**Title:** Is the adiposity-associated FTO gene variant related to all-cause mortality independent of adiposity? Meta-analysis of data from 169,551 Caucasian adults.

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Conflict of interest statement

No conflict of interest was declared.

# Is the adiposity-associated *FTO* gene variant related to all-cause mortality independent of adiposity? Meta-analysis of data from 169,551 Caucasian adults

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## Summary

Previously, a single nucleotide polymorphism (SNP), rs9939609, in the *FTO* gene showed a much stronger association with all-cause mortality than expected from its association with body mass index (BMI), body fat mass index (FMI) and waist circumference (WC). This finding implies that the SNP has strong pleiotropic effects on adiposity and adiposity-independent pathological pathways that leads to increased mortality. To investigate this further, we conducted a meta-analysis of similar data from 34 longitudinal studies including 169,551 adult Caucasians among whom 27,100 died during follow-up. Linear regression showed that the minor allele of the *FTO* SNP was associated with greater BMI ($n$ = 169,551; 0.32 kg $m^{-2}$; 95% CI 0.28–0.32, $P < 1 \times 10^{-32}$), WC ($n$ = 152,631; 0.76 cm; 0.68–0.84, $P < 1 \times 10^{-32}$) and FMI ($n$ = 48,192; 0.17 kg $m^{-2}$; 0.13–0.22, $P = 1.0 \times 10^{-13}$). Cox proportional hazard regression analyses for mortality showed that the hazards ratio (HR) for the minor allele of the *FTO* SNPs was 1.02 (1.00–1.04, $P$ = 0.097), but the apparent excess risk was eliminated after adjustment for BMI and WC (HR: 1.00; 0.98–1.03, $P$ = 0.662) and for FMI (HR: 1.00; 0.96–1.04, $P$ = 0.932). In conclusion, this study does not support that the *FTO* SNP is associated with all-cause mortality independently of the adiposity phenotypes.

### Keywords

*FTO*; meta-analysis; mortality; obesity

## Introduction

In 2007, genome-wide association studies discovered the first obesity susceptibility locus, the ‘fat mass and obesity associated gene’ (*FTO*) (1,2). A cluster of common single nucleotide polymorphisms (SNPs) in the first intron of *FTO* was identified as those carrying the association. Each additional minor A-allele of the rs9939609 SNP in the *FTO* cluster is associated with increased body mass index (BMI) by ~0.40 kg $m^{-2}$ (1) and an increased risk of obesity by 20–30% (1,3). This *FTO* SNP appears to influence primarily the size of the overall fat mass irrespective of the body fat distribution (4). Thus, it is expected that the *FTO* SNP would also be associated with the various health-damaging effects of adiposity. Indeed, several analyses using the *FTO* SNP as instrumental variable of adiposity confirmed the causality of the association between adiposity and its detrimental health effects (5–7).

It would therefore be expected that the *FTO* SNP is also associated with all-cause mortality. In observational studies, an underestimation of the strength of the association between adiposity and mortality is suspected because of the likely confounding and reverse causality, as indicated in a previous study using the BMI of children as instrumental variable for BMI of the parents (8). However, a recent study indicated that the *FTO* rs9939609 may have a much stronger association with all-cause mortality than could plausibly be attributed to such biases (9). While the SNP showed the expected association with BMI, it was also associated with a doubling of mortality (dominant genetic model), even after adjusting for waist circumference (WC), fat mass index (FMI = body fat mass/height$^2$; kg m$^{-2}$) and BMI at younger ages. No distinct associations were found with any of the major causes of death or preceding disease incidence that could explain the finding.

On this basis, we speculate that the *FTO* SNP or some other SNPs tightly linked to it in the genomic region may have a major pleiotropic effect influencing pathways implicated in the disease processes leading to increased risk of dying independent of body weight regulation. To test the hypothesis that the *FTO* SNP rs9939609 (or any proxy SNP, $r^2 > 0.80$) was associated with all-cause mortality, with and without adjustment for BMI, WC and FMI, we conducted a meta-analysis based on individual data of Caucasian men and women from multiple studies.

## Populations and methods

### Study selection

We planned a meta-analysis based on novel analyses of longitudinal data. We performed a PubMed search using the search terms '*FTO* and obesity' to identify potential contributing studies. Further studies were identified via the network of collaborators who joined the meta-analysis and through e-mail within the GIANT (www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium) and CHARGE (web.chargeconsortium.com) consortia. All studies that included Caucasian populations and that were able to provide baseline data on BMI and the *FTO* SNP genotype, as well as mortality data during the following observation time, were considered as eligible (Fig. 1). This resulted in a total of 34 studies (Table S1) (10–42). They included 37 cohorts, as in three studies data were analysed separately for cohorts established on the basis of follow-up of previously formed cases and controls (Health Professionals Follow-up Study (HPFS) (26), Nurses' Health Studies (NHS) (32) and Stockholm Heart Epidemiology program (SHEEP) (39)).

