Interactions between Zinc Transporter-8 Gene (SLC30A8) and Plasma Zinc Concentrations for Impaired Glucose Regulation and Type 2 Diabetes

Running title: Zinc-SLC30A8 Interaction for T2D

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The first two authors contributed equally to this study.
ABSTRACT

Though both SLC30A8 rs13266634 SNP and plasma zinc concentrations have been associated with impaired glucose regulation (IGR) and type 2 diabetes (T2D), their interactions for IGR and T2D remain unclear. Therefore, to assess zinc-SLC30A8 interactions, we performed a case-control study in 1,796 participants: 218 newly diagnosed IGR patients, 785 newly diagnosed T2D patients, and 793 individuals with normal glucose tolerance (NGT). After adjustment for age, sex, BMI, family history of diabetes and hypertension, the multivariable OR of T2D associated with a 10-µg/dl higher plasma zinc level was 0.87 (0.85-0.90). Meanwhile, the OR of SLC30A8 rs13266634 homozygous genotypes CC compared with TT was 1.53 (1.11-2.09) for T2D. Similar associations were found in IGR and IGR&T2D groups. Each 10-µg/dl increment of plasma zinc was associated with 22% (OR, 0.78; 95% CI, 0.72-0.85) lower odds of T2D in TT genotype carriers, 17% (0.83; 0.80-0.87) lower odds in CT genotype carriers, and 7% (0.93; 0.90-0.97) lower odds in CC genotype carriers (P for interaction = 0.01). Our study suggested that the C allele of rs13266634 was associated with higher odds of T2D, and higher plasma zinc was associated with lower odds. The inverse association of plasma zinc concentrations with T2D was modified by SLC30A8 rs13266634. Further studies are warranted to confirm our findings and clarify the mechanisms underlying the interaction between plasma zinc and the SLC30A8 gene in relation to T2D.
According to the International Diabetes Federation (IDF), the number of diabetic patients worldwide rose to 371 million in 2012 and is still increasing in almost all countries (1). Considering the pathogenesis of T2D, it can be described as a complex disorder because of the interplay of multiple genetic and environmental factors (2).

Nutrient status, as a modifiable factor, could influence glucose homeostasis and thus represent an important target for T2D prevention and management (3). Zinc is an essential trace element found in most foods that facilitates catalytic, structural, and transcriptional actions within cells (4). At the same time, zinc is a critical component of the catalytic site of over 300 enzymes, implicated in synthesis, storage, and secretion of insulin, as well as being a signaling molecule after insulin secretion (5). Some studies have investigated possible associations between zinc status and diabetes; most reported that patients with diabetes had lower serum/plasma zinc levels (6-14).

SLC30A8 (the zinc transporter-8 gene) has been suggested as an interesting candidate for studying the interaction with total zinc intake and fasting glucose levels in individuals without T2D (15). SLC30A8 is almost exclusively expressed in pancreatic beta cells and encodes a protein that transports zinc from the cytoplasm into insulin secretory vesicles, an important step in insulin synthesis and secretion (16). Genome-wide association studies (17-21) and a recent meta-analysis of 36 studies have demonstrated that allele C of the SLC30A8 rs13266634 polymorphism is associated with increased risk of impaired glucose regulation (IGR) and T2D (22).

However, to our knowledge, no study has examined the interaction between
SLC30A8 rs13266634 and plasma zinc concentrations in association with T2D. Therefore, the objective of our study was to examine the associations between rs13266634 and plasma zinc concentrations as well as their joint associations and interactions with newly diagnosed IGR and T2D in a hospital-based case-control study conducted among a Chinese Han population.

