**Supplement table 1: Mixed effect model for drinking-discordant MZ twins**

|  |  |  |
| --- | --- | --- |
| Average daily alcohol consumption（g/d） | Model1 | Model2 |
| **≥5** | ≥10 | ≥20 | ≥30 | **≥40** | ≥5 | ≥10 | ≥20 | ≥30 | ≥40 |
| MZ pairs | **57** | 43 | 26 | 22 | **17** | 57 | 43 | 26 | 22 | 17 |
| Most significant *P* value | **5.56E-08** | 6.30E-07 | 1.32E-04 | 1.37E-05 | **6.04E-08** | 4.22E-07 | 1.57E-06 | 5.30E-04 | 4.93E-05 | 3.41E-06 |
| Most significant FDR | **0.017** | 0.279 | 0.995 | 0.999 | **0.027** | 0.112  | 0.693  | 0.999  | 1.000  | 1.000  |
| Significant sites | **3** | 0 | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 0 |

MZ, monozygotic twins; FDR, false discovery rate.

Model 1: mixed effect model with DNA methylation level as dependent variable, and average daily alcohol consumption as independent variable as fixed effect. Twin identity number was entered as random effect. Age, gender, BMI and SVA were adjusted as covariates in the model as fixed effects. Model 2: additional adjusted with smoking based on model 1.

**Supplement table 2: Empirical Bayes paired moderated t-test for drinking-discordant MZ twins**

|  |  |  |
| --- | --- | --- |
| Average daily alcohol consumption（g/d） | Model1 | Model2 |
| ≥5 | ≥10 | ≥20 | ≥30 | ≥40 | ≥5 | ≥10 | ≥20 | ≥30 | ≥40 |
| MZ pairs | 57 | 43 | 26 | 22 | 17 | 57 | 43 | 26 | 22 | 17 |
| Most significant *P* value | 2.75E-06 | 4.13E-07 | 4.76E-07 | 5.31E-07 | 8.54E-07 | 3.31E-06 | 6.24E-07 | 9.68E-07 | 4.36E-06 | 8.47E-06 |
| Most significant FDR | 0.868  | 0.183  | 0.211  | 0.235  | 0.379  | 0.808  | 0.276  | 0.429  | 1.000  | 1.000  |
| Significant sites | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

MZ, monozygotic twins; FDR, false discovery rate.

Model 1: mixed effect model with DNA methylation level as dependent variable, and average daily alcohol consumption as independent variable as fixed effect. Twin identity number was entered as random effect. BMI and SVA were adjusted as covariates in the model as fixed effects. Model 2: additional adjusted with smoking based on model 1.

