816 | AA | AG | GG | 4515 | 0 | 0 | 4 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.00 | 0.000 |
| *ESR1* | IVS1-397T/C (*Pvu* II) | rs2234693 | TT | TC | CC | 1508 | 2172 | 834 | 5 | 0.334 | 0.481 | 0.185 | 0.330 | 0.489 | 0.181 | 0.30 | 0.425 |
| *ESR2* | Val328Val (G1082A) (*Rsa* I) | rs1256049 | GG | GA | AA | 2320 | 1823 | 373 | 3 | 0.514 | 0.404 | 0.083 | 0.512 | 0.407 | 0.081 | 0.58 | 0.284 |
| *FTO* | T/A | rs9939609 | TT | TA | AA | 2881 | 1454 | 183 | 1 | 0.638 | 0.322 | 0.041 | 0.638 | 0.322 | 0.041 | 1.00 | 0.201 |
| *GCK* | G-30A | rs1799884 | GG | GA | AA | 2989 | 1348 | 181 | 1 | 0.662 | 0.298 | 0.040 | 0.657 | 0.307 | 0.036 | 0.065 | 0.189 |
| *GCKR* | A/G | rs780094 | AA | AG | GG | 1379 | 2221 | 917 | 2 | 0.305 | 0.492 | 0.203 | 0.304 | 0.495 | 0.201 | 0.67 | 0.449 |
| *GCKR* | Leu446Pro (T/C) | rs1260326 | TT | TC | CC | 1402 | 2214 | 902 | 1 | 0.310 | 0.490 | 0.200 | 0.308 | 0.494 | 0.198 | 0.61 | 0.445 |
| *GNAS1* | T393C | rs7121 | TT | TC | CC | 1466 | 2233 | 817 | 3 | 0.325 | 0.494 | 0.181 | 0.327 | 0.490 | 0.183 | 0.52 | 0.428 |
| *IGF2BP2* | G/T (at intron) | rs4402960 | GG | GT | TT | 2215 | 1866 | 436 | 2 | 0.490 | 0.413 | 0.097 | 0.486 | 0.422 | 0.092 | 0.14 | 0.303 |
| *IL-1B* | T-31C | rs1143627 | TT | TC | CC | 1331 | 2196 | 990 | 2 | 0.295 | 0.486 | 0.219 | 0.289 | 0.497 | 0.214 | 0.14 | 0.462 |
| *IL-2* | T-330G | rs2069762 | TT | TG | GG | 2033 | 1986 | 494 | 6 | 0.450 | 0.440 | 0.109 | 0.450 | 0.442 | 0.109 | 0.79 | 0.329 |
| *IL-4* | T-33C | rs2070874 | TT | TC | CC | 1997 | 1989 | 531 | 2 | 0.442 | 0.440 | 0.118 | 0.439 | 0.447 | 0.114 | 0.30 | 0.338 |
| *IL-6* | C-634G | rs1800796 | CC | CG | GG | 2611 | 1607 | 298 | 3 | 0.578 | 0.356 | 0.066 | 0.572 | 0.369 | 0.059 | 0.019 | 0.244 |
| *IL-8* | T-251A | rs4073 | TT | TA | AA | 2077 | 1952 | 463 | 27 | 0.462 | 0.435 | 0.103 | 0.462 | 0.435 | 0.103 | 0.89 | 0.320 |
| *IL-10* | T-819C | rs1800871 | TT | TC | CC | 1942 | 2009 | 557 | 11 | 0.431 | 0.446 | 0.124 | 0.427 | 0.453 | 0.120 | 0.29 | 0.346 |
| *IL-13* | C-1111T | rs1800925 | CC | CT | TT | 3009 | 1359 | 147 | 4 | 0.666 | 0.301 | 0.033 | 0.667 | 0.299 | 0.034 | 0.69 | 0.183 |
| *KCNJ11* | Glu23Lys (C/T) | rs5219 | CC | CT | TT | 1822 | 2081 | 615 | 1 | 0.403 | 0.461 | 0.136 | 0.401 | 0.464 | 0.134 | 0.59 | 0.366 |
| *LCAT/SLC12A4* | Ser232Thr (T/A) | rs4986970 | TT | TA | AA | 4516 | 0 | 0 | 3 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.00 | 0.000 |
| *LIPC* | T-514C | rs1800588 | TT | TC | CC | 1178 | 2255 | 1085 | 1 | 0.261 | 0.499 | 0.240 | 0.260 | 0.500 | 0.240 | 0.93 | 0.490 |
| *LIPC* | Val95Met (G/A) | rs6078 | GG | GA | AA | 2659 | 1606 | 252 | 2 | 0.589 | 0.356 | 0.056 | 0.587 | 0.358 | 0.055 | 0.65 | 0.234 |
| *MPO* | G-463A | rs2333227 | GG | GA | AA | 3612 | 852 | 52 | 3 | 0.800 | 0.189 | 0.012 | 0.800 | 0.189 | 0.011 | 0.81 | 0.106 |
| *MTHFD1* | Arg134Lys (C401T) | rs1950902 | CC | CT | TT | 2773 | 1517 | 228 | 1 | 0.614 | 0.336 | 0.050 | 0.611 | 0.341 | 0.048 | 0.28 | 0.218 |
| *MTHFD1* | Arg653Gln (G1958A) | rs2236225 | GG | GA | AA | 2340 | 1790 | 384 | 5 | 0.518 | 0.397 | 0.085 | 0.514 | 0.406 | 0.080 | 0.12 | 0.283 |
| *MTHFR* | Ala222Val (C677T) | rs1801133 | CC | CT | TT | 1763 | 2060 | 694 | 2 | 0.390 | 0.456 | 0.154 | 0.382 | 0.472 | 0.146 | 0.023 | 0.382 |
| *MTHFR* | Glu429Ala (A1298C) | rs1801131 | AA | AC | CC | 3023 | 1323 | 169 | 4 | 0.670 | 0.293 | 0.037 | 0.666 | 0.300 | 0.034 | 0.11 | 0.184 |
| *MTR* | Asp919Gly (A/G) | rs1805087 | AA | AG | GG | 2883 | 1446 | 185 | 5 | 0.639 | 0.320 | 0.041 | 0.638 | 0.321 | 0.040 | 0.82 | 0.201 |
| *MTRR* | Ile22Met (A66G) | rs1801394 | AA | AG | GG | 2155 | 1925 | 436 | 3 | 0.477 | 0.426 | 0.097 | 0.477 | 0.428 | 0.096 | 0.83 | 0.310 |
| *NOS3* | T-786C | rs2070744 | TT | TC | CC | 3602 | 863 | 48 | 6 | 0.798 | 0.191 | 0.011 | 0.799 | 0.190 | 0.011 | 0.70 | 0.106 |
| *PPARD* | T-842C (at exon3) | rs2267668 | TT | TC | CC | 2922 | 1421 | 172 | 4 | 0.647 | 0.315 | 0.038 | 0.647 | 0.315 | 0.038 | 1.00 | 0.195 |
| *PPARD* | T-48444C (at exon3) | rs6902123 | TT | TC | CC | 4343 | 173 | 2 | 1 | 0.961 | 0.038 | 0.000 | 0.961 | 0.038 | 0.000 | 0.69 | 0.020 |
| *PPARD* | Asn163Asn (A65G) (at exon7) | rs2076167 | AA | AG | GG | 2780 | 1530 | 204 | 5 | 0.616 | 0.339 | 0.045 | 0.617 | 0.337 | 0.046 | 0.76 | 0.215 |
| *PPARG* | Pro12Ala (C/G) | rs1801282 | CC | CG | GG | 4236 | 274 | 5 | 4 | 0.938 | 0.061 | 0.001 | 0.938 | 0.061 | 0.001 | 0.80 | 0.031 |
| *PPARG* | His477His (C161T) | rs3856806 | CC | CT | TT | 3231 | 1198 | 86 | 4 | 0.716 | 0.265 | 0.019 | 0.720 | 0.257 | 0.023 | 0.043 | 0.152 |
| *PPARGC1A* | Thr394Thr (C/T) | rs2970847 | CC | CT | TT | 2755 | 1539 | 221 | 4 | 0.610 | 0.341 | 0.049 | 0.609 | 0.343 | 0.048 | 0.76 | 0.219 |
| *PPARGC1A* | Gly482Ser (G/A) | rs8192678 | GG | GA | AA | 1317 | 2247 | 952 | 3 | 0.292 | 0.498 | 0.211 | 0.292 | 0.497 | 0.211 | 0.93 | 0.460 |
| *PRKAA2* | IVS4+961T/C | rs1418442 | TT | TC | CC | 2677 | 1581 | 251 | 10 | 0.594 | 0.351 | 0.056 | 0.591 | 0.355 | 0.053 | 0.38 | 0.231 |
| *PRKAA2* | IVS7+81T/C | rs932447 | TT | TC | CC | 2679 | 1585 | 252 | 3 | 0.593 | 0.351 | 0.056 | 0.591 | 0.356 | 0.053 | 0.38 | 0.231 |
| *PRKAA2* | A/T (at 3'UTR) | rs1342382 | AA | AT | TT | 2739 | 1556 | 218 | 6 | 0.607 | 0.345 | 0.048 | 0.607 | 0.344 | 0.049 | 0.90 | 0.221 |
| *PTGS2(COX2)* | G-765C | rs20417 | GG | GC | CC | 4260 | 247 | 6 | 6 | 0.944 | 0.055 | 0.001 | 0.943 | 0.056 | 0.001 | 0.27 | 0.029 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *PTGS2(COX2)* | C-163G | rs5270 | CC | CG | GG | 4379 | 136 | 3 | 1 | 0.969 | 0.030 | 0.001 | 0.969 | 0.031 | 0.000 | 0.10 | 0.016 |
| *PTPN11* | G33861A | rs2301756 | GG | GA | AA | 2926 | 1418 | 174 | 1 | 0.648 | 0.314 | 0.039 | 0.647 | 0.314 | 0.038 | 0.89 | 0.195 |
| *RETN* | C-420G | rs1862513 | CC | CG | GG | 1956 | 1977 | 585 | 1 | 0.433 | 0.438 | 0.129 | 0.425 | 0.454 | 0.121 | 0.015 | 0.348 |
| *SCARB1* | Val135Ile (G/A) | rs5891 | GG | GA | AA | 4518 | 0 | 0 | 1 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.00 | 0.000 |
| *SCARB1* | Ala350Ala (C1119T) | rs5888 | CC | CT | TT | 2678 | 1619 | 221 | 1 | 0.593 | 0.358 | 0.049 | 0.596 | 0.352 | 0.052 | 0.25 | 0.228 |
| *SCARB1* | G/A (at intron) | rs3782287 | GG | GA | AA | 2676 | 1574 | 268 | 1 | 0.592 | 0.348 | 0.059 | 0.588 | 0.358 | 0.055 | 0.074 | 0.234 |
| *SERPINC1* | C/G | rs677 | CC | CG | GG | 2762 | 1527 | 227 | 3 | 0.612 | 0.338 | 0.050 | 0.609 | 0.342 | 0.048 | 0.41 | 0.219 |
| *SHMT1* | Leu435Phe (C1420T) | rs1979277 | CC | CT | TT | 3755 | 722 | 41 | 1 | 0.831 | 0.160 | 0.009 | 0.830 | 0.162 | 0.008 | 0.36 | 0.089 |
| *SLC19A(RFC1)* | His27Arg (A80G) | rs1051266 | AA | AG | GG | 1536 | 2187 | 793 | 3 | 0.340 | 0.484 | 0.176 | 0.339 | 0.486 | 0.175 | 0.76 | 0.418 |
| *SLC30A8* | Arg325Trp (C/T) | rs13266634 | CC | CT | TT | 1543 | 1992 | 976 | 8 | 0.342 | 0.442 | 0.216 | 0.317 | 0.492 | 0.191 | <0.0001 | 0.437 |
| *SRD5A2* | Leu89Val (C/G) | rs523349 | CC | CG | GG | 1334 | 2286 | 896 | 3 | 0.295 | 0.506 | 0.198 | 0.301 | 0.495 | 0.204 | 0.15 | 0.452 |
| *TAS2R38* | Pro49Ala (C/G) | rs713598 | CC | CG | GG | 1399 | 2219 | 898 | 3 | 0.310 | 0.491 | 0.199 | 0.309 | 0.494 | 0.198 | 0.74 | 0.445 |
| *TAS2R38* | Ala262Val (C/T) | rs1726866 | CC | CT | TT | 1401 | 2218 | 899 | 1 | 0.310 | 0.491 | 0.199 | 0.309 | 0.494 | 0.198 | 0.70 | 0.444 |
| *TAS2R38* | Val296Ile (C/T) | rs10246939 | CC | CT | TT | 1401 | 2218 | 898 | 2 | 0.310 | 0.491 | 0.199 | 0.309 | 0.494 | 0.197 | 0.72 | 0.444 |
| *TCF7L2* | C/T (at intron) | rs7903146 | CC | CT | TT | 4174 | 333 | 11 | 1 | 0.924 | 0.074 | 0.002 | 0.923 | 0.075 | 0.002 | 0.11 | 0.039 |
| *TNF-A* | T-1031C | rs1799964 | TT | TC | CC | 3147 | 1244 | 127 | 1 | 0.697 | 0.275 | 0.028 | 0.696 | 0.277 | 0.027 | 0.75 | 0.166 |
| *TNF-A* | C-857T | rs1799724 | CC | CT | TT | 2990 | 1365 | 162 | 2 | 0.662 | 0.302 | 0.036 | 0.661 | 0.304 | 0.035 | 0.70 | 0.187 |
| *USF1* | C7131T | rs3737787 | CC | CT | TT | 2793 | 1506 | 218 | 2 | 0.618 | 0.333 | 0.048 | 0.616 | 0.338 | 0.046 | 0.43 | 0.215 |
| *VDR* | Met1Thr (*Fok* I) (C/T) | rs2228570 | CC | CT | TT | 1768 | 2105 | 643 | 3 | 0.391 | 0.466 | 0.142 | 0.390 | 0.469 | 0.141 | 0.68 | 0.375 |