### Genotyping

If available, genotyping of the rs9939609 SNP was preferred, but a proxy SNP in high linkage disequilibrium with the rs9939609 was used instead in the following studies: EPIC Norfolk (rs1121980; $r^2 = 0.84$) (18), EPIC-Potsdam (rs9935401; $r^2 = 0.98$) (19) and Heinz Nixdorf Recall Study (rs8050136; $r^2 = 1$) (25). In The Health ABC study (http://www.grc.nia.nih.gov/branches/leps/healthabc/), the genotype was generated by 1,000 genome imputation. Only genotype data that met the local quality control criteria, i.e. genotyping call rate, concordance in duplicate samples and tests for deviations from Hardy–Weinberg equilibrium, were used in the analyses.

### Measurement of adiposity phenotype

BMI was calculated for each individual on the basis of measured height and weight (exceptions were the use of self-reported data, which have been validated, from The Danish 1905-Cohort (15), the Danish Diet, Cancer, and Health Cohort (DCH) (16), the NHS (32) and the HPFS (26)). Furthermore, 32 of the 37 cohorts included data on WC (10,12,14,16–35,37–42), which were measured at the same time as height and weight, and 10 studies had information on total body fat mass measured by bio-impedance from which FMI was calculated (10,14,19,21,23, 24,29,30,42).

### Study-specific analyses

All the study-specific analyses were planned to be performed by local analysts according to a centrally prepared analysis plan for the covariates and through usage of a hierarchical set of Stata scripts (Stata 12.1; StataCorp LP, College Station, TX, USA; 2012), developed by the Data Hub at the Institute of Preventive Medicine, Copenhagen, Denmark. The local analysts were asked to check the accuracy of their variable assignments and additional checks were later performed at the Data Hub. Any detected ambiguities were clarified with the respective study investigators before the final meta-analysis stage. All the derived effect estimates of the local analyses along with their standard errors were automatically saved in corresponding Stata datasets that were returned to the Data Hub. Alternatively, data were sent directly and analysed using the same scripts by the Data Hub analyst (LHÄ), which was performed for nine studies (12,16,21,23,24,30,31,40,42).

For the main analyses, the effect of the *FTO* SNP was tested assuming an additive allelic effect. However, because in the previous study the effect of the *FTO* SNP on mortality fitted a dominant genetic model (9), this model was also investigated. In order to take into account the variation in sex and age, all adiposity phenotypes were subsequently analysed adjusting for sex and age using residuals from regressions of the phenotypes on sex, age, age-squared, age × sex or age-squared × sex.

Each study tested the association between the *FTO* geno-type and the adiposity phenotypes using linear regression. Further, each study tested for a main effect on mortality of *FTO* genotype, BMI, WC and FMI, respectively. In addition, the associations of the combined adiposity pheno-types, (BMI and WC) and (FMI and WC), with mortality were estimated. Finally, the association between the *FTO* genotype and mortality was examined adjusting for the single and combined adiposity phenotypes.

Due to the distinct U-shaped association between BMI and mortality (43,44), where the increased mortality may not reflect associations with fat mass (45), individuals with BMI < 20 kg $m^{-2}$ were excluded from the analyses. However, as the nadir of the U curve has often been reported to be around 25 kg $m^{-2}$ (43,44), there may be a J-shaped relationship between BMI above 20 kg $m^{-2}$ and mortality. Similarly, the association between WC and mortality has been reported to be U-shaped (46–48). Studies have shown that WC adjusted for BMI and BMI adjusted for WC have monotonic associations with all-cause mortality in opposite directions; positive and linear for the WC and decreasing and flattening out for the BMI (46,48). Therefore, in the corresponding regression models, both BMI and WC were

simultaneously included as linear, quantitative trait variables. FMI has a linear relationship with mortality (45,49) and was hence included as a quantitative trait variable in separate analyses. In additional analyses, it was also adjusted for WC, which appears to capture the effect of the FMI on mortality (49).