RESEARCH DESIGN AND METHODS

Study population

The study population consisted of 1,796 patients: 218 newly diagnosed IGR patients, 785 newly diagnosed T2D patients, and 793 individuals with normal glucose tolerance (NGT). Those with newly diagnosed IGR and T2D were consecutively recruited from first-time patients attending the outpatient clinics of Department of Endocrinology, Tongji Medical College Hospital, Wuhan, China from December 2004 to November 2007. Concomitantly, we consecutively recruited healthy NGT individuals who were frequency-matched by age (±5 years) and sex to patients from an unselected population undergoing a routine health check-up in the same hospital. The inclusion criteria for patients with newly diagnosed IGR and T2D were: age ≥ 30 years, body mass index (BMI) < 40 kg/m², no history of a diagnosis of diabetes, and no history of receiving pharmacological treatment for hyperlipidaemia and hypertension. Patients with clinically significant neurological, endocrinological, or other systemic diseases, as well as acute illness and chronic inflammatory or infectious diseases, were excluded from the study. The inclusion criteria for controls
were the same as those for patients, except that the age criterion was ≥ 25 years. The definitions of IFG, IGT, T2D, and NGT met the respective diagnostic criteria recommended by the World Health Organization in 1999 (23). IGR was defined as impaired fasting glucose (IFG) [fasting plasma glucose (FPG) ≥ 6.1 mmol/l and < 7.0 mmol/l and 2-h post-glucose load < 7.8 mmol/l] and/or impaired glucose tolerance (IGT) [FPG < 7.0 mmol/l and 2-h post-glucose load ≥ 7.8 mmol/l and < 11.1 mmol/l]. T2D was diagnosed when FPG ≥ 7.0 mmol/l and/or 2-h post-glucose load ≥ 11.1 mmol/l. A FPG concentration < 6.1 mmol/l, and a 2-h oral glucose tolerance test (OGTT) plasma glucose concentration < 7.8 mmol/l was considered normal glucose tolerance (NGT). All the participants enrolled were of Chinese Han ethnicity. They gave written informed consent to the study and had taken no medication known to affect glucose tolerance or insulin secretion before participation. The study was approved by the ethics committee of the Tongji Medical College.

**Body composition and blood parameters**

Personal information on demography was collected through questionnaires, including gender, age, history of disease (hypertension and hyperlipemia), and family history of diabetes. Anthropometric measurements included height (m) and weight (kg) using standardized techniques. BMI was calculated as weight divided by the square of height (kg/m²). After a 10-h overnight fast, all participants underwent a 75-g OGTT, and venous blood samples were collected at 0 and 2 h for determination of FPG, OGTT2h, fasting plasma insulin (FPI), total cholesterol (TC), triglyceride (TG),
homoeostasis model assessment of insulin resistance (HOMA-IR), homoeostasis model assessment of beta cell function (HOMA-beta), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), as described in our previous study (24).

Measurement of plasma zinc concentrations

Plasma zinc concentrations were measured in the MOE Key Lab of Environment and Health and School of Public Health at Tongji Medical College of Huazhong University of Science and Technology, using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700 Series, Japan). For quality assurance the CRM (certified reference material) ClinChek No. 8883 and No. 8884 human plasma controls were used. For No. 8883 we determined a concentration of: 917 ± 67 µg/L (certified: 925 ± 185 µg/L) and for No. 8884 we measured: 1314 ± 114 µg/L (certified: 1363 ± 273 µg/L). The limit of detection was 0.0012 µg/L, and all study participants had plasma zinc levels above the limit of detection. The concentration of our lowest standard solution (0.02 µg/L) was set as the LOQ (limit of quantitation) for measurement.

Genotyping

The SLC30A8 polymorphism rs13266634 was genotyped using an allelic discrimination assay-by-design TaqMan method on an ABI 7900HT PCR system (Applied Biosystems, Foster City, CA, USA). The primers and labeled
oligonucleotide probes were designed and supplied by Applied Biosystems. The TaqMan genotyping reaction was amplified (50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min), and the endpoint fluorescent readings were performed by ABI 7900HT data collection and analysis software version 2.2.1 (SDS 2.2.1). The genotype success rate was 98.12% for rs13266634. All the SNPs were in accord with Hardy-Weinberg equilibrium (P >0.05 for all).