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
[a]AA, Aa, aa, and XX indicate homozygotes of major alleles, heterozygotes, homozygotes of minor alleles, and samples for which the genotype could not be determined, respectively.
[b]Based on the Hardy–Weinberg equilibrium.

**Table 5. Minor allele frequencies of 107 selected genetic polymorphisms (106 SNPs and 1 insertion/deletion polymorphism) by study area**

| Gene | Polymorphism | rs number | Minor allele frequency by study area | | | | | | | | | | | $P^a$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total | Chiba | Shizu-oka | Okazaki | ACC | Taka-shima | Kyoto | Toku-shima | Fukuoka | Saga | Amami | |
| *ABCA1* | C-565T | rs2422493 | 0.402 | 0.371 | 0.429 | 0.406 | 0.426 | 0.401 | 0.394 | 0.468 | 0.404 | 0.405 | 0.355 | 0.005 |
| *ABCA1* | G-191C | rs1800976 | 0.402 | 0.370 | 0.429 | 0.406 | 0.425 | 0.403 | 0.403 | 0.468 | 0.408 | 0.405 | 0.356 | 0.006 |
| *ABCA1* | G-17C | rs2740483 | 0.301 | 0.363 | 0.296 | 0.300 | 0.271 | 0.294 | 0.300 | 0.247 | 0.313 | 0.313 | 0.271 | 0.0002 |
| *ABCA1* | Val825Ile (G/A) | rs2066715 | 0.356 | 0.354 | 0.358 | 0.331 | 0.354 | 0.350 | 0.347 | 0.353 | 0.360 | 0.358 | 0.389 | 0.51 |
| *ABCA1* | Val771Met (G/A) | rs2066718 | 0.066 | 0.067 | 0.060 | 0.067 | 0.059 | 0.061 | 0.034 | 0.047 | 0.073 | 0.068 | 0.088 | 0.041 |
| *ABCA1* | Arg1587Lys (G/A) | rs2230808 | 0.391 | 0.393 | 0.391 | 0.384 | 0.392 | 0.394 | 0.347 | 0.326 | 0.369 | 0.388 | 0.441 | 0.027 |
| *ACE* | Insertion/Deletion (I/D) | rs1799752 | 0.365 | 0.373 | 0.369 | 0.346 | 0.363 | 0.365 | 0.313 | 0.353 | 0.357 | 0.341 | 0.425 | 0.003 |
| *ADD1* | Trp460Gly (T/G) | rs4961 | 0.451 | 0.454 | 0.436 | 0.424 | 0.455 | 0.430 | 0.463 | 0.463 | 0.429 | 0.464 | 0.509 | 0.006 |
| *ADH1B* | His47Arg (A/G) | rs1229984 | 0.239 | 0.220 | 0.232 | 0.200 | 0.247 | 0.220 | 0.250 | 0.184 | 0.266 | 0.223 | 0.318 | <0.0001 |
| *ADH1C* | Arg272Gln (C/T) | rs1693482 | 0.075 | 0.053 | 0.068 | 0.054 | 0.178 | 0.056 | 0.053 | 0.058 | 0.065 | 0.053 | 0.072 | <0.0001 |
| *ADIPOQ* | C-11377G | rs266729 | 0.251 | 0.245 | 0.239 | 0.254 | 0.238 | 0.252 | 0.300 | 0.279 | 0.257 | 0.250 | 0.255 | 0.60 |
| *ADIPOQ* | Gly15Gly (T/G) | rs2241766 | 0.290 | 0.285 | 0.304 | 0.285 | 0.309 | 0.301 | 0.316 | 0.295 | 0.287 | 0.284 | 0.251 | 0.18 |
| *ADIPOQ* | G276T | rs1501299 | 0.262 | 0.290 | 0.284 | 0.282 | 0.147 | 0.266 | 0.263 | 0.289 | 0.286 | 0.308 | 0.236 | <0.0001 |
| *ADIPOQ* | IVS-3971A/G | rs822396 | 0.061 | 0.053 | 0.049 | 0.069 | 0.057 | 0.079 | 0.050 | 0.047 | 0.073 | 0.057 | 0.056 | 0.063 |
| *ADIPOR1* | G-8503A | rs6666089 | 0.032 | 0.029 | 0.040 | 0.034 | 0.028 | 0.027 | 0.044 | 0.026 | 0.029 | 0.024 | 0.040 | 0.29 |
| *ADIPOR1* | C5843T | rs1342387 | 0.483 | 0.484 | 0.473 | 0.476 | 0.481 | 0.480 | 0.478 | 0.426 | 0.495 | 0.483 | 0.504 | 0.78 |
| *ADIPOR1* | C10224G | rs7539542 | 0.216 | 0.226 | 0.230 | 0.201 | 0.202 | 0.223 | 0.219 | 0.263 | 0.233 | 0.215 | 0.194 | 0.21 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *ADRB2* | Gln27Glu (C/G) | rs1042714 | 0.065 | 0.073 | 0.051 | 0.061 | 0.066 | 0.093 | 0.081 | 0.079 | 0.072 | 0.063 | 0.039 | 0.0001 |
| *ADRB3* | Trp64Arg (T/C) | rs4994 | 0.195 | 0.184 | 0.173 | 0.195 | 0.184 | 0.195 | 0.147 | 0.168 | 0.193 | 0.218 | 0.240 | 0.001 |
| *AGT* | Thr174Met (C/T) | rs4762 | 0.105 | 0.104 | 0.106 | 0.101 | 0.106 | 0.092 | 0.094 | 0.095 | 0.123 | 0.105 | 0.115 | 0.68 |
| *AGT* | Thr235Met (C/T) | rs699 | 0.187 | 0.181 | 0.183 | 0.208 | 0.189 | 0.195 | 0.219 | 0.211 | 0.173 | 0.178 | 0.170 | 0.31 |
| *AGTR1(ATR1)* | A1166C (at 3'UTR) | rs5186 | 0.087 | 0.089 | 0.093 | 0.083 | 0.088 | 0.076 | 0.081 | 0.089 | 0.080 | 0.082 | 0.104 | 0.61 |
| *ALDH2* | Glu487Lys (G/A) | rs671 | 0.254 | 0.232 | 0.274 | 0.316 | 0.298 | 0.254 | 0.226 | 0.300 | 0.269 | 0.267 | 0.112 | <0.0001 |
| *APOA1* | Ala61Thr (C/T) | rs12718465 | 0.055 | 0.051 | 0.049 | 0.056 | 0.061 | 0.055 | 0.088 | 0.016 | 0.046 | 0.043 | 0.079 | 0.0005 |
| *APOA1* | Arg184Pro (G/C) | rs5078 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| *APOA5* | T-1131C | rs662799 | 0.345 | 0.355 | 0.336 | 0.322 | 0.337 | 0.326 | 0.338 | 0.342 | 0.342 | 0.337 | 0.412 | 0.002 |
| *APOA5* | Gly185Cys (G553T) | rs2075291 | 0.069 | 0.084 | 0.062 | 0.057 | 0.073 | 0.068 | 0.063 | 0.074 | 0.064 | 0.079 | 0.065 | 0.36 |
| *APOE* | T-203G | rs405509 | 0.305 | 0.289 | 0.297 | 0.294 | 0.302 | 0.256 | 0.306 | 0.295 | 0.318 | 0.308 | 0.383 | <0.0001 |
| *APOE* | Arg158Cys (C/T) (at exon2) | rs7412 | 0.043 | 0.045 | 0.048 | 0.048 | 0.035 | 0.042 | 0.050 | 0.042 | 0.044 | 0.048 | 0.030 | 0.49 |
| *APOE* | Cys112Arg (T/C) (at exon4) | rs429358 | 0.101 | 0.105 | 0.105 | 0.101 | 0.123 | 0.101 | 0.078 | 0.068 | 0.109 | 0.102 | 0.075 | 0.026 |
| *ARNTL(BMAL1)* | A/G | rs7950226 | 0.404 | 0.424 | 0.405 | 0.420 | 0.395 | 0.402 | 0.391 | 0.347 | 0.427 | 0.431 | 0.344 | 0.002 |
| *ART4(DOK1)* | Leu208Leu (G/A) | rs3088189 | 0.107 | 0.114 | 0.114 | 0.130 | 0.084 | 0.113 | 0.078 | 0.068 | 0.105 | 0.108 | 0.109 | 0.022 |