**Supplement table 3: Association results from sensitivity analyses in the validation stage with both twins having methylation data at the same wave in SATSA samples**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Model 1**  |  | **Model 2**  |
|  | $$β$$ | **95% CI** | ***P* value** |  | $$β$$ | **95% CI** | ***P* value** |
| ***Overall samples*** |
| cg21716444 | -3.72E-06 | -1.09E-05,3.47E-06 | 0.310 |  | -4.32E-06 | -1.15E-05,2.89E-06 | 0.240 |
| cg07326074 | 1.64E-05 | -1.00E-05,4.27E-05 | 0.224 |  | 1.74E-05 | -9.02E-06,4.39E-05 | 0.197 |
| cg04163847 | 1.45E-05 | -9.23E-05,1.21E-04 | 0.790 |  | 1.44E-05 | -9.25E-05,1.21E-04 | 0.791 |
| cg23306464 | 2.15E-06 | -1.64E-05,2.07E-05 | 0.821 |  | 3.05E-06 | -1.55E-05,2.16E-05 | 0.747 |
| Only MZ |
| cg21716444 | -4.42E-06 | -1.42E-05,5.35E-06 | 0.375 |  | -4.74E-06 | -1.45E-05,5.06E-06 | 0.343 |
| cg07326074 | 2.21E-05 | -1.38E-05,5.81E-05 | 0.227 |  | 2.26E-05 | -1.35E-05,5.86E-05 | 0.220 |
| cg04163847 | 4.24E-05 | -8.43E-05,1.69E-04 | 0.512 |  | 4.19E-05 | -8.49E-05,1.69E-04 | 0.517 |
| cg23306464 | 1.03E-05 | -1.12E-05,3.17E-05 | 0.348 |  | 9.98E-06 | -1.15E-05,3.15E-05 | 0.362 |
| Only DZ |
| cg21716444 | -6.84E-07 | -1.15E-05,1.01E-05 | 0.901 |  | -1.90E-06 | -1.28E-05,8.98E-06 | 0.732 |
| cg07326074 | -1.26E-07 | -3.87E-05,3.85E-05 | 0.995 |  | 1.63E-06 | -3.73E-05,4.05E-05 | 0.935 |
| cg04163847 | -2.99E-05 | -1.97E-04,1.38E-04 | 0.726 |  | -3.17E-05 | -2.00E-04,1.36E-04 | 0.711 |
| cg23306464 | -6.80E-06 | -3.69E-05,2.33E-05 | 0.658 |  | -3.59E-06 | -3.38E-05,2.66E-05 | 0.816 |

SATSA: the Swedish Adoption/Twin Study of Aging; MZ: monozygotic twins; DZ: dizygotic twins; 95% CI: 95% confidence interval.

Model 1: mixed effect model with methylation level as dependent variable, and average daily alcohol consumption as independent variable as fixed effect. Person’s identity number and twin identity number was entered as random effect. Age, gender, type of chip and BMI were adjusted as covariates in the model as fixed effects. Model 2: additional adjusted with smoking based on model 1. β means the change of methylation level for every 1 g/d amount of change in alcohol drinking.

**Supplement table 4: Association results from cross-sectional sensitivity analyses in the validation stage in SATSA samples**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Model 1**  |  | **Model 2** |
|  | $$β$$ | **95% CI** | ***P* value** |  | $$β$$ | **95% CI** | ***P* value** |
| ***Overall samples***  |
| cg21716444 | -2.40E-06 | -1.03E-05,5.48E-06 | 0.551 |  | -2.75E-06 | -1.06E-05,5.12E-06 | 0.493 |
| cg07326074 | 1.82E-05 | -9.88E-06,4.64E-05 | 0.204 |  | 1.86E-05 | -9.59E-06,4.67E-05 | 0.196 |
| cg04163847 | 2.42E-05 | -7.37E-05,1.22E-04 | 0.627 |  | 2.49E-05 | -7.32E-05,1.23E-04 | 0.619 |
| cg23306464 | 4.62E-06 | -1.29E-05,2.22E-05 | 0.606 |  | 5.60E-06 | -1.19E-05,2.31E-05 | 0.530 |
| ***Only MZ*** |
| cg21716444 | -4.04E-06 | -1.71E-05,9.06E-06 | 0.545 |  | -4.23E-06 | -1.72E-05,8.76E-06 | 0.523 |
| **cg07326074** | 2.93E-05 | -5.34E-06,6.39E-05 | 0.097 |  | 2.93E-05 | -5.34E-06,6.39E-05 | 0.097 |
| cg04163847 | 5.07E-05 | -7.56E-05,1.77E-04 | 0.431 |  | 5.07E-05 | -7.56E-05,1.77E-04 | 0.432 |
| cg23306464 | 1.30E-05 | -1.45E-05,4.05E-05 | 0.355 |  | 1.28E-05 | -1.47E-05,4.03E-05 | 0.361 |
| ***Only DZ*** |
| cg21716444 | -1.93E-06 | -1.21E-05,8.28E-06 | 0.711 |  | -2.59E-06 | -1.29E-05,7.68E-06 | 0.621 |
| cg07326074 | 1.30E-05 | -3.08E-05,5.67E-05 | 0.561 |  | 1.46E-05 | -2.93E-05,5.86E-05 | 0.514 |
| cg04163847 | -4.58E-05 | -1.94E-04,1.02E-04 | 0.544 |  | -4.76E-05 | -1.97E-04,1.02E-04 | 0.532 |
| cg23306464 | -5.84E-06 | -2.98E-05,1.82E-05 | 0.634 |  | -1.70E-06 | -2.55E-05,2.21E-05 | 0.889 |