**Statistical analysis**

Descriptive statistics were calculated for all demographic and clinical characteristics of the study subjects. Comparisons of NGT, IGR, and T2D were performed by Chi-square (categorical variables), t test (continuous variables, normal distribution), or Mann-Whitney U test (continuous variables, skewed distribution). For calculation of the odds ratios (ORs) for IGR and T2D, plasma zinc concentrations were considered continuous variables and categorized in tertiles according to the NGT group: category 1, < 129.45 µg/dl; category 2, 129.45-197.58 µg/dl and category 3, ≥ 197.58 µg/dl. The distributions of genotype were analyzed for deviation from Hardy-Weinberg equilibrium using a likelihood ratio test. Binary logistic regression analysis was used to assess the associations of New-IGR and T2D with plasma zinc concentrations and rs13266634 polymorphisms. ORs and 95% confidence intervals (CIs) were adjusted for known risk factors for T2D including age, sex, BMI, hypertension, and family history of diabetes. In addition, we examined the association
between plasma zinc concentration (tertiles) and IGR or T2D stratified by rs13266634 polymorphisms (TT, CT, or CC genotypes). We also estimated the joint association of plasma zinc concentrations (tertiles) and rs13266634 polymorphisms (TT, CT, or CC genotypes) with IGR or T2D.

To test the interaction between plasma zinc concentrations and rs13266634 polymorphisms in association with T2D, we introduced a multiplicative interaction term of genotypes (per rs13266634 C allele) and plasma zinc tertile as continuous variables and added these variables to the same aforementioned multivariate model. Likelihood ratio test with one degree of freedom was used to assess the significance of the interaction between plasma zinc concentration and the genotypes, with a comparison of the likelihood scores of the two models with and without the interaction term.

Statistical power for the rs13266634-zinc interaction with T2D was calculated using QUANTO 1.2.4 (http://hydra.usc.edu/gxe). Assuming an OR of 0.31 per plasma zinc tertile and an OR of 1.27 per rs13266634 C allele (allele frequency 55%), our study had 80% power to detect an interaction OR of at least 1.35 for T2D.

All data analyses were carried out with SPSS13.0 software packages. P values presented are two-tailed with significance level of 0.05.

RESULTS

Anthropometric and metabolic characteristics

Anthropometric and metabolic characteristics of the 1,796 participants with NGT,
T2D, and IGR are reported in Table 1 and Supplementary Table 1. Compared to controls, the individuals with IGR and T2D were older and had higher BMI, greater prevalence of family history of diabetes and hypertension, higher levels of TC, TG, FPG, FPI, and OGTT2h, and lower plasma zinc concentrations. In the insulin sensitivity indexes, we noted lower HOMA-beta and higher HOMA-IR in T2D, but the HOMA-IR in IGR was higher only compared with controls.

**Associations of plasma zinc concentrations with IGR and T2D**

Table 2 presents ORs for IGR and T2D associated with the levels of plasma zinc concentrations as continuous variables and categorized into tertiles according to their distribution in the controls. Lower odds for New-IGR and T2D versus controls were associated with higher plasma zinc concentrations, with a likely dose-response trend. After adjustment for age, sex, BMI, family history of diabetes, and hypertension, the multivariable OR for T2D across 10 µg/dl higher plasma zinc was 0.87 (95% CI: 0.85-0.90). Meanwhile, when comparing the highest with the lowest tertile of plasma zinc, higher plasma zinc concentrations were associated with decreased odds of T2D (adjusted OR = 0.06; 95% CI: 0.04-0.11). Similar results were obtained in IGR and IGR&T2D groups.

**Associations of rs13266634 polymorphism with IGR and T2D**

Minor allele frequencies (T allele) in NGT, IGR, and T2D groups were 47.80, 42.20, and 41.97, respectively (Supplementary Table 2). Compared to the T allele,
after adjustment for age, sex, BMI, family history of diabetes and hypertension, the C allele was associated with increased odds of T2D (OR = 1.27; 95% CI: 1.08-1.51). The adjusted OR of T2D was 1.53 (95% CI: 1.11-2.09) for homozygous CC genotypes compared with TT. Similar associations were found in IGR and IGR&T2D groups.