The Cox proportional hazards regression model was used for the analyses of mortality and the outcome is expressed as hazard ratios (HR) per unit difference of the covariates. The participants were followed from date of blood collection for DNA (or from the date of anthropometric measurements if that took place later) until death, censoring because of loss to follow-up, emigration or end of follow-up. To ensure optimal adjustment for age as a major determinant of mortality, we used age as the underlying time scale in the Cox regression. This implied entrance of the individual into the estimation of the HR at the age when the follow-up began and hence from the age at which the individual was considered at risk of dying. Moreover, as the possible pleiotropic effect might be most distinct in the older segments of the cohorts (because of the higher mortality), we also performed subgroup analyses restricting the minimum age-at-risk to 60, 65 and 70 years, respectively. All Cox regression analyses were adjusted for sex and lack of difference in association between the two sexes was confirmed. The proportional hazard assumptions were assessed by a test based on Schoenfeld residuals (50) using the Stata *estat phtest* command; only one of 37 studies (EPIC-Potsdam) showed a significant deviation ($P = 0.01$).

### Meta-analysis

The locally estimated regression coefficients and standard errors were combined by inverse variance weighted meta-analyses with random effects (51,52), using the Stata *metan* command (53). In all the meta-analyses, between-study heterogeneity was tested for by the Q statistic and quantified by the $I^2$ value; $I^2$ values of <25%, 25–75% and >75% were defined as low, moderate and high heterogeneity, respectively (53,54). If the heterogeneity turned out to be moderate or high, meta-regression analysis was planned in order to search for and adjust for the sources of heterogeneity.

## Results

### Study characteristics

The 37 participating cohorts provided data on 70,020 men of whom 13,857 died during follow-up and 99,531 women of whom 13,243 died during follow-up (Table S1). Regarding the mean follow-up time over cohorts, the median length was 9.4 years (range of means 2.9–20.0 years). Mean baseline age ranged from 38 to 93 years. The minor allele frequency of the *FTO* SNP ranged from 34% to 45%.

### Association of FTO with adiposity phenotypes

The association between the *FTO* SNP and BMI and WC was confirmed; each additional minor allele of the *FTO* SNP was associated with a 0.32 kg $m^{-2}$ higher BMI and with a 0.76 cm higher WC (Table 1). In the 10 cohorts with data on FMI, each *FTO* minor allele was associated with a 0.17 kg $m^{-2}$ higher FMI (Table 1).

### Association of adiposity phenotypes with all-cause mortality

All-cause mortality was positively associated with BMI (HR per unit BMI of 1.02, 95% confidence interval [CI]: 1.01–1.03; $P = 1.0 \times 10^{-8}$), but the association went in the opposite direction when BMI was concurrently adjusted for WC (HR: 0.97, 95% CI: 0.96–0.98; $P = 4.5 \times 10^{-6}$) (Table 2). The mortality was positively associated with WC (HR: 1.01, 95% CI: 1.01–1.02; $P = 5.6 \times 10^{-24}$), also when concurrently adjusted for BMI (HR: 1.02, 95% CI: 1.02– 1.03; $P = 8.8 \times 10^{-18}$) (Table 2). Mortality was positively associated with FMI alone (HR: 1.05 (95% CI: 1.02–1.08; $P = 0.003$), but negatively associated when adjusted for WC (HR: 0.97, 95% CI: 0.95–0.99; $P = 0.009$) (Table 2).

### Associations of FTO with all-cause mortality

FTO was associated (albeit non-significantly) with all-cause mortality with a HR of 1.02 (95% CI: 1.00–1.04; $P = 0.097$) per minor allele. Adjustment for BMI and WC attenuated the estimate to a HR of 1.00 (95% CI: 0.98–1.03; $P = 0.662$) (Fig. 3).

In the subset of 10 studies including 48,192 individuals with FMI data (6,436 deaths), each minor *FTO* allele was associated with mortality with a HR of 1.01 (95% CI: 0.96–1.06; $P = 0.731$) and a HR of 1.00 (95% CI: 0.96– 1.04; $P = 0.932$) when adjusted for FMI (Fig. 4).

The analyses assuming a dominant genetic model showed no association between *FTO* and mortality (HR of 1.00, 95% CI: 0.98–1.03; $P = 0.901$). The age-restricted subgroup analyses (observations left-truncated with delayed entry at 60, 65 and 70 years, respectively) and the sex-specific analyses showed results consistent with the non-censored analyses (Tables S2 & S3).

The heterogeneity in all meta-analyses was very low, so there was no reason to conduct a comprehensive meta-regression analysis. The possible modification of the *FTO*–mortality association by mean age at events in each cohort was addressed by a meta-regression analysis, which did not show a significant relationship.

## Discussion

In this meta-analysis, combining data of up to 169,551 adults of whom 27,100 died during follow-up, we found a very modest and statistically insignificant effect of the adiposity-associated *FTO* SNPs on all-cause mortality. When assuming an additive genetic effect, each minor allele increased mortality by ~2% with CIs ranging from ~0% to ~4% (Fig. 2). When adjusting for the adiposity phenotypes, there was virtually no association between the *FTO* SNPs and all-cause mortality.

