**SUPPLEMENTARY MATERIAL**

**Supplementary Figure 1. Promoter capture Hi-C interactions between promoters and the enhancer in FTO intron 1 in differentiated human skeletal muscle myotubes**

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**Supplementary Figure 1.** Promoter Capture Hi-C (PC-HiC) in the *FTO* region in myotubes derived from human skeletal muscle cells. Significant PC-HiC interactions are illustrated with arches. The top y axis is the interaction enrichment score. Black bars indicate locations of promoter and enhancer PC-HiC fragments. Cis-regulatory activity was measured by H3K27ac ChIP-Seq. The position of rs9939609 is indicated by the dashed line.

**Supplementary Table 1. Definition of register outcomes**

|  |  |  |
| --- | --- | --- |
| **Endpoint** | **ICD-8** | **ICD-10** |
| Peripheral Artery Disease | 25006, 4402, 4439 | E105, E115, E125, E135, E145, I702, I739 |
| Hypertension | 400-404, 42600 | I10-I13, I15, I270, I674 |
| Ischemic Heart Disease | 410, 412-414 | I20-I25, T822, Z951 |
| Ischemic Stroke | 433-434, 436 | I63-I64 |
| CVD any event | 0742, 0930, 25006, 2891-2893, 390-391, 3920, 393-396, 3970, 3979, 398, 400-404, 410, 412-414, 420-426, 42700, 42710, 450, 4272, 4275, 42792, 42798, 428, 42999, 430-431, 433-434, 436-438, 440-444, 447-448, 450-454, 456-457, 4580-4581, 4589, 7824 | E105, E115, E125, E135, E145, I00-I01, I020, I029, I05-I13, I15, I20-I28, I30-I52, I60-I74, I77-I83, I85-I89, I95, I97-I99, K550-K551, K559, T822, Z951 |
| Type 2 Diabetes | 250 | E11 |

**Supplementary Table 2. Interaction between *FTO* rs9939609 and insulin sensitivity and physical activity, respectively, on BMI**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ***FTO* effect, kg/m2** | ***FTO* effect, kg/m2** | **Effect modification, kg/m2** | **p** |
|  | Low | High |  |  |
| PA | 0.68 (0.08) | 0.36 (0.05) | -0.32 (0.10) | 0.0013 |
| IS | 0.61 (0.07) | 0.30 (0.05) | -0.31 (0.09) | 0.00028 |

Effect sizes (standard errors) and effect modifications were calculated with an additive genetic model adjusted for sex, age as a restricted cubic spline with four knots, and cohort. IS, insulin sensitivity; PA, physical activity.

**Supplementary Table 3. Joint two-way interaction analyses of the effect of physical activity and insulin sensitivity on the association between *FTO* rs9939609 and BMI.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ***FTO* effect, kg/m2** | ***FTO* effect, kg/m2** | **Effect modification, kg/m2** | **p** |
| **IS\PA** | Low | High | PA |  |
| Low | 0.73 (0.09) | 0.53 (0.08) | -0.20 (0.09) | 0.023 |
| High | 0.45 (0.08) | 0.25 (0.06) | IS |  |
|  |  |  | -0.28 (0.09) | 0.0011 |

Effect sizes (standard errors) and effect modifications were calculated with an additive genetic model adjusted for sex, age as a restricted cubic spline with four knots, and cohort. IS, insulin sensitivity; PA, physical activity.

**Supplementary Table 4. Three-way interaction analysis of the effect of physical activity and insulin sensitivity on the association between *FTO* rs9939609 and BMI.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ***FTO* effect, kg/m2** | ***FTO* effect, kg/m2** | **Effect modification, kg/m2** | **p** |
| **IS\PA** | Low | High | PA |  |
| Low | 0.60 (0.12) | 0.60 (0.09) | 0.01 (0.15) | 0.964 |
| High | 0.52 (0.10) | 0.22 (0.06) | IS |  |
|  |  |  | -0.08 (0.15) | 0.603 |
|  |  |  | PA and IS |  |
|  |  |  | -0.30 (0.18) | 0.097 |

Effect sizes (standard errors) and effect modifications were calculated with an additive genetic model adjusted for sex, age as a restricted cubic spline with four knots, and cohort. The effect modifications for PA and IS, respectively, were estimated comparing the corresponding low-high group with the low-low reference, whereas the PA and IS effect modification was estimated as an addition to these individual effect modifications. IS, insulin sensitivity; PA, physical activity.