Interaction between plasma zinc concentrations and rs13266634 polymorphism on IGR and T2D

Plasma zinc levels were shown for NGT and T2D according to tertiles of plasma zinc and rs13266634 genotypes (Supplementary figure 1); the inverse association between plasma zinc and T2D was modified by rs13266634 genotypes (Table 4). The association between plasma zinc and T2D was weaker in CC genotype carriers and CT genotype carriers than in TT genotype carriers. The adjusted ORs and 95% CIs of T2D per each 10 µg/dl increment of plasma zinc were 0.93 (0.90-0.97), 0.83 (0.80-0.87), and 0.78 (0.72-0.85), respectively (P for interaction = 0.01). A similar interaction was found for IGR&T2D, but not for IGR alone. When the joint effects were examined, individuals with TT genotype and the highest tertile of plasma zinc concentration had a much lower risk of IGR&T2D (OR = 0.008; 95% CI: 0.001-0.061) than those with CC genotype in the lowest zinc tertile (reference) (Figure 1).

DISCUSSION

To our knowledge, this was the first study to examine the interaction between
plasma zinc concentrations and SLC30A8 genetic variation for IGR and T2D. We found that elevated plasma zinc concentrations were associated with lower odds of newly diagnosed IGR and T2D, with a dose-response trend. As for the SLC30A8 rs13266634, we confirmed the association of the risk allele C with newly diagnosed IGR and T2D in the Chinese Han population. As expected, a strong interaction was observed for the SLC30A8 rs13266634 variant and plasma zinc concentrations in relation to T2D and IGR&T2D. The inverse association between increasing plasma zinc concentrations and T2D could significantly attenuate in the CT and CC genotype groups compared with in the TT genotype group. We also observed that the association between the CC genotype and T2D was mitigated by increasing plasma zinc concentrations.

Several mechanisms may explain the important role of plasma zinc in the etiology of T2D. The pancreatic β cell is one of the cell types that contain the most zinc, because insulin is synthesized and stored in the pancreatic β cell in a solid form, as a zinc-insulin (2:6) crystal (25). Meanwhile, as an enzymatic cofactor, zinc is also implicated in all processes of synthesis, storage, and secretion of insulin, as well as being a signaling molecule after insulin secretion (5, 26). Some of the insulinomimetic effects of zinc, such as attenuating hyperglycemia and increasing lipogenesis, can be explained by its influence on insulin signaling at several levels, inducing phosphorylation of the β subunit of the insulin receptor as well as of Akt (27, 28). Moreover, another line of evidence indicates that zinc may protect insulin and β cells from death by suppression of proinflammatory cytokines, such as interleukin-1β
(29) and nuclear factor kappa B (30), as well as playing a structural role in antioxidant enzymes (31).

Considering that plasma zinc concentration is a reliable biomarker that can be used to reflect zinc status or zinc intake (32), our findings are in line with results from a large 24-year prospective study among women, demonstrating a significant inverse association between total zinc intake and risk of T2D (33). A recent systematic review of the relationship between zinc intake and serum/plasma zinc showed that population differences affected the distribution of plasma zinc concentrations (34). In that review, the means of plasma zinc in different populations range from 8.5 to 17.48 µmol/l (55.2 to 113.6 µg/dl), but unfortunately no study was carried out in China. Nonetheless, the zinc concentration in our study was largely comparable to that in the literature. Modest to substantial differences in plasma zinc concentration have been reported in previous studies of patients with T2D compared with control groups (ranging from 4.2% to more than 50% of the levels in the control group) (6, 9, 14). We observed a relatively large between-group difference (35% of the levels in the control group), which could be the result of imbalanced zinc homeostasis in patients with T2D compared with controls. However, a previous cohort study found reduced zinc intake was in women who subsequently developed T2D than those who did not (33). It has been shown that intestinal secretion of zinc can be enhanced by inflammation (35), and inflammation has been implicated in the pathogenesis of T2D (36). In addition, our controls are younger than those with diabetes, and the levels of plasma zinc could decrease with aging because of changes in maximal absorption and
entire saturation response of zinc (35, 37), although age was adjusted in our analyses.