Our results do not support the findings from the previous study, which reported a statistically significant very strong positive association between the *FTO* rs9939609 SNP and all-cause mortality (9). In the previous study, *FTO* minor allele carriers had almost twice the mortality rate of the homozygous carriers of the major allele when analysed in a dominant genetic effect model (9). The results from the present meta-analysis provide evidence that if *FTO* has an effect on mortality that is not attributable to its association with the adiposity phenotypes, it is – almost without doubt – much smaller than that found in the

previous study (as indicated by the CI). Hence, the results of the previous study are likely to be spurious, possibly reflecting random sampling errors irrespective of its statistical strength and otherwise consistent associations between the *FTO* SNP and the adiposity phenotypes and their associations with all-cause mortality (4,9). On the other hand, several previous studies have investigated the association between *FTO* and cardiovascular disease and the results were recently pooled in a meta-analysis (55). The overall conclusion was that *FTO* was associated with an increased cardiovascular risk independent of its association with BMI (55). Whether *FTO* has adiposity-independent effects on mortality from specific causes remains an important challenge for future research to elucidate.

The association between the *FTO* SNP and mortality was robust as judged from the narrow CIs and the low heterogeneity. Consistent results were also found when restricting the time-at-risk to older ages (above 60, 65 and 70 years, respectively) and to each sex, indicating no effect modifications by age and sex. When assuming a dominant effect for the minor allele, no association was found between the *FTO* SNP and mortality. Moreover, as addressed in the following, the associations between the *FTO* SNP and the adiposity phenotypes as well as the associations between the adiposity phenotypes and all-cause mortality were generally as expected from previous studies.

We estimated an effect size for BMI per minor allele of *FTO* to 0.32 kg $m^{-2}$, which is similar to the findings in other large-scale studies in Caucasian adults, where effect sizes ranging between 0.26 and 0.39 kg $m^{-2}$ have been reported (1,56–59). The corresponding effect size for WC in the present meta-analysis was 0.76 cm per additional minor allele, which is within the range of 0.73–1.00 cm per additional minor allele previously reported in large-scale studies in Caucasian adults (1,57,60).

Our linear estimates of associations between BMI and mortality and between WC and mortality are probably biased because of the U- or J-shaped associations (43–46,48,49), but the other estimated associations shown in Table 2 are likely to reflect the expected monotonic, approximate linear associations (45,46,48,49). Thus, previous studies suggest that WC has a strong positive relation to mortality when adjusted for BMI, whereas BMI adjusted for WC is inversely associated with mortality (46–48). In agreement with these relationships, a study found a direct association between fat mass and mortality and an inverse association between lean mass and mortality (45). Further, when adjusting fat mass for WC, the positive association with mortality was eliminated, whereas adjustment of lean mass for WC did not alter the association with mortality (49).

As reported by others studying the relation between the *FTO* SNPs and the health-damaging effects of adiposity (5–7), the present study offers an opportunity to interpret the association of the adiposity phenotypes with all-cause mortality in the conceptual framework of so-called Mendelian randomization analysis by using the *FTO* SNP as an instrumental variable (61). A key requirement of these analyses is that the instrumental variable, here the alleles of the SNP, is associated with the factor to be investigated, here mortality, only through the investigated cause, here the adiposity phenotypes, i.e. there must be no pleiotropic effects. The present results support this assumption for the *FTO* SNP. Such analysis provides a calculation of the association of the adiposity phenotypes with mortality that is presumed to

be unbiased by confounding or reverse causality and may hence be interpreted as evidence for a causal relation (61). For both BMI and FMI, the causal calculation for mortality is a HR of 1.05 per kg $m^{-2}$. This is greater than the observed mortality for BMI (HR of 1.02), but equal to that observed for FMI. The lower mortality observed for BMI than calculated from the instrumental variable analysis may reflect confounding and reverse causality, possibly inducing the inverse relation between the lean body mass component of BMI and mortality (45). The equality of the observed and calculated HR for FMI suggests that the observed association between FMI and mortality is probably unbiased and may reflect a causal relation.

The construction of the present study does not allow us to conduct a proper in-depth Mendelian randomization analysis (61). In spite of the size of the study, it was not originally set up to address such analysis and there remains considerable statistical uncertainty of the components used for the calculations. Furthermore, the inference would be considerably improved by various measures such as inclusion of adiposity-associated SNPs in other genomic regions, integration of the different measures of body composition and shape, calculation of the associations of the adiposity phenotypes with mortality under proper control of the well-known confounding factors (e.g. smoking), analyses taking into account non-linear relations between the adiposity phenotypes and mortality and subdivision of the mortality by age at death and by major causes of death.