**Supplementary Table 5. Association between *FTO* rs9939609 genotype and all-cause mortality and cardiometabolic disease outcomes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Basic adjustment** | | **Basic + BMI adjustment** | |
| **Outcome, n (all/events)** | **HR (95% CI)** | **p** | **HR (95% CI)** | **pBMI** |
| All-Cause Mortality (19,584/1,948) | 1.08 (1.01, 1.15) | 0.019 | 1.08 (1.01, 1.15) | 0.023 |
| Peripheral Artery Disease (19,482/369) | 1.08 (0.93, 1.25) | 0.320 | 1.06 (0.92, 1.23) | 0.413 |
| Hypertension (18,669/2,712) | 1.07 (1.01, 1.13) | 0.019 | 1.03 (0.97, 1.09) | 0.296 |
| Ischemic Heart Disease (18,932/1,278) | 1.04 (0.96, 1.12) | 0.393 | 1.01 (0.94, 1.10) | 0.737 |
| Ischemic Stroke (19,351/781) | 1.11 (1.01, 1.23) | 0.037 | 1.11 (1.00, 1.22) | 0.049 |
| CVD any event (17,084/4,409) | 1.03 (0.99, 1.08) | 0.125 | 1.01 (0.97, 1.06) | 0.620 |
| Type 2 Diabetes (19,282/726) | 1.18 (1.07, 1.31) | 0.0015 | 1.06 (0.96, 1.18) | 0.237 |

Hazard ratios (HR) with 95% confidence intervals (95% CI) and p-values were estimated with an additive genetic model with basic adjustment for sex, age-at-examination as a restricted cubic spline with four knots, and cohort (p) or additionally adjusting for BMI (as a restricted cubic spline with four knots; pBMI).

**Supplementary Table 6. Effect modification of insulin sensitivity and physical activity, respectively, on the association between *FTO* rs9939609 and all-cause mortality and cardiometabolic disease outcomes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **HR (95% CI)** | **Effect modification (95% CI)** | **p** |
| **All-Cause Mortality** | | | |  |
| PA | Low | 1.12 (1.00, 1.26) |  |  |
|  | High | 1.07 (0.99, 1.15) | 0.95 (0.83, 1.10) | 0.504 |
| IS | Low | 1.07 (0.97, 1.17) |  |  |
|  | High | 1.08 (0.99, 1.18) | 1.01 (0.89, 1.15) | 0.849 |
| **Hypertension** | | |  |  |
| PA | Low | 1.14 (1.04, 1.26) |  |  |
|  | High | 1.04 (0.97, 1.11) | 0.91 (0.81, 1.02) | 0.111 |
| IS | Low | 1.10 (1.01, 1.19) |  |  |
|  | High | 1.04 (0.96, 1.12) | 0.95 (0.85, 1.06) | 0.335 |
| **Ischemic Stroke** | | |  |  |
| PA | Low | 0.91 (0.74, 1.11) |  |  |
|  | High | 1.20 (1.06, 1.34) | 1.32 (1.05, 1.66) | 0.019 |
| IS | Low | 0.94 (0.80, 1.11) |  |  |
|  | High | 1.22 (1.08, 1.39) | 1.30 (1.05, 1.60) | 0.015 |
| **Type 2 Diabetes** | | |  |  |
| PA | Low | 1.30 (1.09, 1.55) |  |  |
|  | High | 1.13 (0.99, 1.28) | 0.87 (0.70, 1.08) | 0.194 |
| IS | Low | 1.21 (1.07, 1.36) |  |  |
|  | High | 1.05 (0.84, 1.32) | 0.87 (0.68, 1.12) | 0.283 |

Hazard ratios (HR) with 95% confidence intervals (95% CI), effect modifications and p-values were estimated with an additive genetic model with basic adjustment for sex, age-at-examination as a restricted cubic spline with four knots, and cohort. IS, insulin sensitivity; PA, physical activity.

**Supplementary Table 7. Effect of physical activity and insulin sensitivity on *FTO* expression in muscle**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Group | | Sample size (n) | *FTO* expression | β (SE) | p | prs9939609 |
| PA | Low | 31 | 8.12 (0.03) | -0.02 (0.02) | 0.402 | 0.387 |
|  | High | 109 | 8.12 (0.01) |  |  |  |
| IS | Low | 46 | 8.11 (0.03) | 0.02 (0.02) | 0.334 | 0.219 |
|  | High | 94 | 8.13 (0.01) |  |  |  |

Mean (standard error, SE) of quantile normalized, log2-transformed *FTO* expression according to PA or IS group. Differences in expression across PA and IS groups, respectively, were assessed with linear regression adjusting for age, BMI categorized as normal weight, overweight, or obesity, and 15 probabilistic estimations of expression residual factors (p), as well as additionally for the *FTO* rs9939609 genotype (prs9939609). IS, insulin sensitivity; PA, physical activity.