| *ART4(DOK1)* | Asp265Asn (G/A) | rs11276 | 0.107 | 0.114 | 0.113 | 0.130 | 0.084 | 0.113 | 0.078 | 0.068 | 0.105 | 0.108 | 0.109 | 0.023 |
| *BHMT* | Arg239Gln (G742A) | rs3733890 | 0.221 | 0.235 | 0.236 | 0.241 | 0.229 | 0.203 | 0.209 | 0.226 | 0.243 | 0.217 | 0.164 | 0.0005 |
| *CBS* | Tyr233Tyr (C699T) | rs234706 | 0.024 | 0.027 | 0.033 | 0.026 | 0.020 | 0.021 | 0.028 | 0.011 | 0.034 | 0.017 | 0.017 | 0.073 |
| *CD14* | T-260C/T-159C | rs2569190 | 0.462 | 0.460 | 0.467 | 0.467 | 0.439 | 0.499 | 0.428 | 0.395 | 0.468 | 0.448 | 0.471 | 0.088 |
| *CDKAL1* | G/C | rs7754840 | 0.407 | 0.428 | 0.390 | 0.430 | 0.392 | 0.411 | 0.363 | 0.374 | 0.424 | 0.435 | 0.367 | 0.009 |
| *CDKN2A/B* | T/C | rs10811661 | 0.451 | 0.465 | 0.450 | 0.466 | 0.446 | 0.470 | 0.453 | 0.411 | 0.441 | 0.452 | 0.419 | 0.42 |
| *CETP* | A-629C | rs1800775 | 0.445 | 0.445 | 0.463 | 0.442 | 0.436 | 0.441 | 0.469 | 0.405 | 0.462 | 0.422 | 0.458 | 0.52 |
| *CETP* | Ile405Val (A/G) | rs5882 | 0.492 | 0.537 | 0.376 | 0.452 | 0.523 | 0.498 | 0.509 | 0.458 | 0.495 | 0.556 | 0.506 | <0.0001 |
| *CETP* | TaqIB (C/T) | rs708272 | 0.405 | 0.404 | 0.401 | 0.419 | 0.419 | 0.384 | 0.375 | 0.363 | 0.378 | 0.443 | 0.398 | 0.067 |
| *CETP* | G/T | rs3764261 | 0.233 | 0.183 | 0.340 | 0.295 | 0.203 | 0.188 | 0.209 | 0.158 | 0.204 | 0.223 | 0.240 | <0.0001 |
| *CHRNB2* | G-42A | rs2072658 | 0.204 | 0.236 | 0.214 | 0.185 | 0.215 | 0.222 | 0.203 | 0.253 | 0.202 | 0.203 | 0.140 | <0.0001 |
| *CHRNB2* | C/T (at 3'UTR) | rs2072660 | 0.243 | 0.258 | 0.225 | 0.264 | 0.224 | 0.241 | 0.216 | 0.237 | 0.214 | 0.233 | 0.292 | 0.001 |
| *COMT* | Val158Met (G/A) | rs4680 | 0.336 | 0.310 | 0.328 | 0.336 | 0.323 | 0.359 | 0.303 | 0.295 | 0.336 | 0.315 | 0.406 | <0.0001 |
| *CYP1A1* | Ile462Val (A/G) | rs1048943 | 0.239 | 0.222 | 0.235 | 0.256 | 0.222 | 0.244 | 0.250 | 0.268 | 0.222 | 0.225 | 0.274 | 0.069 |
| *CYP1A2* | A734C | rs762551 | 0.358 | 0.336 | 0.349 | 0.356 | 0.344 | 0.367 | 0.352 | 0.384 | 0.351 | 0.363 | 0.391 | 0.38 |
| *CYP11B2* | T-344C | rs1799998 | 0.331 | 0.334 | 0.318 | 0.339 | 0.289 | 0.294 | 0.346 | 0.284 | 0.329 | 0.325 | 0.434 | <0.0001 |
| *CYP17A1* | T-34C | rs743572 | 0.466 | 0.453 | 0.460 | 0.449 | 0.485 | 0.492 | 0.500 | 0.353 | 0.467 | 0.452 | 0.482 | 0.018 |
| *ESR1* | IVS1-351A/G (*Xba* I) | rs11155816 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| *ESR1* | IVS1-397T/C (*Pvu* II) | rs2234693 | 0.425 | 0.436 | 0.422 | 0.422 | 0.416 | 0.452 | 0.334 | 0.421 | 0.430 | 0.438 | 0.415 | 0.062 |
| *ESR2* | Val328Val (G1082A) (*Rsa* I) | rs1256049 | 0.284 | 0.268 | 0.281 | 0.297 | 0.272 | 0.308 | 0.272 | 0.284 | 0.314 | 0.293 | 0.250 | 0.054 |
| *FTO* | T/A | rs9939609 | 0.201 | 0.172 | 0.195 | 0.220 | 0.190 | 0.188 | 0.169 | 0.158 | 0.206 | 0.186 | 0.277 | <0.0001 |
| *GCK* | G-30A | rs1799884 | 0.189 | 0.193 | 0.179 | 0.183 | 0.186 | 0.182 | 0.138 | 0.147 | 0.304 | 0.173 | 0.159 | <0.0001 |
| *GCKR* | A/G | rs780094 | 0.449 | 0.428 | 0.436 | 0.440 | 0.439 | 0.425 | 0.350 | 0.453 | 0.450 | 0.445 | 0.562 | <0.0001 |
| *GCKR* | Leu446Pro (T/C) | rs1260326 | 0.445 | 0.430 | 0.434 | 0.439 | 0.438 | 0.416 | 0.353 | 0.421 | 0.447 | 0.432 | 0.559 | <0.0001 |
| *GNAS1* | T393C | rs7121 | 0.428 | 0.448 | 0.427 | 0.408 | 0.463 | 0.422 | 0.469 | 0.342 | 0.454 | 0.418 | 0.391 | 0.002 |
| *IGF2BP2* | G/T (at intron) | rs4402960 | 0.303 | 0.328 | 0.306 | 0.292 | 0.298 | 0.296 | 0.309 | 0.268 | 0.300 | 0.304 | 0.306 | 0.81 |
| *IL-1B* | T-31C | rs1143627 | 0.462 | 0.444 | 0.463 | 0.477 | 0.439 | 0.454 | 0.491 | 0.484 | 0.477 | 0.462 | 0.475 | 0.52 |
| *IL-2* | T-330G | rs2069762 | 0.329 | 0.330 | 0.336 | 0.316 | 0.311 | 0.323 | 0.309 | 0.305 | 0.350 | 0.321 | 0.365 | 0.21 |
| *IL-4* | T-33C | rs2070874 | 0.338 | 0.317 | 0.337 | 0.315 | 0.333 | 0.291 | 0.313 | 0.284 | 0.336 | 0.337 | 0.457 | <0.0001 |
| *IL-6* | C-634G | rs1800796 | 0.244 | 0.247 | 0.206 | 0.250 | 0.234 | 0.226 | 0.225 | 0.232 | 0.223 | 0.240 | 0.338 | <0.0001 |
| *IL-8* | T-251A | rs4073 | 0.320 | 0.334 | 0.321 | 0.318 | 0.320 | 0.304 | 0.331 | 0.266 | 0.339 | 0.329 | 0.306 | 0.56 |
| *IL-10* | T-819C | rs1800871 | 0.346 | 0.329 | 0.346 | 0.320 | 0.340 | 0.313 | 0.356 | 0.268 | 0.354 | 0.359 | 0.425 | <0.0001 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *IL-13* | C-1111T | rs1800925 | 0.183 | 0.182 | 0.178 | 0.197 | 0.185 | 0.178 | 0.213 | 0.158 | 0.172 | 0.190 | 0.176 | 0.73 |
| *KCNJ11* | Glu23Lys (C/T) | rs5219 | 0.366 | 0.386 | 0.364 | 0.382 | 0.343 | 0.374 | 0.363 | 0.316 | 0.361 | 0.366 | 0.368 | 0.52 |
| *LCAT/SLC12A4* | Ser232Thr (T/A) | rs4986970 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| *LIPC* | T-514C | rs1800588 | 0.490 | 0.522 | 0.478 | 0.496 | 0.484 | 0.497 | 0.522 | 0.484 | 0.502 | 0.503 | 0.429 | 0.006 |
| *LIPC* | Val95Met (G/A) | rs6078 | 0.234 | 0.232 | 0.229 | 0.221 | 0.269 | 0.259 | 0.247 | 0.237 | 0.208 | 0.253 | 0.182 | <0.0001 |
| *MPO* | G-463A | rs2333227 | 0.106 | 0.107 | 0.105 | 0.107 | 0.102 | 0.113 | 0.100 | 0.100 | 0.106 | 0.093 | 0.119 | 0.84 |