As ZnT8 is primarily expressed in β cells and is especially required for the normal accumulation of zinc in β-cell granules, many studies have elucidated the role of ZnT8 and its encoding gene, SLC30A8 (16, 38). It has been shown both in vitro and in animal studies that alterations in ZnT8 expression strongly modulate insulin secretion (38-40). Meanwhile, other observations suggest that the variant rs13266634 in SLC30A8 impairs islet ZnT8 expression, insulin secretion, or glucose homeostasis (41-43). However, a meta-analysis of 32 epidemiologic studies suggests that the same variant does not affect insulin secretion from human islets as well as islet expression of SLC30A8 (44). In the current study, the rs13266634 CC genotype was associated with a 72.8% higher risk of IGR and a 52.5% higher risk of T2D than was the TT genotype. However, compared with genotype TT, genotype CT was not associated with an increased risk of IGR or T2D. Furthermore, it was surprising that this association was stronger in IGR than in T2D, but the sample size of participants with IGR was limited.

As has been previously reported in a 14-cohort meta-analysis, total zinc intake has a stronger protective association with fasting glucose levels in individuals carrying the glucose-raising A allele of rs11558471 in SLC30A8 than those carrying C allele (15), but no study has reported on the interaction of zinc and SLC30A8 rs13266634 variants with IGR or T2D. When comprehensively analyzing the effects of plasma zinc and the SLC30A8 rs13266634 variant on IGR and T2D, we found that the inverse association of plasma zinc with T2D was attenuated by the rs13266634 C
allele, but the inverse association was not abolished even in the CC genotype group. Moreover, the combination of TT genotype and the highest tertile of plasma zinc concentration was associated with a much lower odds of IGR&T2D than the combined CC genotype and the lowest tertile, which confirmed that both the rs13266634 C allele and decreased plasma zinc could be associated with IGR&T2D. However, as the plasma zinc concentration increased, the association between CC genotype and T2D was substantially attenuated. This result is broadly consistent with the findings of Kanoni et al. (15), that higher total zinc intake blunted the glucose-raising effect of SLC30A8 variant. One potential explanation for this interaction is that higher plasma zinc might abolish the impairments of islet ZnT8 expression, insulin secretion, and glucose homeostasis induced by the rs13266634 C allele (31), so that there was still an inverse association between higher plasma zinc and T2D even in the CC genotype group. Additional studies on these topics are needed to provide evidence that variation in SLC30A8 influences the regulation of zinc transporter activity or the modulation of islet zinc content, which would support the biological plausibility of the statistical interaction we report here.

Our participants with T2D were confined to the newly diagnosed and drug naive to exclude the effects of drug interventions, because some drugs may change the status of metabolism including zinc. Moreover, we used plasma zinc concentration as a biomarker to measure zinc status to avoid possible bias through dietary assessment, such as systematic measurement error in diet exposure and the influence of other nutrients on the bioavailability of zinc (35).
Although our study showed a strong association between the SLC30A8 rs13266634 variant and plasma zinc concentration on IGR and T2D, it has several limitations. First, the cross-sectional nature of our study does not allow us to infer any causality between plasma zinc and IGR or T2D, and plasma zinc level may be reduced by the development of IGR and T2D. Therefore, these findings should be confirmed in further prospective cohort studies. Furthermore, although plasma zinc concentration has been suggested to be a reliable biomarker to reflect zinc status or zinc intake in individuals with either a low or a high supply of dietary zinc (32), plasma zinc concentration can change in response to factors unrelated to zinc status or dietary zinc intake, including tissue catabolism, infection, or inflammation. In addition, all our participants were of Chinese Han ethnicity, which minimizes the confounding effects by ethnic background. Whether these results can be generalized to other populations requires further study.

CONCLUSIONS

The C allele of SLC30A8 rs13266634 was associated with increased ORs for newly detected IGR and T2D, while elevated plasma zinc concentration showed an inverse association. The association between plasma zinc levels and T2D was stronger among individuals carrying the TT genotype of SLC30A8 rs13266634 than those carrying the CT or CC genotypes. The current findings may contribute to our understanding of the etiologic role of zinc in T2D development and imply that zinc intervention for the prevention of T2D may need to be personalized according to
SLC30A8 genotypes. Further studies are warranted to confirm our findings and clarify the mechanisms underlying the interactions between zinc and the SLC30A8 gene for T2D.