SATSA: the Swedish Adoption/Twin Study of Aging; MZ: monozygotic twins; DZ: dizygotic twins; 95% CI: 95% confidence interval.

Model 1: mixed effect model with methylation level as dependent variable, and daily alcohol consumption as independent variable as fixed effect. Twin identity number was entered as random effect. Age, gender, type of chip and BMI were adjusted as covariates in the model as fixed effects. Model 2: additional adjusted with smoking based on model 1. β means the change of methylation level for every 1 g/d amount of change in alcohol drinking.

**Supplement table 5: Association between alcohol predictor and alcohol consumption in SATSA samples**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Model 1**  |  | **Model 2** |
|  | $$β$$ | **95% CI** | ***P* value** |  | $$β$$ | **95% CI** | ***P* value** |
| **Overall samples**  | 2.44E-03 | 1.73E-03,3.15E-03 | 0.000 |  | 2.44E-03 | 1.73E-03,3.15E-03 | 0.000 |
| **Only MZ**  | 1.77E-03 | 7.76E-04,2.76E-03 | 0.000 |  | 1.78E-03 | 7.93E-04,2.77E-03 | 0.000 |
| **Only DZ** | 3.07E-03 | 2.06E-03,4.07E-03 | 0.000 |  | 3.07E-03 | 2.06E-03,4.09E-03 | 0.000 |

SATSA: the Swedish Adoption/Twin Study of Aging; MZ: monozygotic twins; DZ: dizygotic twins; 95% CI: 95% confidence interval.

Model 1: mixed effect model with alcohol predictor (calculated from 92 CpG sites referred to Liu et al. study) as dependent variable, and daily alcohol consumption as independent variable as fixed effect. Twin identity number was entered as random effect. Age, gender, type of chip and BMI were adjusted as covariates in the model as fixed effects. Model 2: additional adjusted with smoking based on model 1. β means the change of alcohol predictor for every 1 g/d amount of change in alcohol drinking.

**Supplement table 6: Association between alcohol predictor and alcohol consumption in CNTR samples**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Model 1**  |  | **Model 2** |
|  | $$β$$ | **95% CI** | ***P* value** |  | $$β$$ | **95% CI** | ***P* value** |
| **ME model**  | 3.04E-04 | 2.86E-04,3.22E-04 | 0.632 |  | 2.78E-04 | 2.61E-04,2.93E-04 | 0.664 |
| **Twin paired analysis** | 6.28E-05 | 5.90E-05,6.65E-05 | 0.928 |  | 5.50E-05 | 5.17E-05,5.82E-05 | 0.937 |

CNTR: Chinese National Twin Registry (CNTR); 95% CI: 95% confidence interval.

Model 1: mixed effect model with alcohol predictor (calculated from 144 CpG sites referred to Liu et al. study) as dependent variable, and daily alcohol consumption as independent variable as fixed effect. Twin identity number was entered as random effect. Age, gender and BMI were adjusted as covariates in the model as fixed effects. Model 2: additional adjusted with smoking based on model 1. β means the change of alcohol predictor for every 1 g/d amount of change in alcohol drinking.