| *MTHFD1* | Arg134Lys (C401T) | rs1950902 | 0.218 | 0.214 | 0.216 | 0.218 | 0.212 | 0.229 | 0.184 | 0.195 | 0.217 | 0.239 | 0.215 | 0.66 |
| *MTHFD1* | Arg653Gln (G1958A) | rs2236225 | 0.283 | 0.271 | 0.273 | 0.269 | 0.265 | 0.280 | 0.284 | 0.279 | 0.278 | 0.301 | 0.331 | 0.036 |
| *MTHFR* | Ala222Val (C677T) | rs1801133 | 0.382 | 0.387 | 0.402 | 0.388 | 0.411 | 0.393 | 0.375 | 0.442 | 0.410 | 0.364 | 0.288 | <0.0001 |
| *MTHFR* | Glu429Ala (A1298C) | rs1801131 | 0.184 | 0.195 | 0.189 | 0.202 | 0.193 | 0.193 | 0.225 | 0.191 | 0.147 | 0.222 | 0.105 | <0.0001 |
| *MTR* | Asp919Gly (A/G) | rs1805087 | 0.201 | 0.188 | 0.210 | 0.186 | 0.183 | 0.179 | 0.184 | 0.232 | 0.193 | 0.176 | 0.299 | <0.0001 |
| *MTRR* | Ile22Met (A66G) | rs1801394 | 0.310 | 0.292 | 0.314 | 0.298 | 0.315 | 0.296 | 0.306 | 0.337 | 0.318 | 0.297 | 0.346 | 0.24 |
| *NOS3* | T-786C | rs2070744 | 0.106 | 0.111 | 0.125 | 0.117 | 0.112 | 0.098 | 0.119 | 0.122 | 0.109 | 0.102 | 0.068 | 0.004 |
| *PPARD* | T-842C (at exon3) | rs2267668 | 0.195 | 0.175 | 0.199 | 0.201 | 0.183 | 0.205 | 0.213 | 0.221 | 0.192 | 0.190 | 0.210 | 0.55 |
| *PPARD* | T-48444C (at exon3) | rs6902123 | 0.020 | 0.015 | 0.020 | 0.030 | 0.025 | 0.017 | 0.019 | 0.016 | 0.019 | 0.019 | 0.012 | 0.15 |
| *PPARD* | Asn163Asn (A65G) (at exon7) | rs2076167 | 0.215 | 0.190 | 0.212 | 0.231 | 0.206 | 0.221 | 0.225 | 0.234 | 0.209 | 0.205 | 0.236 | 0.30 |
| *PPARG* | Pro12Ala (C/G) | rs1801282 | 0.031 | 0.031 | 0.027 | 0.028 | 0.037 | 0.047 | 0.022 | 0.011 | 0.029 | 0.032 | 0.025 | 0.064 |
| *PPARG* | His477His (C161T) | rs3856806 | 0.152 | 0.153 | 0.159 | 0.143 | 0.149 | 0.167 | 0.159 | 0.147 | 0.177 | 0.166 | 0.099 | 0.0002 |
| *PPARGC1A* | Thr394Thr (C/T) | rs2970847 | 0.219 | 0.229 | 0.223 | 0.237 | 0.219 | 0.222 | 0.209 | 0.237 | 0.208 | 0.243 | 0.168 | 0.005 |
| *PPARGC1A* | Gly482Ser (G/A) | rs8192678 | 0.460 | 0.472 | 0.447 | 0.444 | 0.431 | 0.448 | 0.463 | 0.442 | 0.471 | 0.438 | 0.538 | <0.0001 |
| *PRKAA2* | IVS4+961T/C | rs1418442 | 0.231 | 0.216 | 0.228 | 0.232 | 0.206 | 0.217 | 0.184 | 0.237 | 0.205 | 0.237 | 0.318 | <0.0001 |
| *PRKAA2* | IVS7+81T/C | rs932447 | 0.231 | 0.216 | 0.228 | 0.232 | 0.207 | 0.218 | 0.184 | 0.237 | 0.205 | 0.239 | 0.318 | <0.0001 |
| *PRKAA2* | A/T (at 3'UTR) | rs1342382 | 0.221 | 0.238 | 0.220 | 0.200 | 0.250 | 0.225 | 0.204 | 0.211 | 0.233 | 0.197 | 0.212 | 0.072 |
| *PTGS2(COX2)* | G-765C | rs20417 | 0.029 | 0.028 | 0.033 | 0.031 | 0.033 | 0.027 | 0.025 | 0.053 | 0.026 | 0.030 | 0.017 | 0.22 |
| *PTGS2(COX2)* | C-163G | rs5270 | 0.016 | 0.017 | 0.023 | 0.017 | 0.020 | 0.015 | 0.009 | 0.011 | 0.009 | 0.011 | 0.016 | 0.32 |
| *PTPN11* | G33861A | rs2301756 | 0.195 | 0.194 | 0.199 | 0.172 | 0.167 | 0.185 | 0.184 | 0.205 | 0.173 | 0.159 | 0.320 | <0.0001 |
| *RETN* | C-420G | rs1862513 | 0.348 | 0.351 | 0.328 | 0.330 | 0.343 | 0.330 | 0.334 | 0.316 | 0.340 | 0.366 | 0.411 | 0.002 |
| *SCARB1* | Val135Ile (G/A) | rs5891 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| *SCARB1* | Ala350Ala (C1119T) | rs5888 | 0.228 | 0.240 | 0.212 | 0.226 | 0.228 | 0.225 | 0.253 | 0.205 | 0.240 | 0.240 | 0.213 | 0.59 |
| *SCARB1* | G/A (at intron) | rs3782287 | 0.234 | 0.225 | 0.223 | 0.243 | 0.227 | 0.249 | 0.297 | 0.189 | 0.207 | 0.219 | 0.263 | 0.008 |
| *SERPINC1* | C/G | rs677 | 0.219 | 0.220 | 0.197 | 0.209 | 0.238 | 0.223 | 0.238 | 0.211 | 0.220 | 0.227 | 0.217 | 0.58 |
| *SHMT1* | Leu435Phe (C1420T) | rs1979277 | 0.089 | 0.072 | 0.089 | 0.071 | 0.093 | 0.092 | 0.084 | 0.095 | 0.084 | 0.091 | 0.117 | 0.026 |
| *SLC19A(RFC1)* | His27Arg (A80G) | rs1051266 | 0.418 | 0.415 | 0.435 | 0.412 | 0.426 | 0.423 | 0.397 | 0.395 | 0.430 | 0.454 | 0.352 | 0.001 |
| *SLC30A8* | Arg325Trp (C/T) | rs13266634 | 0.437 | 0.439 | 0.449 | 0.443 | 0.438 | 0.418 | 0.472 | 0.453 | 0.434 | 0.451 | 0.411 | 0.53 |
| *SRD5A2* | Leu89Val (C/G) | rs523349 | 0.452 | 0.437 | 0.468 | 0.430 | 0.458 | 0.458 | 0.391 | 0.399 | 0.449 | 0.471 | 0.464 | 0.13 |
| *TAS2R38* | Pro49Ala (C/G) | rs713598 | 0.445 | 0.448 | 0.439 | 0.419 | 0.427 | 0.454 | 0.434 | 0.479 | 0.445 | 0.435 | 0.491 | 0.080 |
| *TAS2R38* | Ala262Val (C/T) | rs1726866 | 0.444 | 0.449 | 0.438 | 0.419 | 0.427 | 0.454 | 0.434 | 0.479 | 0.444 | 0.435 | 0.491 | 0.078 |
| *TAS2R38* | Val296Ile (C/T) | rs10246939 | 0.444 | 0.448 | 0.438 | 0.419 | 0.427 | 0.454 | 0.434 | 0.479 | 0.444 | 0.435 | 0.491 | 0.079 |
| *TCF7L2* | C/T (at intron) | rs7903146 | 0.039 | 0.051 | 0.039 | 0.042 | 0.043 | 0.037 | 0.034 | 0.042 | 0.025 | 0.034 | 0.043 | 0.27 |
| *TNF-A* | T-1031C | rs1799964 | 0.166 | 0.175 | 0.148 | 0.165 | 0.157 | 0.153 | 0.153 | 0.237 | 0.151 | 0.180 | 0.188 | 0.024 |
| *TNF-A* | C-857T | rs1799724 | 0.187 | 0.172 | 0.171 | 0.173 | 0.166 | 0.161 | 0.194 | 0.184 | 0.203 | 0.199 | 0.255 | <0.0001 |
| *USF1* | C7131T | rs3737787 | 0.215 | 0.229 | 0.223 | 0.220 | 0.234 | 0.217 | 0.263 | 0.216 | 0.196 | 0.204 | 0.178 | 0.020 |
| *VDR* | Met1Thr (*Fok* I) (C/T) | rs2228570 | 0.375 | 0.344 | 0.393 | 0.368 | 0.380 | 0.381 | 0.391 | 0.437 | 0.402 | 0.377 | 0.343 | 0.048 |