AUTHOR CONTRIBUTIONS

Z.S., W.B., Y.Z., Y.R., X.W., Y.J., Y.S., P.Y., C.S., F.H. and L.L. designed and conducted research; Z.S. and W.B. analyzed data; Z.S., W.B., and L.L. wrote the paper; and L.L. had primary responsibility for final content. Z.S. and W.B. contributed equally to the manuscript. All authors read and approved the final manuscript. L.L. is the guarantor of this work and has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

ACKNOWLEDGMENTS

No potential conflicts of interest relevant to this article were reported.

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References


Table 1 Anthropometric and metabolic characteristics of NGT and T2D groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NGT (n=793)</th>
<th>T2D (n=785)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>490 (61.79)</td>
<td>442 (56.31)</td>
<td>0.027</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.48 (11.59)</td>
<td>50.98 (10.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.95 (3.87)</td>
<td>24.97 (3.97)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of diabetes, n (%)</td>
<td>60 (7.56)</td>
<td>111 (14.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>144 (18.14)</td>
<td>263 (33.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>4.56 (0.63)</td>
<td>9.82 (3.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OGTT2h (mmol/L)</td>
<td>6.44 (1.08)</td>
<td>16.49 (5.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FPI (µU/mL)</td>
<td>7.07 (4.04-9.93)</td>
<td>8.80 (5.44-13.79)</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-beta</td>
<td>88.00 (50.63-126.94)</td>
<td>33.08 (15.06-62.50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.81 (0.88-2.76)</td>
<td>3.60 (2.18-5.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.34 (0.91)</td>
<td>4.79 (1.43)</td>
<td>&lt;0.001</td>
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<tr>
<td>TG (mmol/L)</td>
<td>1.34(1.16)</td>
<td>1.99(1.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.34(0.58)</td>
<td>1.43(0.86)</td>
<td>0.084</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.69(0.86)</td>
<td>2.59(1.24)</td>
<td>0.199</td>
</tr>
<tr>
<td>Zinc (µg/dl)</td>
<td>161.10 (114.56-221.08)</td>
<td>104.96 (85.72-133.69)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL-C, high-density lipoprotein cholesterol; HOMA-beta, homeostasis model assessment of beta cell function; HOMA-IR, homeostasis model assessment of insulin resistance; IGR, impaired glucose regulation; LDL-C, low-density lipoprotein cholesterol; NGT, normal glucose tolerance; OGTT2h, 2-h post-glucose load; T2D, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides.

Data were presented as number (percentage) for categorical data, mean (standard deviation) for parametrically distributed data or median (interquartile range) for nonparametrically distributed data.
### Table 2 Associations of plasma zinc concentrations with IGR and T2D.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tertiles of plasma zinc concentration (µg/dl)</th>
<th>Per 10 µg/dl of plasma zinc</th>
<th>P value for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (Lowest) 2 3 (Highest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGR vs. NGT</td>
<td>&lt; 129.45 129.45-197.58 ≥ 197.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases/controls</td>
<td>141/265 65/264 12/264</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude OR (95% CI)</td>
<td>1 0.46 (0.33-0.65) 0.09 (0.05-0.16) 0.86 (0.83-0.89)</td>
<td>0.09 (0.05-0.16) 0.86 (0.83-0.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted OR (95% CI)</td>
<td>1 0.47 (0.32-0.71) 0.10 (0.05-0.21) 0.87 (0.83-0.91)</td>
<td>0.10 (0.05-0.21) 0.87 (0.83-0.91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T2D vs. NGT</td>
<td>559/265 191/264 35/264</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cases/controls</td>
<td>559/265 191/264 35/264</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude OR (95% CI)</td>
<td>1 0.34 (0.27-0.44) 0.06 (0.04-0.09) 0.85 (0.83-0.87)</td>
<td>0.06 (0.04-0.09) 0.85 (0.83-0.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted OR (95% CI)</td>
<td>1 0.36 (0.28-0.48) 0.09 (0.06-0.13) 0.87 (0.85-0.89)</td>
<td>0.09 (0.06-0.13) 0.87 (0.85-0.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(IGR or T2D) vs. NGT</td>
<td>/ / /</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: OR = Odds Ratio, CI = Confidence Interval, NGT = Normal Glucose Tolerance, IGR = Impaired Glucose Regulation, T2D = Type 2 Diabetes
<table>
<thead>
<tr>
<th>No. of cases/controls</th>
<th>700/265</th>
<th>256/264</th>
<th>47/264</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude OR (95% CI)</td>
<td>1</td>
<td>0.37 (0.29-0.46)</td>
<td>0.07 (0.05-0.10)</td>
</tr>
<tr>
<td>Adjusted OR(^a) (95% CI)</td>
<td>1</td>
<td>0.38 (0.29-0.50)</td>
<td>0.09 (0.06-0.13)</td>
</tr>
</tbody>
</table>