The key strength of the present study is that it is strictly hypothesis-driven and designed for and capable of testing the proposed hypothesis. The meta-analysis was based on analysis of original individual participant data according to a standardized plan in all eligible cohorts. This analytical standardization across studies minimized study heterogeneity and the usage of all identified data minimized bias related to study selection, which might otherwise have caused serious publication bias because of the difficulty in publishing null results. However, as the study was conducted among Caucasians only, it is difficult to generalize the results to other ethnic groups.

We conclude that the *FTO* SNP is not associated with all-cause mortality independently of the adiposity phenotypes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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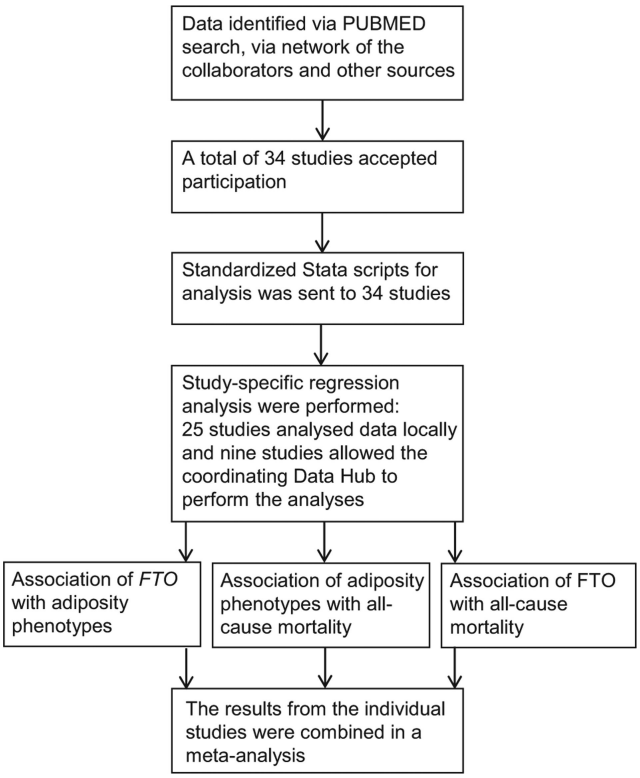


**Figure 1.**

Study design of the *FTO*–mortality meta-analysis. Eligible studies were identified by a literature search, as well as through network of the collaborators and calls in the international consortia GIANT and CHARGE (labelled ‘other sources’ in the figure). Of all studies that were invited, 34 studies of adults ($n$ = 169,551) participated in the meta-analysis. Standardized Stata scripts were sent to each of the studies, 25 studies analysed data locally and nine studies were analysed centrally at the coordinating Data Hub. All the local estimates were meta-analysed.