Abbreviations: ACC, Aichi Cancer Center; SNP, single nucleotide polymorphism.
[a]*P* for difference among study areas.

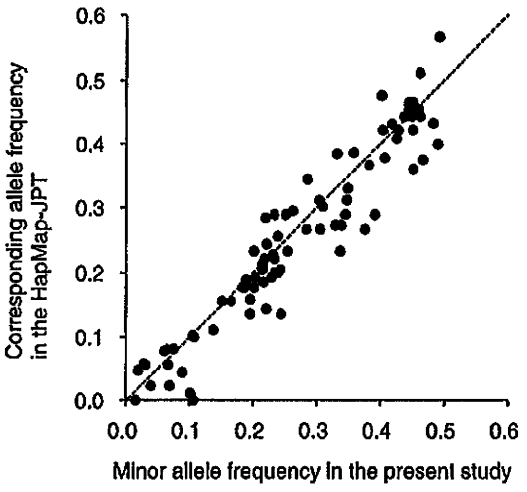


Figure. Scatter plot of allele frequencies for 88 polymorphisms in the data set from the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study and the HapMap-JPT data set registered at the US National Library of Medicine (http://www.ncbi.nlm.nih.gov/snp). Points on the identity (dotted) line represent allele frequencies that are identical in the 2 populations.

available.

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Conflicts of interest: None declared.

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# ESTABLISHMENT AND ANALYSIS OF *SLC22A12* (URAT1) KNOCKOUT MOUSE

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□ *In order to elucidate the mechanisms of post-exercise acute renal failure, one of the complications of hereditary renal hypouricemia, we have targeted the mouse* Slc22a12 *gene by the exchange of exons 1–4 with pMC1neo-polyA. The knockout mice revealed no gross anomalies. The concentration ratio of urinary urate/creatinine of the knockout mice was significantly higher than that of wildtype mice, indicating an attenuated renal reabsorption of urate. The plasma levels of urate were around 11 μM and were similar among the genotypes. Although the fractional excretion of urate of knockout mice was tend to higher than that of wildtype mice, the urate reabsorption ability remained in the kidney of knockout mice, indicating a urate reabsorptive transporter other than Urat1.*

**Keywords** Urat1; knockout mice; urate; allantoin

## INTRODUCTION

Post-exercise acute renal failure is a serious complication of hereditary renal hypouricemia 1 (OMIM: #220150), which is caused by mutation in *SLC22A12*, the gene encoding the renal urate transporter Urat.[1] In order to elucidate the pathological mechanisms and possible prevention of post-exercise acute renal failure, we have established *Slc22a12* (Urat1) knockout mice as an animal model of hereditary renal hypouricemia.

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## MATERIALS AND METHODS

### Generation of Urat1 Knockout Mice

A 13.6 kb DNA clone containing *Slc22a12* gene was isolated from a mouse 129/sv genomic DNA library (MoBioTec, Germany) using a digoxigenin-labeled probe prepared by PCR with sense primer (5′-ccctctctctcctgggttagctcacagtac-3′) and antisense primer (5′-tgtgatgagcctgctttccccttggctctg-3′). A replacement targeting vector was constructed by incorporating a 1.2 kb segment of inverted directed pMC1neo-polyA and a 1.9 kb segment of pSK-DTA (Figure 1A) was linearized by BlpI digestion and electroporated into embryonic stem (ES) cells obtained from mouse strain 129/sv. A homologous recombinant ES clone was selected by genomic Southern analysis using probe 1 prepared with sense primer (5′-gactgtctagaggcggccttaactag-3′) and antisense primer (5′-ctgctgagccgcaggagcccacagacgccgctgg-3′). Successfully transfected ES cells were injected into developing blastocysts from C57BL/6J mice to obtain

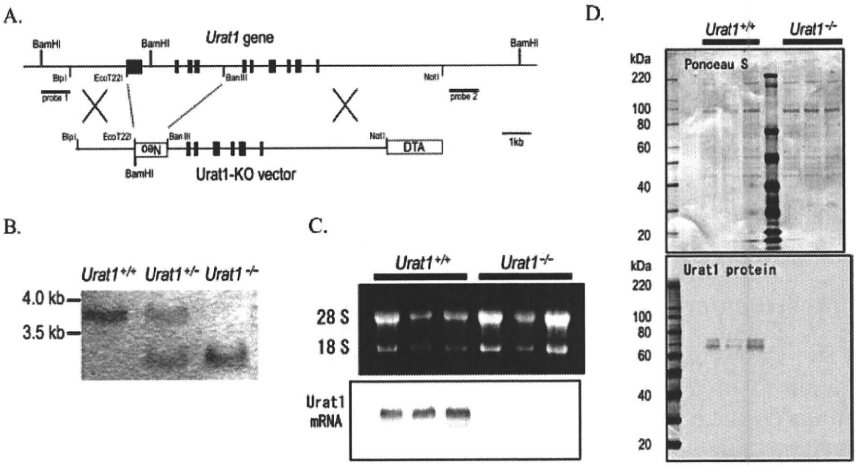


**FIGURE 1** Establishment of *Slc22a12* (Urat1) knockout mice. A) Schema of targeted disruption of *Slc22a12*. Homologous recombination was performed to replace a 3.4 kb segment including exons 1–4 of *Slc22a12* with Neo cassette resulting in formation of a null allele. The positions of probe 1 for genomic Southern analysis are indicated. B) Genomic Southern analysis for genotyping of a typical litter from a cross between mice heterozygous for the Urat1 null allele. BamHI-digested genomic DNA was analyzed with probe 1 as indicated in panel A. C) Northern analysis. Total RNA from the kidneys of 3 WT (WT 1–3) and 3 Urat1 KO (KO 1–3) mice was electrophoresed through a denaturing gel (upper panel) and then transferred to a nylon membrane and hybridized to a digoxigenin-labeled mouse Urat1 cDNA (lower panel). D) Western analysis. Membrane fractions of kidneys from 3 WT (WT 1–3) and 3 Urat1 KO (KO 1–3) mice were separated by SDS-PAGE and then transferred to a nitrocellulose membrane (upper panel, stained with Ponceau-S). Mouse Urat1 protein was detected with the anti-Urat1 antibody.

chimeric mice, which were bred with C57BL/6J mice to obtain mice heterozygous for Uratl deficiency. Gene targeting of Uratl-/- offspring obtained by mating of heterozygous mice was confirmed by genomic Southern analysis using probe 1.

### Northern Analysis

Total RNAs (2 $\mu$g each) prepared by ISOGEN kit (Nippongene, Japan) from the kidneys of three Uratl+/+ and three Uratl−/− mice were mixed with ethidium bromide and separated on a formaldehyde denaturing agarose gel for obtaining a picture. The separated total RNA was transferred on a nylon membrane (Hybond-$N^+$, GE, UK) by capillary blotting with 20 × SSC, hybridized at 55°C with a digoxigenin-labeled full length mouse Uratl cDNA probe, and washed in 0.1 × SSC at 65°C for the detection of mouse Uratl mRNA.[2]

### Western Analysis

Membrane proteins (2 $\mu$g) of the mouse kidney prepared as described before[2] were separated by SDS-PAGE under non-reducing conditions with NuPAGE Bis-Tris 4–12% gel (Invitrogen, Tokyo, Japan) and transferred to a nitrocellulose membrane using iBlot module (Invitrogen). Mouse Uratl protein was detected using the ECL kit (GE) with a novel affinity-purified anti-mouse Uratl antibody that was obtained from the serum of a rabbit immunized by KLH-conjugated mouse Uratl C-terminal peptide, CHDT-PDGSILMSTRL (Sigma Genosys, Japan). The nitrocellulose membrane was stained with Ponceau S to check for blotting failure.

### Measurement of Allantoin, Urate, and Creatinine by HPLC

The Uratl mutation was backcrossed onto the C57BL/6J strain for four generations to produce Uratl−/− offspring with a similar genetic background. The 24-hour urine samples of seven 13-week old wildtype mice (WT) and eight 13-week old knockout mice (KO) were collected using metabolic cages (Tecniplast, Italy). The blood of them were collected with heparin from the abdominal vein of the mice anesthetized with ether and 50 mg/kg pentobarbital i.p. 40 $\mu$l of acetonitrile (ACN) and 10 $\mu$l of plasma sample or 100 times diluted urine sample were delivered into Ultrafree-MC 0.22 $\mu$m PVDF membrane filter unit (Millipore, USA) to remove proteins by centrifugation. The ACN and any aqueous material were evaporated to dryness in a centrifuge evaporator (Ikemoto-Rika, Japan). The residue containing the analyte was resuspended in 10 $\mu$l of HPLC mobile phase (20 mM ammonium formate). Separation was achieved at a flow rate of 0.200 mL/min on a 250

mm × 2 mm, 3 μm particle size ODS column, Unison UK-C18, (Imtakt, Japan) at 25°C (Hitachi LaChrom Elite PDA system).