\(^a\) Binary logistic regression analysis was adjusted for age, sex, BMI, family history of diabetes and hypertension.
Table 3 Odd ratios for the association between IGR/T2D and plasma zinc concentrations, according to rs13266234 genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Tertiles of plasma zinc concentrations</th>
<th>Per 10 µg/dl of plasma zinc</th>
<th>P value for trend</th>
<th>P value for interaction#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (Lowest)</td>
<td>2</td>
<td>3 (Highest)</td>
<td></td>
</tr>
<tr>
<td>IGR vs. NGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1.00</td>
<td>0.50 (0.20-1.21)</td>
<td>0.06 (0.01-0.47)</td>
<td>0.88 (0.80-0.96)</td>
</tr>
<tr>
<td>CT</td>
<td>1.00</td>
<td>0.46 (0.24-0.90)</td>
<td>0.07 (0.02-0.26)</td>
<td>0.87 (0.81-0.93)</td>
</tr>
<tr>
<td>CC</td>
<td>1.00</td>
<td>0.45 (0.23-0.88)</td>
<td>0.13 (0.04-0.47)</td>
<td>0.86 (0.80-0.92)</td>
</tr>
<tr>
<td>T2D vs. NGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1.00</td>
<td>0.35 (0.20-0.61)</td>
<td>Not estimated*</td>
<td>0.78 (0.72-0.85)</td>
</tr>
<tr>
<td>CT</td>
<td>1.00</td>
<td>0.32 (0.21-0.50)</td>
<td>0.06 (0.03-0.13)</td>
<td>0.83 (0.80-0.87)</td>
</tr>
<tr>
<td>CC</td>
<td>1.00</td>
<td>0.40 (0.25-0.64)</td>
<td>0.18 (0.09-0.35)</td>
<td>0.93 (0.90-0.97)</td>
</tr>
<tr>
<td>(IGR or T2D) vs. NGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Not estimated
<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>1.00</td>
<td>0.36 (0.21-0.62)</td>
<td>&lt; 0.001</td>
<td>0.01</td>
<td>0.001-0.09</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CT</td>
<td>1.00</td>
<td>0.35 (0.23-0.53)</td>
<td>&lt; 0.001</td>
<td>0.06</td>
<td>0.03-0.12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CC</td>
<td>1.00</td>
<td>0.41 (0.26-0.64)</td>
<td>&lt; 0.001</td>
<td>0.17</td>
<td>0.09-0.32</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The ORs and 95% CIs were adjusted for age, sex, BMI, family history of diabetes and hypertension.

* The ORs and 95% CIs were not estimated because there was no DM case in this category.

# The interaction was testing whether the association of IGR/T2M with plasma zinc level is modified by genotype.
FIGURE LEGENDS

Figure 1 Joint association of plasma zinc concentrations and SLC30A8 rs13266634 genotypes in relation to IGR&T2D.

The analysis was adjusted for age, sex, BMI, family history of diabetes and hypertension.

SUPPLEMENTARY MATERIALS

Supplementary Table 1 Anthropometric and metabolic characteristics of NGT and IGR groups.

Supplementary Table 2 Associations of the SNP rs13266234 with IGR and T2D.

Supplementary figure 1 Plasma zinc levels for NGT and T2D according to tertiles of plasma zinc and rs13266634 genotypes.