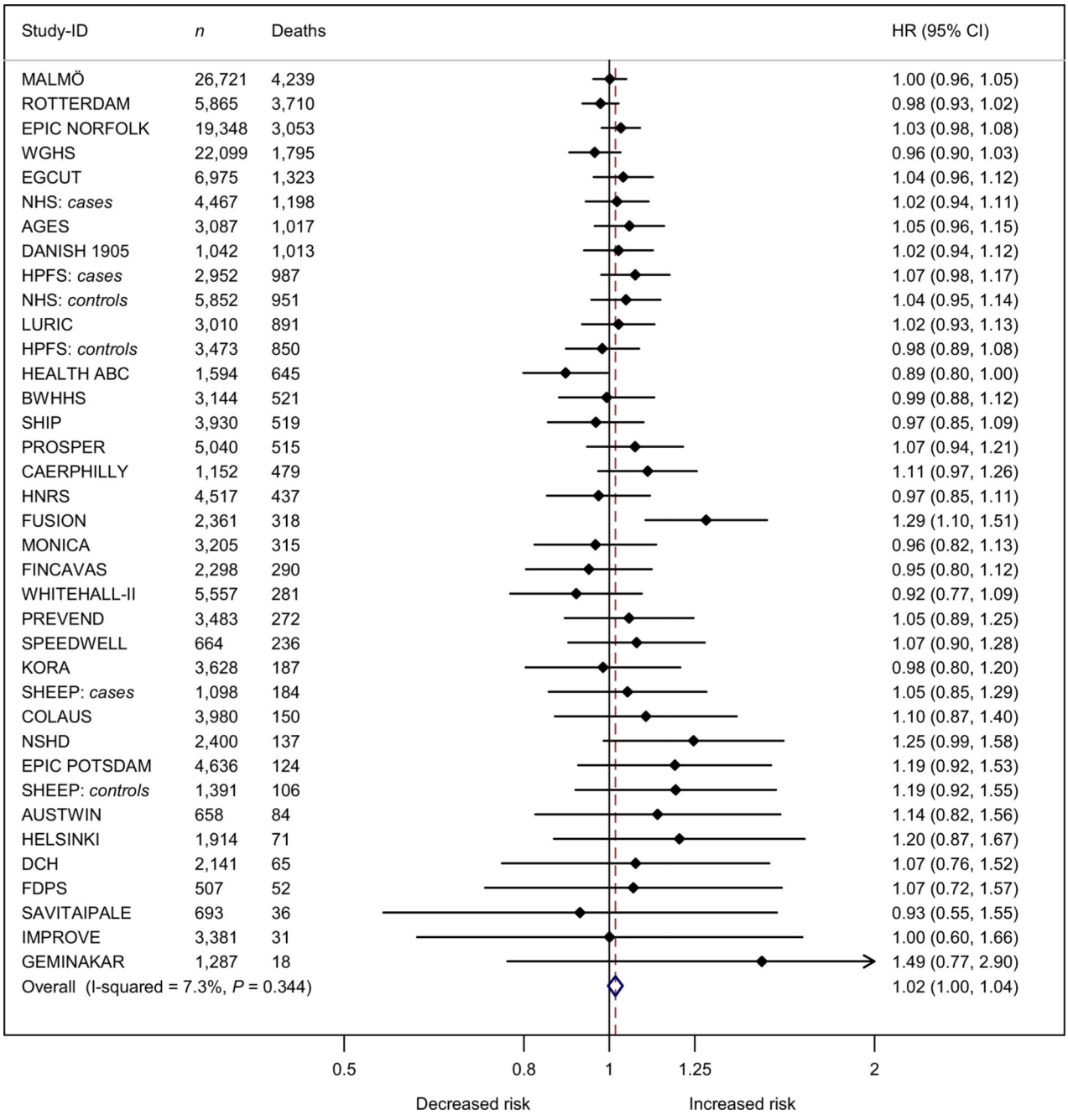


**Figure 2.**

Forest plot of the effect of *FTO* rs9939609 on all-cause mortality in a random effects meta-analysis of 169,551 Caucasian adults sorted by number of deaths. The studies are sorted by decreasing sample size (the largest at the top). Details of the studies are given in Table S1. The overall estimate equalled a HR of 1.016 (0.997–1.035), $P = 0.097$.

95% CI, 95% confidence intervals; Deaths, number of deaths; HR, estimated hazard ratio of all-cause mortality per minor allele of the rs9939609 or a proxy ($r^2 > 0.8$); *n*, number of individuals.