## RESULTS

### Confirmation of Targeting of *Slc22a12* (Urat1)

Homologous recombination was used to replace a segment of exons 1–4 of *Slc22a12* with pMC1neo-polyA, effectively generating a null allele (Figure 1A). Mice heterozygous for this allele were bred to yield knockout type offspring, which were typed by genomic Southern analysis of genomic DNA using probe 1 (Figure 1B). The 3.7 kb of wild allele and 3.0 kb of targeted allele were detected and coincided with the predicted fragment sizes of 3,738 bp of wildtype allele and 2,912 bp of targeted allele from the gene database (Genbank #AC124394). Thus, the *Slc22a12* gene was targeted successfully. The Urat1−/− mice revealed no gross anomalies and grew and bred normally.

The absence of Urat1 mRNA was demonstrated in the total RNA of knockout mice by Northern analysis (Figure 1C, lower panel). The possibility of degradation of total RNA in the knockout mouse sample was eliminated by demonstrating that the ribosomal RNAs were intact (Figure 1C, upper panel), therefore, the loss of expression of the Urat1 gene in the kidney of knockout mice was confirmed. Moreover, the absence of Urat1 protein was demonstrated in the membrane fraction of the kidney of knockout mice by Western blotting using a novel anti-mouse Urat1 antibody (Figure 1D, lower panel). The possibility of incomplete transfer of knockout mouse samples was also eliminated by the staining of the intact protein on the membrane (Figure 1D, upper panel), therefore, the novel anti-mouse Urat1 antibody recognizes mouse Urat1 molecule specifically.

### Urate Excretion in *Slc22a12* (Urat1) Knockout Mice

For the detection of allantoin, urate and creatinine, a novel HPLC method was developed for the simultaneous detection of the three analytes and reduction of organic solvent use. Detection of allantoin, urate, and creatinine peaks were achieved at 235 nm at 3.40 ± 0.02, 5.40 ± 0.02, and 6.10 ± 0.02 minutes, respectively. The purity of the peaks was confirmed by a PDA spectrum (data not shown). The coefficient values (CV) of this method were 0.4~4.2% for urate (0.05~10 mg/dL), 0.4~6.4% for creatinine (0.01~10 mg/dL), and 0.1~3.7% for allantoin (0.5~50 mg/dL). The linearity of standard curves were also confirmed ($r^2 = 1.00$) at the same range of concentrations (Figure 2A).

There was a significant increase in the amount of urinary urate excretion (255.7 ± 4.3 mmol/mol Cr in WT and 355.6 ± 35.8 mmol/mol Cr in

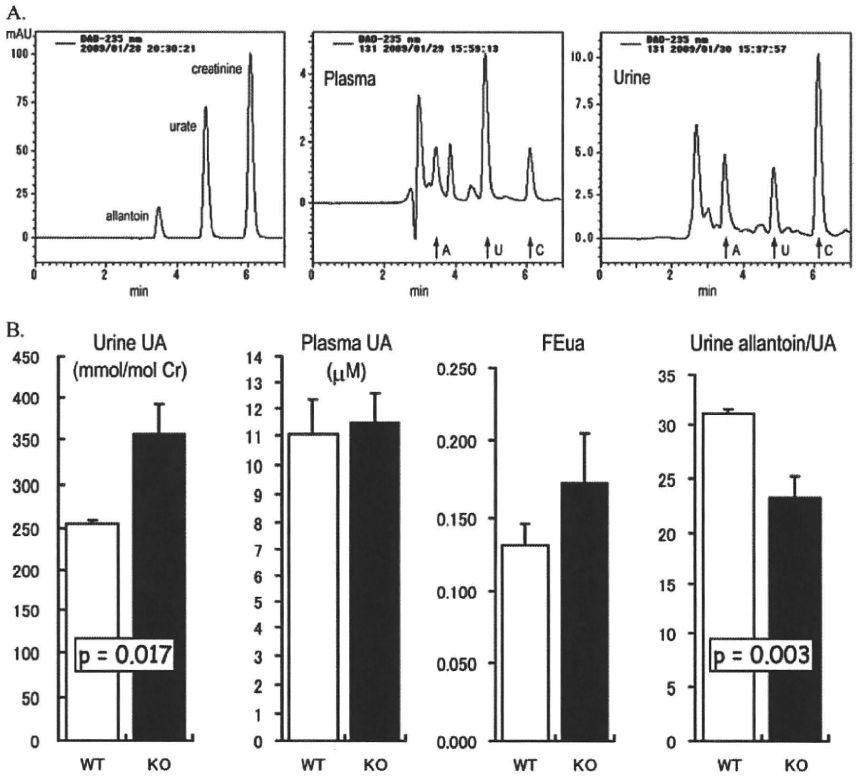


**FIGURE 2** Renal urate handling of *Slc22a12* (Urat1) knockout mice. A) Typical chromatograms of standard (left), plasma (middle), and diluted urine (right) samples. The peaks of allantoin (A), urate (U), and creatinine (C) are indicated in the chromatograms. B) Renal urate handling of WT (open column, n = 7) and KO mice (closed column, n = 8). Urinary concentrations of urate are presented normalized to that of creatinine. Plasma urate concentrations were measured from blood samples collected with heparin under anesthesia. The fractional excretion of urate (FEua) was calculated as the ratio of urine urate normalized to urine creatinine to plasma urate normalized to plasma creatinine. The urinary allantoin-to-urate ratios were calculated. Data represent mean ± SE.

KO, p = 0.017 by Student's *t* test) and a significant decrease in the urinary allantoin/urate ratio in knockout mice (31.0 ± 0.5 in WT and 23.2 ± 2.0 in KO, p = 0.003 by Student's *t* test), indicating an attenuated renal reabsorption of urate and a decreased allantoin production from reabsorbed urate. However, plasma levels of urate were around 11 μM and were similar among the genotypes (11.1 ± 1.3 μM in WT and 11.5 ± 1.1 μM in KO, p = 0.815 by Student's *t* test). It was also failed to demonstrate the difference of FEua among the genotypes (0.13 ± 0.01 in WT and 0.17 ± 0.03 in KO, p = 0.273 by Student's *t* test; Figure 2B).

## DISCUSSION

Eraly et. al. already reported on another RST (Urat1) knockout mouse, in which the third exon of the RST gene (*Slc22a12*) was replaced by a $\beta$-galactosidase transgene flanked by upstream splice acceptor sites and downstream transcriptional as well as translational stop signals.[3] Both our knockout mice and Eraly's mice were demonstrated by Northern analysis to be defective in the transcription of *Slc22a12*, and were successfully produced as *Slc22a12* knockout mice. Both our knockout mice and Eraly's RST-null mice appeared grossly normal.

Our novel anti-Urat1 antibody was verified to recognize the Urat1 molecule in the kidney specifically. Although we have tried to produce anti-Urat1 antibody with three antigen peptides (two N-terminal peptides and one C-terminal peptide) using two rabbits for each antigen, only one antibody recognized the Urat1 molecule specifically. Thus, knockout mice seem to be useful for verification of peptide antibodies.

Although the defect of URAT1 causes renal hypouricemia in humans, both Eraly's and our study revealed that there was no difference in plasma urate levels between WT and KO mice. Urate oxidase in mouse liver degrades reabsorbed urate from the kidney and this action likely extinguishes the difference in plasma urate levels between the WT and KO mice. The significant elevation of the daily urate excretion in KO mice was not accompanied by a change in the excretion of purine metabolites; urinary excretions of urate and allantoin were $1.745 \pm 0.155$ $\mu$mol/g/day in WT and $1.661 \pm 0.156$ $\mu$mol/g/day in KO. Therefore, the significant elevation of the daily urate excretion in KO mice was not due to an increase of purine metabolism but to a decrease of urate reabsorption in the kidney. Although a decrease of urate degradation in the liver also induces the elevation of urinary urate excretion, renal specific transporter Urat1 seems to have no effect on liver function.

The plasma urate level of wildtype mice in Eraly's paper was about 0.11 $\mu$M, two orders of magnitude lower than the urate level of our wildtype mice, 11.1 $\mu$M, which was almost the same as that reported by Dan et. al.[4] If the plasma urate level in Eraly's paper is a misprint of 0.11 mM (110 $\mu$M), it may be the same mistake that urate levels in mouse blood have been reported incorrectly one order of magnitude higher than our and Dan's data. Dan also described that xanthine oxidase activity in the rodent serum caused the urate level to increase up to 10 times of the real value in vitro after collection of blood sample.[4]

Both Eraly's and our study revealed that FEua of Urat1 (RST) knockout mice was below 1.0; therefore, an urate reabsorptive transporter (or transporters) other than Urat1 must be expressed in the kidney. (This is coincident with the FEua of the renal hypouricemia patients with a homozygous URAT1 defect, which was below 1.0.[5]) The urate reabsorptive transporter Glut 9[6,7] appears a likely candidate for such a role.

In conclusion, we succeeded in targeting the Urat1 gene in mice. After back-crossing, we will start to study the mechanism and prevention of post-exercise acute renal failure.