|  |  |
| --- | --- |
| **Supplementary Table 7. The CpGs used in the alcohol predictor** |  |
| **IlmnID** | **Chr** | **Position** | **UCSC Gene** | **used in SATSA** | **used in CNTR** |
| cg27537125 | 1 | 25349681 |  | YES | YES |
| cg20970369 | 1 | 111744108 | *DENND2D* | YES | YES |
| cg19266329 | 1 | 145456128 |  | YES | YES |
| cg19238380 | 1 | 156093948 | *LM0* | YES | YES |
| cg18933331 | 1 | 110186418 |  | YES | YES |
| cg16290996 | 1 | 173835989 | *GAS5* | NO | YES |
| cg16246545 | 1 | 120255941 | *PHGDH* | NO | YES |
| cg16014412 | 1 | 9687471 |  | YES | YES |
| cg13222915 | 1 | 184598594 |  | YES | YES |
| cg10401367 | 1 | 151031295 | *CDC42SE1* | NO | YES |
| cg07173823 | 1 | 45190519 | *C1orf228* | NO | YES |
| cg04843111 | 1 | 156617074 | *BCAN* | YES | YES |
| cg03725309 | 1 | 109757585 | *SARS* | NO | YES |
| cg02995045 | 1 | 6419906 | *ACOT7* | NO | YES |
| cg01236573 | 1 | 26248897 |  | NO | YES |
| cg00907427 | 1 | 23668691 | *HNRNPR* | NO | YES |
| cg00567854 | 1 | 203273693 | *BTG2* | YES | YES |
| cg00045114 | 1 | 172674159 |  | NO | YES |
| cg24242519 | 2 | 16846569 | *FAM49A* | YES | YES |
| cg21187770 | 2 | 26205876 | *KIF3C* | YES | YES |
| cg19704902 | 2 | 8445893 |  | YES | YES |
| cg18018313 | 2 | 227657192 | *IRS1* | YES | YES |
| cg09999582 | 2 | 103352711 | *MFSD9* | YES | YES |
| cg06846495 | 2 | 232326668 | *NCL* | NO | YES |
| cg05903736 | 2 | 240197131 | *HDAC4* | YES | YES |
| cg01054402 | 2 | 85551850 | *TGOLN2* | YES | YES |
| cg00883689 | 2 | 54802904 | *SPTBN1* | NO | YES |
| cg25109663 | 3 | 114102785 | *ZBTB20* | NO | YES |
| cg23482898 | 3 | 12858887 | *CAND2* | YES | YES |
| cg17185710 | 3 | 151984822 | *MBNL1* | NO | YES |
| cg14576319 | 3 | 171175713 | *TNIK* | YES | YES |
| cg12825509 | 3 | 185648568 | *TRA2B* | NO | YES |
| cg12340144 | 3 | 38388808 | *XYLB* | NO | YES |
| cg12197470 | 3 | 159755869 |  | YES | YES |
| cg09882118 | 3 | 48508272 | *TREX1* | YES | YES |
| cg06657944 | 3 | 176915062 | *TBL1XR1* | YES | YES |
| cg05670953 | 3 | 37033903 | *MLH1* | YES | YES |
| cg01526748 | 3 | 191930926 | *FGF12* | NO | YES |
| cg00085438 | 3 | 152049980 | *MBNL1* | NO | YES |
| cg06690548 | 4 | 139162808 | *SLC7A11* | NO | YES |
| cg05205842 | 4 | 38673130 | *KLF3* | YES | YES |
| cg04487857 | 4 | 139096540 | *SLC7A11* | NO | YES |
| cg22160883 | 5 | 138609222 | *MATR3* | YES | YES |
| cg19459094 | 5 | 176856845 | *GRK6* | YES | YES |
| cg12807764 | 5 | 146864669 |  | NO | YES |
| cg22871253 | 6 | 159238744 | *EZR* | NO | YES |
| cg18842187 | 6 | 33647826 | *ITPR3* | NO | YES |