Plasma zinc levels were presented as median (interquartile range).
Joint association of plasma zinc concentrations and SLC30A8 rs13266634 genotypes in relation to IGR&T2D.
Supplementary Table 1  Anthropometric and metabolic characteristics of NGT and IGR groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NGT(n=793)</th>
<th>IGR(n=218)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>490/303</td>
<td>121/97</td>
<td>0.09</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.48(11.59)</td>
<td>49.68(11.34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.95(3.87)</td>
<td>25.53(4.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of diabetes, n (%)</td>
<td>60(7.56)</td>
<td>35(15.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>144(18.14)</td>
<td>65(29.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>4.56(0.63)</td>
<td>5.98(0.63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OGTT2h(mmol/L)</td>
<td>6.44(1.08)</td>
<td>9.13(1.34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FPI (µU/mL)</td>
<td>7.07(4.04-9.93)</td>
<td>9.36(6.24-14.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-beta</td>
<td>88.00(50.63-126.94)</td>
<td>80.93(48.65-125.81)</td>
<td>0.523</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.81(0.88-2.76)</td>
<td>2.42(1.61-3.91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.34(0.91)</td>
<td>4.55(1.29)</td>
<td>0.018</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.34(1.16)</td>
<td>1.74(1.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.34(0.58)</td>
<td>1.41(0.84)</td>
<td>0.307</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.69(0.86)</td>
<td>2.51(1.06)</td>
<td>0.054</td>
</tr>
<tr>
<td>Zinc (µg/dl)</td>
<td>161.10 (114.56-221.08)</td>
<td>112.96 (90.87-143.40)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
### Supplementary Table 2 Associations of the SNP rs13266234 with IGR and T2D.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>Crude OR (95% CI)</th>
<th>P value</th>
<th>Adjusted OR(^a) (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGR vs. NGT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td>218</td>
<td>793</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>252(57.8)</td>
<td>826(52.20)</td>
<td>1.26(1.02-1.56)</td>
<td>0.034</td>
<td>1.41(1.09-1.82)</td>
<td>0.009</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td></td>
<td>86(39.45)</td>
<td>336(42.37)</td>
<td>1.11(0.75-1.37)</td>
<td>0.609</td>
<td>1.08(0.67-1.73)</td>
<td>0.766</td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td>83(38.07)</td>
<td>245(30.90)</td>
<td>1.47(0.98-2.18)</td>
<td>0.06</td>
<td>1.728(1.07-2.79)</td>
<td>0.025</td>
</tr>
<tr>
<td><strong>T2D vs. NGT</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Alleles</td>
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<td>785</td>
<td>793</td>
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<td></td>
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</tr>
<tr>
<td>C</td>
<td></td>
<td>911(58.03)</td>
<td>826(52.20)</td>
<td>1.27(1.11-1.46)</td>
<td>0.001</td>
<td>1.27(1.08-1.51)</td>
<td>0.005</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td></td>
<td>319(40.64)</td>
<td>336(42.37)</td>
<td>1.18(0.92-1.53)</td>
<td>0.191</td>
<td>1.20(0.89-1.63)</td>
<td>0.234</td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td>296(37.71)</td>
<td>245(30.90)</td>
<td>1.51(1.16-1.96)</td>
<td>0.002</td>
<td>1.53(1.11-2.09)</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>(IGR or T2D) vs. NGT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>1163(57.98)</td>
<td>826(52.20)</td>
<td>1.27(0.11-1.45)</td>
<td>&lt;0.001</td>
<td>1.31(1.11-1.53)</td>
<td>0.001</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>CT</td>
<td></td>
<td>405(40.38)</td>
<td>336(42.37)</td>
<td>1.17(0.92-1.48)</td>
<td>0.204</td>
<td>1.20(0.90-1.60)</td>
<td>0.22</td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td>379(37.79)</td>
<td>245(30.90)</td>
<td>1.50(1.17-1.92)</td>
<td>0.001</td>
<td>1.57(1.16-2.11)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

\(^a\) Binary logistic regression analysis was adjusted for age, sex, BMI, family history of diabetes and hypertension.
Plasma zinc levels for NGT and T2D according to tertiles of plasma zinc and rs13266634 genotypes.

76x55mm (300 x 300 DPI)