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原著1

# 尿酸トランスポーターURAT1トランスジェニックマウスにおける尿酸の体内動態

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尿酸はヒトにおけるプリン体の最終代謝産物であり，主に腎臓から排泄される．腎臓での尿酸の排泄亢進や排泄低下によって腎性低尿酸血症や高尿酸血症が引き起こされるため，腎臓での尿酸輸送を理解することは臨床的に重要である．尿酸トランスポーターURAT1は，腎臓近位尿細管管腔側において尿酸再吸収を行う分子である．このURAT1は尿酸降下薬の作用点であり，また遺伝子変異により腎性低尿酸血症をきたす．よってURAT1は血中尿酸値に大きく影響を及ぼす因子であると考えられるが，その発現が尿酸値や他の尿酸トランスポーターにどのような影響を及ぼすのか明らかになっていない．そこで，URAT1トランスジェニック（Tg）マウスを用いて，尿酸値測定およびマイクロアレイと定量PCRによる遺伝子発現変化の解析を行った．

RT-PCRの結果から，*Urat1* mRNAが腎臓特異的に発現しており，Tgマウスでは野生型と比べて発現量の上昇が見られた．ウエスタンブロットおよび免疫組織染色により，導入したHA-mURAT1タンパク質が腎臓の近位尿細管管腔側に発現していることを確認した．以上の結果から，導入したHA-mURAT1がすでに報告されているmURAT1と同様の発現分布を示し，URAT1が過剰発現していることを確認できた．そこで，このマウスをモデルとして解析を行った．血中および尿中尿酸値を測定した結果，どちらの尿酸値にも変化が見られなかった．マイクロアレイおよび定量PCRにて検討した結果，*Urat1*遺伝子はTgマウスにおいて発現が有意に増加していたが，他の尿酸トランスポーターや尿酸代謝酵素の遺伝子の発現量に変化は見られなかった．以上の結果より，URAT1過剰発現は，マウス生体内での尿酸動態および他の尿酸トランスポーターや尿酸代謝酵素の遺伝子発現に大きな影響を及ぼさないことが示された．

## 緒　言

尿酸はヒトにおけるプリン体の最終代謝産物である．体内で合成された内因性プリン体や食事から得られる外来性プリン体は，ヒポキサンチン，キサンチンを経て尿酸に代謝される．多くの哺乳類では，尿酸はウリカーゼにより水溶性が高いアラントインへ代謝されるが，ヒトを含む霊長類ではウリカーゼを遺伝的に欠損しているため，尿酸がプリン体の最終代謝産物となる[1]．従って，ヒトの血中尿酸値は他の哺乳類に比べて高値を示すことが知られている．

尿酸は主に肝臓で合成され，その約70％が腎臓，残りの約30%が小腸から排泄される．腎臓において糸球体濾過された尿酸は，尿細管において再吸収と分泌が行われ，最終的に糸球体ろ過量の約10％が尿中に排泄される．血中尿酸値は生体内における尿酸の産生と排泄のバランスによって決定されるが，痛風患者の半数以上が尿酸排泄の低下が原因であり，尿酸産生亢進との混合型を合わせると85％以上の患者に尿酸排泄の低下が見られる[2]．血液中の尿酸値は通常2.8-

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4.1 mg/dlの範囲に保たれており，主に腎臓における尿酸輸送が血中尿酸値を調節していると考えられている[3]．従って，主要排泄器官である腎臓での尿酸輸送を理解することは重要である．

URAT1は，*SLC22A12*遺伝子によりコードされるOATファミリーに属するトランスポーターであり，主に腎近位尿細管の管腔側膜に発現する．URAT1は細胞内の乳酸やニコチン酸，pyrazine carboxylic acid（PZA）などの有機酸との交換により尿酸を尿細管から再吸収する[4]．尿酸排泄促進薬（痛風治療薬）であるベンズブロマロンやプロベネシドは，URAT1の阻害が主な作用機序と考えられている[5]．また，抗結核薬であるピラジナミドは，URAT1がピラジナミド活性代謝物であるPZAと尿酸とを交換輸送することにより，尿酸の再吸収を促進する[5,6]．さらに，*URAT1*遺伝子変異により尿酸再吸収が阻害されると，腎性低尿酸血症が引き起こされることが知られている[4]．従って，URAT1は血中尿酸値に大きく影響を及ぼすトランスポーターとして重要であり，腎臓における尿酸輸送を理解する上で不可欠な因子である．

これまでin vitroで尿酸輸送能を持つトランスポーターは数々報告されている[7]が，*in vivo*でその輸送が証明された，つまり遺伝子変異によって病態を引き起こすことが示された尿酸トランスポーターは，URAT1[4]，URATv1[8]，ABCG2[9]の3つである．URAT1は尿酸再吸収における管腔側の尿酸の取り込みにおいて重要であるが，実際にURAT1が血中尿酸値にどれほど影響を及ぼすのかは未だ明らかになっていない．そこで本研究では，生体内での尿酸輸送機構を明らかにすることを目的として，URAT1の過剰発現マウスを作出し，尿酸輸送におけるURAT1の役割解明を行った．

## 方　法

### 1．HA-mURAT1 トランスジェニック（Tg）マウスの作製

Tgマウス作製にあたり，導入したURAT1を検出するため，hemagglutinin（HA）エピトープタグを挿入することにした．HA-mURAT1 Tgマウスは，フェニックスバイオ（株）に作出を依頼した．マウス*Urat1*遺伝子をコードするゲノムクローンのコード配列の開始コドン直後にHAエピトープタグ配列を挿入した組換えBACクローンを構築した．この組換えゲノムクローンをJcl:BDF1マウスの受精卵に導入してTgマウスを作出することにより，マウス個体本来の発現制御下でタグ付きmURAT1を発現する動物モデルを得た[10]．本研究の動物実験は，杏林大学における動物実験規定に則って行った．

### 2．RT（reverse-transcription）- PCR

約12週令のマウスからエーテル麻酔下で腎臓を摘出し，腎臓の皮質部位を採取した．フェノール・クロロホルム抽出により，total RNAを得た．逆転写反応およびPCR反応は，それぞれSuper Script III First-Strand Synthesis System，Go Taq Polymerase（Invitrogen）を用い，そのプロトコールに従って行った．

### 3．腎皮質タンパク質の調製

TS buffer（5mM Tris-HCl，pH7.4，250mM sucrose）にプロテアーゼインヒビター（1 μg/ml leupeptin，1 μ pepstatinA，1 μg/ml aprotinin，10 μ/ml PMSF）を加え，採取した腎臓皮質をホモジナイズした．4℃，800×gで10分間遠心を行い，上清をさらに4℃，100,000×gで90分間，遠心した．沈殿物にTS bufferを加え，ホモジナイザーにて懸濁した．

### 4．血漿・尿中の生化学的検査

エーテル麻酔下でマウスを開胸し，ヘパリン処理を施したシリンジを用いて心臓から血液を採取した．800×gで10分間遠心を行い，上清の血漿を得た．また膀胱穿刺により，尿を採取した．生化学測定はオリエンタル酵母株式会社，長浜LSLに委託した．

### 5．DNAマイクロアレイ解析

CodeLink Gene Expression Sytem（Applied

Microarrays社）の製品説明書に従って行った．bioarray chamber はarray WoRxe（GE Healthcare製）を用いて走査し，CodeLink Expression Analysis Version 4.2ソフトウエアを用いてmRNA発現量解析を行った．Student's t-testを用い，$p<0.05$を統計的に有意と判定した．Fold changeは，野生型（WT）マウスでのある遺伝子のmRNA発現量を1とした時，Tgマウスの同じ遺伝子のmRNA発現の変化量で示した（n=3）．

6．定量PCR解析

マウス腎臓皮質からtotal RNAを調製し，逆転写反応によってcDNAを調製した．定量PCR反応は，THUNDERBIRD SYBR qPCR Mix（TOYOBO）を用いた．測定はABI PRISM 7700 Sequence Detector（ABI社，ソフトウエア：Sequence Detector Software 1.91allias）で行った．発現量解析はΔΔ Ct法により行った[11]．

## 結　果

1．RT-PCR法による*mUrat1* mRNA発現の確認

マウス*Urat1*（*mUrat1*）のmRNA発現をRT-PCR法にて検討を行った（Fig. 1 A）．ポジティブコントロールとして，RT反応液の代わりに*mUrat1* cDNAを，ネガティブコントロールとして，逆転写反応を行わなかったものをそれぞれ用いた．その結果，WTマウス，Tgマウスともに，腎臓において*mUrat1* mRNAの発現が確認できた．肝臓においては*mUrat1*のバンドは確認できなかった．脳，小腸においても同様にRT-PCRを行ったが，*mUrat1* mRNAの発現は見られなかった（データ示さず）．以上の結果から，*mUrat1* mRNAは腎臓特異的に発現しており，また過剰発現させても腎臓以外に発現することはないことが示された．腎臓において，*mUrat1* mRNAの発現がWTマウスに比べてTgマウスで増加していた．また，雄性マウスの方が雌性マウスに比べて*mUrat1* mRNAの発現増加が顕著であった．

2．導入したHA-URAT1タンパク質の腎臓における発現

抗HA抗体によるウエスタンブロットを用いて，導入したHA-mURAT1のタンパク質発現を確認した（Fig. 1 B）．mURAT1は糖タンパク質であるため，コアとなるタンパク質が合成された後，小胞体でハイマンノース型，ゴルジ体でコンプレックス型の糖鎖修飾を受け原形質膜に到達する．Tgマウスではハイマンノース型およびコンプレックス型糖鎖修飾を受けたHA-mURAT1のタンパク質発現が確認できた．また，抗HA抗体を用いた免疫組織染色の結果から，導入したHA-mURAT1が腎近位尿細管の管腔側に発現していることを確認した（データ示さず）．

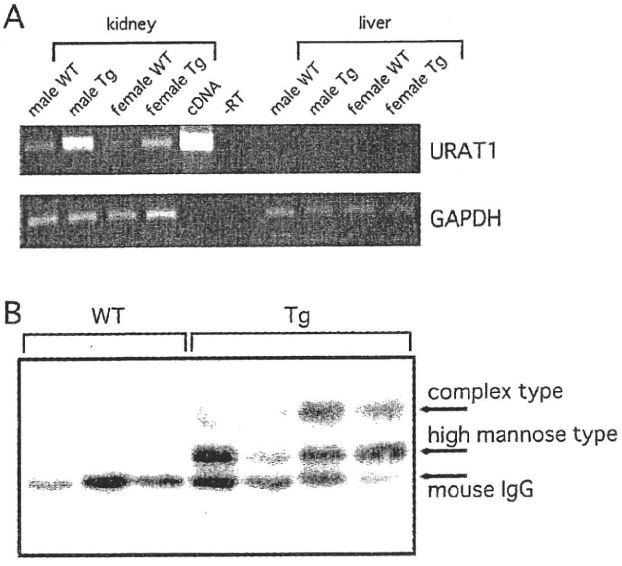


**Fig1.**

Expression of URAT1 mRNA and protein A. mURAT1 mRNA expression in the kidney and the liver was examined by RT-PCR. 1mg total RNA was reverse-transcribed and amplified with PCR. B. HA-URAT1 protein expression was detected by Western blotting. 50 mg protein was loaded on SDS-PAGE and HA-URAT1 was detected with anti-HA antibody. HA-URAT1 modified with high-mannose and complex type carbohydrate chains was expressed in Tg mice.