| cg18336453 | 6 | 43082296 | *PTK7* | YES | YES |
| cg11682350 | 6 | 33240284 | *VPS52* | YES | YES |
| cg11414821 | 6 | 30652771 | *KIAA1949* | YES | YES |
| cg09326440 | 6 | 30458161 | *HLA-E* | YES | YES |
| cg08335767 | 6 | 34206495 | *HMGA1* | YES | YES |
| cg04737759 | 6 | 155253937 |  | NO | YES |
| cg02104962 | 6 | 31548314 |  | YES | YES |
| cg00252472 | 6 | 150739173 |  | NO | YES |
| cg21429551 | 7 | 30635762 | *GARS* | NO | YES |
| cg21248060 | 7 | 1039957 | *C7orf50* | YES | YES |
| cg20249277 | 7 | 112120877 | *C7orf53* | NO | YES |
| cg18032812 | 7 | 56951337 |  | YES | YES |
| cg17192599 | 7 | 151504864 | *PRKAG2* | YES | YES |
| cg16882373 | 7 | 26191458 | *NFE2L3* | YES | YES |
| cg16492851 | 7 | 1062681 | *C7orf50* | YES | YES |
| cg05143694 | 7 | 132299913 | *PLX04* | NO | YES |
| cg04907244 | 7 | 22894795 | *SNORD93* | YES | YES |
| cg20699548 | 8 | 71060638 | *NCOA2* | YES | YES |
| cg11253416 | 8 | 82643680 | *CHMP4C* | NO | YES |
| cg11130692 | 8 | 141643919 | *EIF2C2* | YES | YES |
| cg02719954 | 8 | 23830907 |  | NO | YES |
| cg02707799 | 8 | 41814854 | *MYST3* | NO | YES |
| cg07957491 | 9 | 134407190 | *UCK1* | YES | YES |
| cg06638811 | 9 | 137030555 | *RNU6ATAC* | NO | YES |
| cg03997643 | 9 | 137030726 | *RNU6ATAC* | YES | YES |
| cg00232160 | 9 | 129468157 |  | NO | YES |
| cg23444264 | 10 | 72219751 |  | YES | YES |
| cg19939077 | 10 | 81108060 | *PPIF* | YES | YES |
| cg17485681 | 10 | 73565625 | *CDH23* | YES | YES |
| cg16407699 | 10 | 74020428 |  | YES | YES |
| cg11613559 | 10 | 121577971 | *INPP5F* | YES | YES |
| cg10317175 | 10 | 25247855 |  | NO | YES |
| cg07504977 | 10 | 102131012 |  | NO | YES |
| cg07104958 | 10 | 46168551 | *ANUBL1* | YES | YES |
| cg06996423 | 10 | 105236233 | *CALHM3* | YES | YES |
| cg05457221 | 10 | 134272437 |  | YES | YES |
| cg03599037 | 10 | 82172508 | *C10orf58* | YES | YES |
| cg23299618 | 11 | 45102239 |  | YES | YES |
| cg22986999 | 11 | 68781976 | *MRGPRF* | YES | YES |
| cg18282388 | 11 | 129716470 | *TMEM45B* | YES | YES |
| cg17058475 | 11 | 68607737 | *CPT1A* | YES | YES |
| cg16977872 | 11 | 64008132 | *FKBP2* | YES | YES |
| cg12616487 | 11 | 62379063 | *ROM1* | YES | YES |
| cg11376147 | 11 | 57261198 | *SLC43A1* | YES | YES |
| cg10816169 | 11 | 66080868 |  | NO | YES |
| cg10665488 | 11 | 796569 | *SLC25A22* | NO | YES |
| cg10010533 | 11 | 82445170 | *FAM181B* | YES | YES |
| cg08962271 | 11 | 67273496 | *PITPNM1* | YES | YES |
| cg08115371 | 11 | 79115706 | *ODZ4* | NO | YES |
| cg03331229 | 11 | 102821111 | *MMP13* | NO | YES |
| cg26829071 | 12 | 131590596 | *GPR133* | YES | YES |