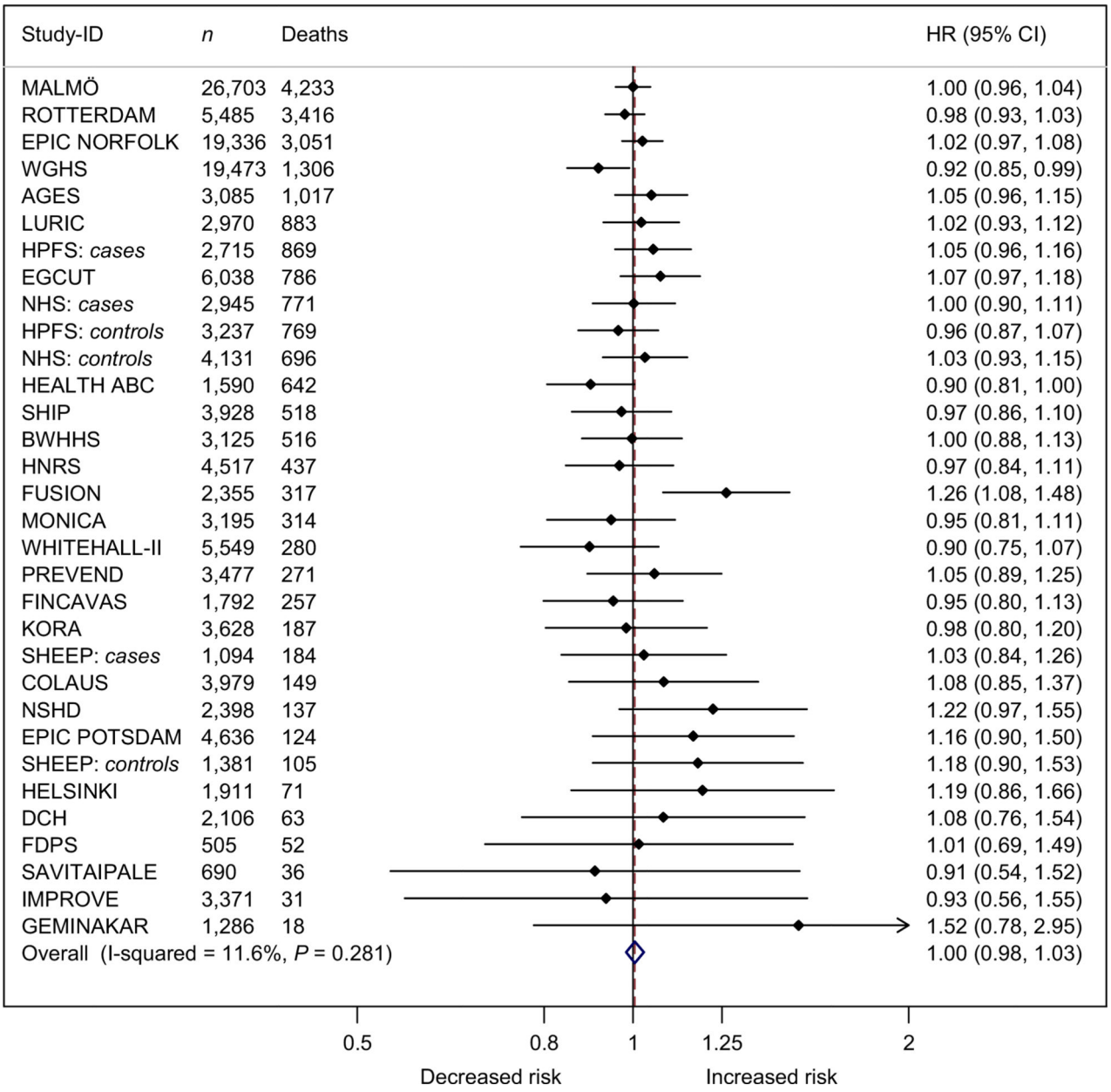


**Figure 3.**

Forest plot of the effect of *FTO* rs9939609 on all-cause mortality adjusted for body mass index and waist circumference in a random effects meta-analysis of 152,631 Caucasian adults sorted by number of deaths. The studies are sorted by decreasing sample size (the largest at the top). Details of the studies are given in Table S1. The overall estimate equalled a HR 1.004 (0.983–1.027), $P = 0.662$.

95% CI, 95% confidence intervals; Deaths, number of deaths; HR, estimated hazard ratio of all-cause mortality per minor allele of the rs9939609 or a proxy ($r^2 > 0.8$); *n*, number of individuals.