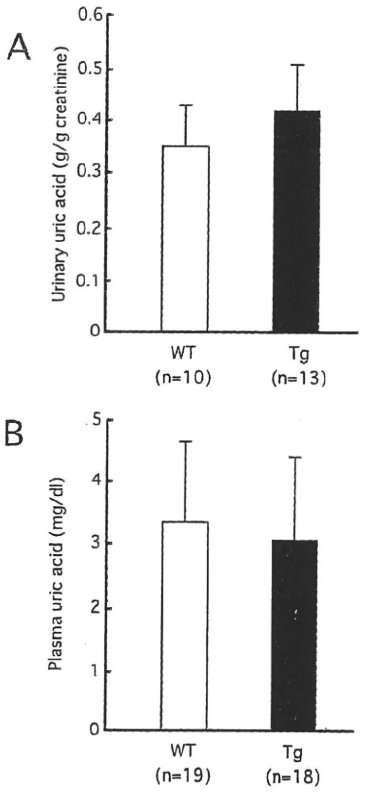


**Fig2.**
Urate concentration in plasma and urine Plasma (A) and Urine (B) urate concentration was measured by enzyme method.

**Table 1. mRNA expression of urate transporters in the kidney cortex of mURAT1 Tg mice.** mRNA levels of urate transporters were analysed by quantitative PCR. The levels of mURAT1 Tg mice were expressed as relative ratio to WT mice (n=3).

| Urate transporters | Relative ratio to WT | p-value |
|---|---|---|
| Urat1 | 5.01 | < 0.001 |
| Uratv1 (Glut9) short | 1.07 | 1.01 |
| Uratv1 (Glut9) long | 1.09 | 0.75 |
| ABCG2 | 0.68 | 1.35 |
| Npt1 | 0.98 | 0.76 |
| Npt4 | 1.21 | 1.01 |
| Mrp4 | 0.65 | 0.59 |
| Oat1 | 0.71 | 0.59 |
| Oat3 | 1.71 | 0.84 |

**Table 2. mRNA expression levels of urate metabolic enzymes in the kidney cortex (A) and in the liver (B) of mURAT1 Tg mice.** mRNA expression levels of enzymes involved in urate metabolism were compared by DNA microarray analysis. Expression levels of these enzymes in Tg mice were presented as fold change relative to those of WT mice (n=3).

| A. Enzymes involved in urate metabolism (kidney) | Fold change | p-value |
|---|---|---|
| Urat1 | 3.64 | 0.020 |
| Xanthine dehydrogenase | 0.772 | 0.416 |
| Hypoxanthine guanine phosphoribosyl transferase1 | 0.921 | 0.709 |
| Adenosine deaminase | 0.861 | 0.203 |
| Purine-nucleoside phosphorylase | 0.52 | 0.051 |
| Urate oxidase | 1.14 | 0.627 |
| phosphoribosyl pyrophosphate synthetase I | 0.909 | 0.582 |
| phosphoribosyl pyrophosphate amidotransferase | 0.960 | 0.785 |

| B. Enzymes involved in urate metabolism (liver) | Fold change | p-value |
|---|---|---|
| Xanthine dehydrogenase | 0.951 | 0.713 |
| Hypoxanthine guanine phosphoribosyl transferase1 | 0.781 | 0.519 |
| Adenosine deaminase | 0.882 | 0.812 |
| Purine-nucleoside phosphorylase | 0.960 | 0.832 |
| Urate oxidase | 1.27 | 0.267 |
| phosphoribosyl pyrophosphate synthetase 1 | 0.992 | 0.980 |
| phosphoribosyl pyrophosphate amidotransferase | 1.26 | 0.38 |

### 3. 血漿中および尿中の尿酸値測定

mURAT1を過剰発現させたことによる尿酸値の変動を検討するために，血漿中および尿中の尿酸値の測定を行った（Fig. 2）．尿酸値の測定は，URAT1の発現が顕著である雄性マウスを用いた．WTマウスおよびTgマウスの血漿尿酸値を測定した結果，WTマウス（3.33±1.34mg／dl，n＝19）とTgマウス（3.05±1.36mg／dl，n＝18）の間には有意な差は認められなかった．また，それぞれの尿中尿酸値を測定した結果，WTマウス（0.35±0.08g／g cre，n＝10）とTgマウス（0.42±0.09g／g cre，n＝13）の間には有意な差は認められなかった．以上の結果から，URAT1を過剰発現させたにもかかわらず，WTマウスとTgマウスの血中尿酸値および尿中尿酸排泄量に変化は見られなかった．

### 4. 腎臓における尿酸トランスポーターの発現変動

URAT1を過剰発現させても，血中・尿中尿酸値に変化が見られなかった．１つの理由として，他の尿酸トランスポーターが代償的にその発現変化を起こしていると考え，定量PCRを用いて尿酸を運ぶとされているトランスポーターの

mRNAの定量を行った（Table 1）．それぞれの尿酸トランスポーターのマウス腎臓皮質におけるmRNA発現量は，ΔΔCt法を用いて相対発現量変化として解析した[11]．*mUrat1*遺伝子は，Tgマウスにおいて有意に発現増加が認められ，導入したHA-*mUrat1*が腎臓に過剰発現していることが確認された．しかしながら，他の尿酸トランスポーター遺伝子に関しては，その発現量に有意な変化は見られなかった．

5．尿酸代謝酵素遺伝子の発現変動（Table 2）

血中・尿中尿酸値に変化が見られなかった他の理由として，尿酸代謝酵素の発現変化を考え，DNAマイクロアレイを用いて尿酸代謝酵素mRNAの発現量を比較した．総遺伝子数36227に関して腎臓皮質におけるmRNAの発現解析を行った．その結果，Tgマウスの方がWTマウスに比べて2倍以上発現量が増加していたものが55遺伝子，1/2以下に減少していたものが38遺伝子存在した．DNAマイクロアレイにおいても，*mUrat1* mRNAの発現量増加が確認できた．しかしながら，尿酸代謝酵素や他の尿酸トランスポーターに関しては，有意な発現量の変化は見られなかった．同様に，尿酸の主要代謝器官である肝臓についてもDNAマイクロアレイ解析を行ったが，尿酸代謝酵素の発現に有意な変化は見られなかった．

## 考　案

本研究では，腎臓における尿酸輸送機構を解明することを目的として，URAT1過剰発現マウスを作出し，その解析を行った．

食物から摂取した尿酸や生体内で合成された尿酸は糸球体濾過を受けた後，腎近位尿細管において再吸収，分泌，分泌後再吸収を受け，最終的に約10%のみが排泄される．この過程の再吸収を担っている分子の1つがURAT1であり，その遺伝的機能欠損によって尿酸再吸収が阻害され，腎性低尿酸血症を引き起こす[4]．また，尿酸降下薬として用いられているベンズブロマロンやプロベネシドがこのURAT1を標的としていることからも，URAT1が血中尿酸値の調節に重要な役割を果たしていると考えられる[5]．そこで我々は，URAT1過剰発現マウスを作出し，その血中・尿中尿酸値に対する寄与に関して検討を行った．URAT1が尿酸再吸収を行うトランスポーターのため，再吸収が亢進し血中尿酸値が上昇すると予測したが，血中尿酸値に変化が見られなかった．マウスにはヒトと異なり，尿酸をアラントインに代謝する酵素ウリカーゼが発現しているため，血中尿酸値の変化が見られない可能性がある[12]．そこで，尿中尿酸値の測定も行った．実際，URAT1，OAT1，OAT3のノックアウトマウスでは，血中尿酸値に変化が見られないものの，尿中尿酸排泄量に変化が見られている[13]．しかしながら，URAT1 Tgマウスでは，尿中尿酸値にも変化が見られなかった．

そこで，URAT1を過剰発現させたことによって代償的に他の尿酸トランスポーターや尿酸代謝酵素が発現変化を起こし，尿酸値に変化が見られなかった可能性を考え，それらの遺伝子発現をDNAマイクロアレイおよび定量PCRで解析した．しかしながら，既知の尿酸トランスポーターや尿酸代謝酵素のmRNAの発現に変化は見られなかった．このように，今回の研究ではmRNAレベルでの発現の定量・比較検討のみを行ったが，mRNAの発現レベルはタンパク質のそれと比例しないこともある．また我々の研究グループは，URAT1がスカフォールドタンパクPDZK1と結合することによって細胞膜上で安定化され，尿酸輸送活性が上昇することを示した[14]．こういった，PDZK1や未知の結合タンパク質との相互作用によって過剰発現させたURAT1または他の尿酸トランスポーターが機能調節されている可能性もある．さらに，他のトランスポーターの様にリン酸化やニトロソ化などの翻訳後修飾によって機能調節されている可能性もある．従って，今後はタンパク質レベルでの発現・機能調節について検討することが必要であろう．

2008年に我々は，グルコーストランスポーターGLUT9（SLC2A9）が腎臓近位尿細管の血管側に存在する電位依存性尿酸トランスポーター