SUPPLEMENTARY DATA

**Protein biomarkers for insulin resistance and type 2 diabetes risk in two large community cohorts**
Nowak *et al.*

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**Supplementary Table 1. Insulin resistance genetic risk score components and association with lnHOMA-IR**

| SNP | Gene | Chromosome | Position (Genome Reference Consortium Human Build 38) | IR increasing allele | other allele | SNP score - lnHOMA-IR association in MAGIC* |
|---|---|---|---|---|---|---|
| rs4846565 | *LYPLAL1* | 1 | 219548762 | G | A | |
| rs10195252 | *GRB14* | 2 | 164656581 | T | C | |
| rs2943645 | *IRS1* | 2 | 226234464 | T | C | |
| rs17036328 | *PPARG* | 3 | 12348985 | T | C | |
| rs3822072 | *FAM13A1* | 4 | 88820118 | A | G | $\beta = 0.012 \pm 0.001$, F=71.619, P = 2.6E-17 |
| rs6822892 | *PDGFC* | 4 | 156813523 | A | G | |
| rs4865796 | *ARL15* | 5 | 53976834 | A | G | |
| rs459193 | *ANKRD55/MAP3K1* | 5 | 56510924 | G | A | |
| rs2745353 | *RSPO3* | 6 | 127131790 | T | C | |
| rs731839 | *PEPD* | 19 | 33408159 | G | A | |

F - F-statistic associated with the regression coefficient, IR - insulin resistance, P - P-value, SNP - single nucleotide polymorphism $\beta \pm$ SE indicates the per-allele effect on lnHOMA-IR *SNP effects based on data from PMID 20081858

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**Supplementary Table 2. Instrumental variable analysis for insulin resistance causally affecting biomarkers**

| | Association of IR genetic risk score with biomarker in PIVUS/ULSAM* | | IV estimator*** | |
|---|---|---|---|---|
| **Biomarker** | **β ± SE**** | **P-value** | **β ± SE** | **P-value** |
| **Leptin** | -0.017 ± 0.012 | 0.174 | -1.450 ± 1.081 | 0.180 |
| **t-PA** | 0.037 ± 0.014 | 0.008 | 3.207 ± 1.271 | 0.012 |
| **Renin** | 0.005 ± 0.014 | 0.687 | 0.473 ± 1.174 | 0.687 |
| **IL-1ra** | -0.025 ± 0.014 | 0.079 | -2.141 ± 1.245 | 0.085 |
| **HGF** | 0.010 ± 0.014 | 0.482 | 0.852 ± 1.216 | 0.483 |
| **Cathepsin D** | 0.011 ± 0.014 | 0.410 | 0.991 ± 1.207 | 0.412 |
| **FABP-4** | -0.015 ± 0.013 | 0.243 | -1.308 ± 1.130 | 0.247 |

FABP-4 - fatty acid binding protein 4, HGF - hepatocyte growth factor, IL-1ra - interleukin-1 receptor antagonist, t-PA - tissue plasminogen activator*all 10 IR-risk SNPs were directly genotyped with the Illumina Cardio-MetaboChip array in PIVUS and ULSAM **β ± SE indicates the per-allele effect on normalized protein expression value of biomarker (SD-unit) ***β ± SE indicates the causal effect of one unit change in lnHOMA-IR on normalized protein expression value of biomarker (SD-unit)

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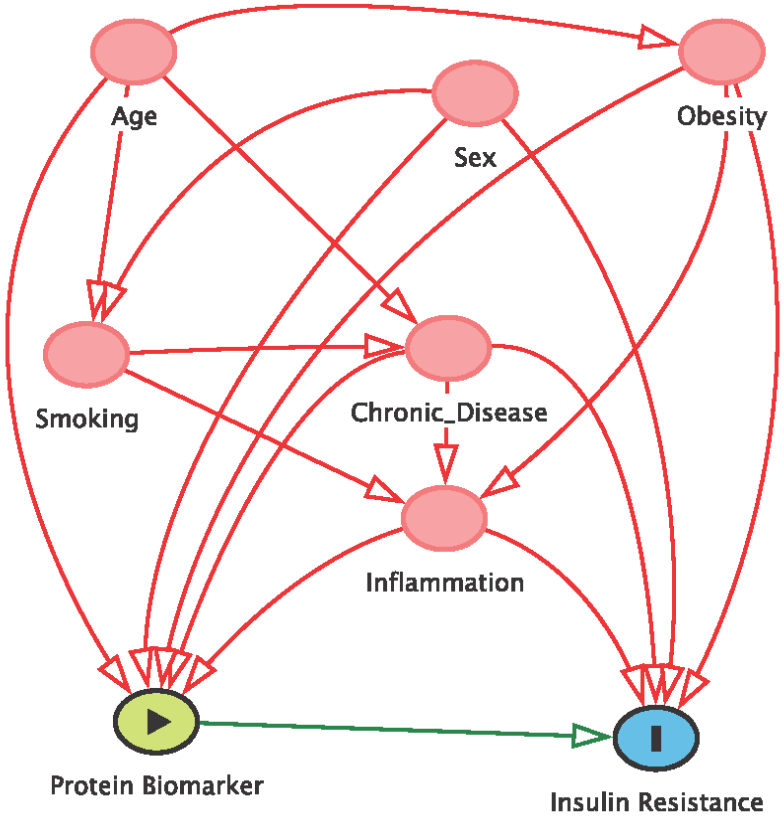
**Supplementary Table 3. Instrumental variable analysis for biomarkers causally affecting insulin resistance**