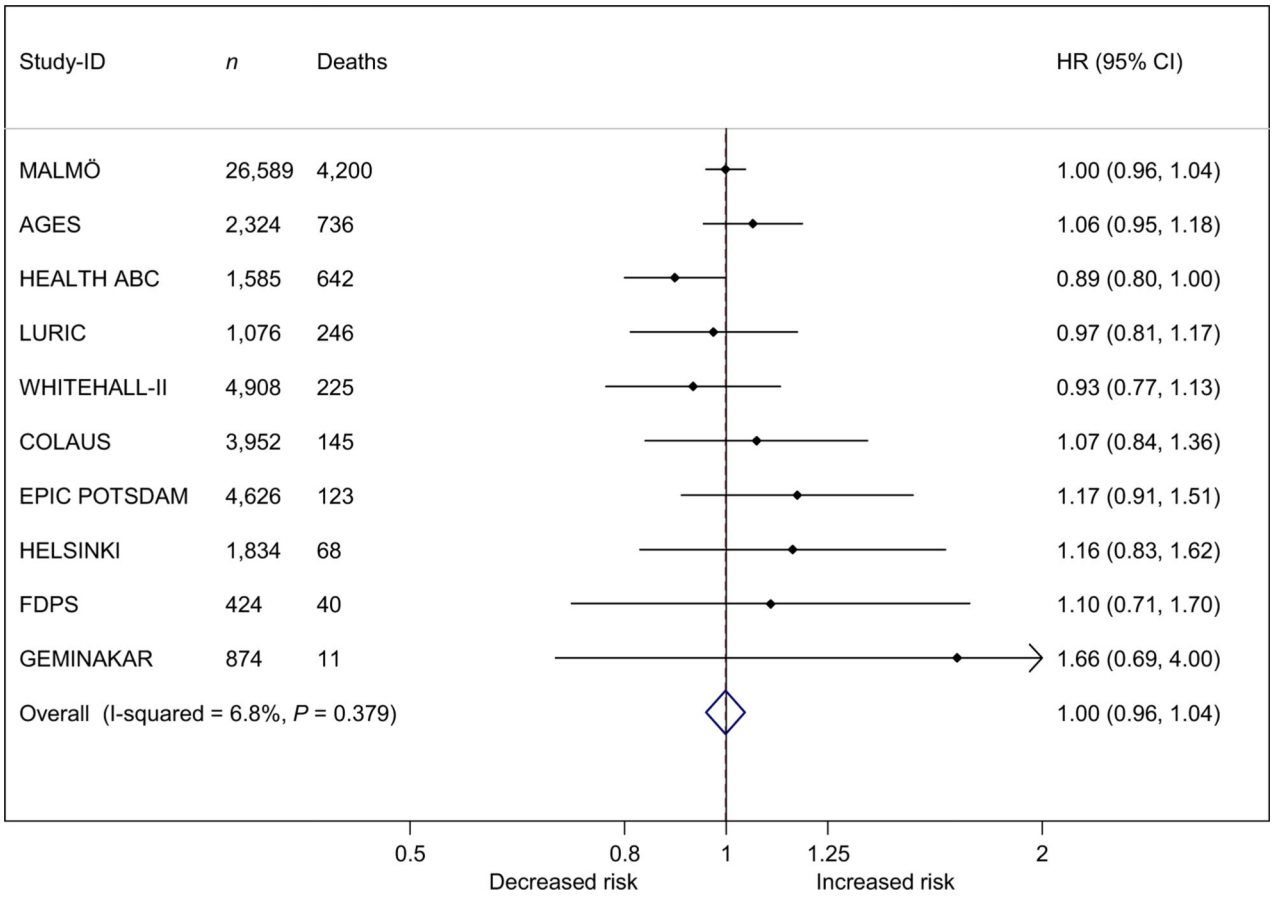


**Figure 4.**

Forest plot of the effect of *FTO* rs9939609 on all-cause mortality adjusted for fat mass index in a random effects meta-analysis of 48,192 Caucasian adults sorted by number of deaths. The studies are sorted by decreasing sample size (the largest at the top). Details of the studies are given in Table S1. The overall estimate equalled a HR 0.998 (0.956–1.042), $P = 0.932$.

95% CI, 95% confidence intervals; Deaths, number of deaths; HR, estimated hazard ratio of all-cause mortality per minor allele of the rs9939609 or a proxy ($r^2 > 0.8$); *n*, number of individuals.

**Table 1**

Association of the minor (A) allele of the rs9939609 SNP in *FTO* with BMI, WC and FMI, respectively, in a random effects meta-analysis of Caucasian adults

| Phenotype | *n* | β (95% CI) | *P*-value |
|---|---|---|---|
| BMI (kg $m^{-2}$) | 169,551 | 0.32 (0.28-0.32) | $<1 \times 10^{-32}$ |
| WC (cm) | 152,631 | 0.76 (0.68-0.84) | $<1 \times 10^{-32}$ |
| FMI (kg $m^{-2}$) | 48,192 | 0.17 (0.13-0.22) | $1.0 \times 10^{-13}$ |

β, estimated difference in phenotype per minor allele of the rs9939609 or a proxy ($r^2 > 0.8$); BMI, body mass index; CI, confidence interval; FMI, fat mass index; *n*, number of individuals; SNP, single nucleotide polymorphism; WC, waist circumference.

## Table 2

Association of BMI, WC and FMI with all-cause mortality in a random effects meta-analysis of Caucasian adults

| Phenotype | *n* | Deaths | HR (95% CI) | *P*-value |
|---|---|---|---|---|
| BMI (kg $m^{-2}$)[a] | 169,551 | 27,100 | 1.02 (1.01-1.03) | $1.0 \times 10^{-8}$ |
| BMI (kg $m^{-2}$)IWC | 152,631 | 22,506 | 0.97 (0.96-0.98) | $4.5 \times 10^{-6}$ |
| WC (cm)[b] | 152,631 | 22,506 | 1.01 (1.01-1.02) | $5.6 \times 10^{-24}$ |
| WC (cm)IBMI | 152,631 | 22,506 | 1.02 (1.02-1.03) | $8.8 \times 10^{-18}$ |
| FMI (kg $m^{-2}$) | 48,192 | 6,436 | 1.05 (1.02-1.08) | 0.003 |
| FMI (kg $m^{-2}$)IWC | 48,167 | 6,433 | 0.97 (0.95-0.99) | 0.009 |

BMI, body mass index; CI, confidence interval; FMI, fat mass index; HR, estimated hazard ratio of all-cause mortality per unit of the phenotype; *n*, number of individuals; WC, waist circumference; I, means adjusted for; e.g. BMI (kg $m^{-2}$)IWC is BMI adjusted for waist circumference.

[a] Due to the assumption of linearity of the BMI-mortality association for BMI ≥ 20 kg $m^{-2}$, the association between high levels of BMI and mortality is potentially underestimated.

[b] Due to the assumption of linearity of the WC-mortality association, the association between high levels of WC and mortality is potentially underestimated.