| cg23975840 | 12 | 117042895 |  | YES | YES |
| cg14741228 | 12 | 31476479 | *FAM60A* | YES | YES |
| cg13442969 | 12 | 68044208 | *DYRK2* | YES | YES |
| cg02499214 | 12 | 122230490 | *RHOF* | YES | YES |
| cg01687189 | 12 | 102225365 | *GNPTAB* | NO | YES |
| cg24754633 | 14 | 91831327 | *CCDC88C* | NO | YES |
| cg20303561 | 14 | 91881497 | *CCDC88C* | NO | YES |
| cg18125510 | 14 | 100841768 | *WARS* | NO | YES |
| cg16702313 | 14 | 74251926 | *C14orf43* | YES | YES |
| cg15821562 | 14 | 90083275 | *FOXN3* | NO | YES |
| cg00986580 | 14 | 22951241 |  | YES | YES |
| cg23893460 | 15 | 72492422 | *PKM2* | YES | YES |
| cg02394812 | 15 | 44092488 | *SERINC4* | NO | YES |
| cg23654112 | 16 | 2525928 | *TBC1D24* | YES | YES |
| cg23166988 | 16 | 2070861 |  | YES | YES |
| cg12688670 | 16 | 29801602 | *KIF22* | YES | YES |
| cg10121429 | 16 | 75466707 | *CFDP1* | NO | YES |
| cg09175009 | 16 | 10912699 | *FAM18A* | YES | YES |
| cg08916477 | 16 | 30391350 | *42614* | YES | YES |
| cg08766770 | 16 | 28123059 | *XPO6* | YES | YES |
| cg06469895 | 16 | 69418206 | *TERF2* | YES | YES |
| cg05683445 | 16 | 28303182 | *SBK1* | YES | YES |
| cg04491089 | 16 | 84151300 | *MBTPS1* | YES | YES |
| cg27492942 | 17 | 36885965 | *CISD3* | YES | YES |
| cg21870662 | 17 | 2241570 | *SGSM2* | YES | YES |
| cg21626848 | 17 | 39969267 | *SC65* | YES | YES |
| cg17757575 | 17 | 34068891 | *RASL10B* | YES | YES |
| cg16696035 | 17 | 17126176 | *FLCN* | YES | YES |
| cg15253293 | 17 | 79366853 |  | YES | YES |
| cg11237406 | 17 | 4649333 | *ZMYND15* | NO | YES |
| cg09155905 | 17 | 33447236 | *FNDC8* | YES | YES |
| cg08677210 | 17 | 55550613 | *MSI2* | YES | YES |
| cg06940168 | 17 | 64370665 | *PRKCA* | NO | YES |
| cg01620757 | 17 | 4635229 | *MED11* | NO | YES |
| cg22332722 | 18 | 25754857 | *CDH2* | NO | YES |
| cg03524354 | 19 | 54301639 | *NLRP12* | NO | YES |
| cg02489552 | 19 | 15121531 | *CCDC105* | YES | YES |
| cg01859460 | 19 | 2273050 | *OAZ1* | YES | YES |
| cg01614597 | 19 | 14185403 | *LOC113230* | YES | YES |
| cg08559712 | 20 | 16030674 | *MACROD2* | NO | YES |
| cg03184011 | 20 | 39945503 |  | YES | YES |
| cg14588638 | 22 | 29665400 | *EWSR1* | YES | YES |
| cg09365259 | 22 | 18594196 | *TUBA8* | YES | YES |
| cg04134965 | 22 | 44423205 | *PARVB* | NO | YES |
| cg03954858 | 22 | 40404957 | *FAM83F* | YES | YES |
| cg03013276 | 22 | 24058932 | *LOC91316* | YES | YES |

The alcohol predictor was generated by 144 target CpGs and 92 CpGs of them were applied to the SATSA sample to predict their alcohol consumption. All the CpGs were used in the CNTR sample.