| | Biomarker | Genetic instrumental variable | | | | | | SNP - Biomarker association | | | SNP - lnHOMA-IR association | | IV estimator | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SNP | chr | pos | Closest gene | EA | other allele | β ± SE* | P-value | $r^2$ | β ± SE** | P-value | β ± SE*** | P-value |
| Published GWAS¶ | IL-1ra | rs4251961 | 2 | 113116890 | *IL-1RN* | T | C | 0.082 ± 0.009 | 2.8E-21 | NR | 0.001 ± 0.004 | 0.818 | 0.012 ± 0.051 | 0.812 |
| | | rs6759676 | 2 | 113078771 | *IL1F10* | C | T | 0.075 ± 0.009 | 1.7E-17 | NR | 0.002 ± 0.004 | 0.658 | 0.024 ± 0.053 | 0.653 |
| | | Combined score | | | | | | 0.079 ± 0.006 | 5.9E-35 | 2.0% | 0.001 ± 0.003 | 0.624 | 0.018 ± 0.037 | 0.624 |
| | HGF | rs5745687 | 7 | 81729735 | *HGF* | C | T | 0.099 ± 0.011 | 3.6E-19 | 2.1% | 0.016 ± 0.008 | 0.062 | 0.162 ± 0.083 | 0.051 |
| | t-PA | rs9399599 | 6 | 147382163 | *STXBP5* | T | A | 0.032 ± 0.004 | 2.9E-14 | 0.3% | 0.001 ± 0.004 | 0.821 | 0.028 ± 0.125 | 0.822 |
| | | rs3136739 | 8 | 42347562 | *POLB* | A | G | 0.063 ± 0.010 | 1.3E-09 | 0.2% | 0.003 ± 0.012 | 0.801 | 0.046 ± 0.191 | 0.809 |
| | | rs7301826 | 12 | 130806556 | *STX2* | C | T | 0.027 ± 0.004 | 1.0E-09 | 0.3% | 0.002 ± 0.004 | 0.694 | 0.059 ± 0.152 | 0.697 |
| | | Combined score | | | | | | 0.032 ± 0.003 | 7.0E-32 | 0.8% | 0.001 ± 0.003 | 0.633 | 0.042 ± 0.087 | 0.633 |
| PIVUS/ULSAM GWAS | Cathepsin D | rs17571¶¶ | 11 | 1761364 | *CTSD* | G | A | 0.708 ± 0.059 | 9.49E-33 | 7.0% | -0.006 ± 0.008 | 0.426 | -0.009 ± 0.011 | 0.425 |

GWAS - genome wide association study, HGF - hepatocyte growth factor, IL-1ra - interleukin-1 receptor antagonist, NR - not reported, t-PA - tissue plasminogen activator, chr - chromosome, pos - position (Genome Reference Consortium Human Build 38), EA - effect-allele (biomarker-increasing allele), r2 – variance explained. ¶References: IL-1ra - PMID 24969107, HGF - PMID 25552591, t-PA - PMID 24578379. ¶¶proxy-SNP (r2 > 0.8) for rs55861089 (position 1783757, chromosome 11, effect/non-effect alleles A/g). *β ± SE indicates the per-allele effect on protein concentration (ln-tranformed for HGF and t-PA, $log_2$-scaled normalized protein expression value for cathepsin D) **β ± SE indicates the per-allele effect on ln-transformed HOMA-IR***β ± SE indicates the causal effect of one unit change in biomarker (ln-tranformed for HGF and t-PA, SD-unit for cathepsin D) on lnHOMA-IR

SUPPLEMENTARY DATA

**Supplementary Figure 1.** Hypothetical causal diagram of the relationship of protein biomarkers and insulin resistance.



SUPPLEMENTARY DATA

**Supplementary Figure 2. Forest plot to explore potential pleiotropy of the genetic IR score to test the effect of IR on t-PA levels.** Insulin resistance-increasing allele counts for each SNP in ULSAM and PIVUS were extracted. Following cohort-, age-, and sex-adjusted meta-analysis, heterogeneity in MR analysis findings based on the individual ten SNPs was assessed by plotting the respective IV estimators and 95% CI. The Forest plot indicates a lack of significant heterogeneity, which makes a violation of the pleiotropy assumption less likely as an explanation of our positive results.