ARIC Manuscript Proposal # 1577

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1.a. Full Title: Interactions between zinc intake and SNPs and their impact on fasting blood glucose levels in multiple cohorts within the CHARGE and MAGIC consortia.

b. Abbreviated Title (Length 26 characters): Zinc, genes and blood glucose.

2. Writing Group:

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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __SK___ **[please confirm with your initials electronically or in writing]**

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3. Timeline: Cohort-specific data analyses: October 30, 2009
Meta-analysis: November 30, 2009
Manuscript drafting complete: January 30, 2009

4. Rationale:

Zinc is one of the most important trace elements. Zinc plays a significant catalytic role, as it is required for the biological function of more than 300 enzymes. Furthermore, zinc also presents structural and regulatory functions in several proteins involved in DNA replication and reverse transcription, as well as in a number of eukaryotic transcription factors, where the potential binding domains are referred to as zinc fingers. Zinc fingers are involved in cell proliferation, in cell differentiation, in cell growth arrest, in cell division, in signal transmission, in growth factors production, in protoncogenes activation, in chemokine production, in codifying hormone nuclear receptor superfamily, in nuclear transcription factor activation, in mRNA stability and in maintaining the extracellular matrix.

We have previously assessed the differential dietary intake of zinc in European old populations and investigated its impact on zinc and inflammatory markers concentrations, in relation to genetic background (Kanoni S., et al. J. Nutr. Biochem. 2009), as well as its impact on psychological parameters (Marcellini F., et al. Biogerontology, 2006), within the ZINCAGE FP6 project.

Furthermore, there is evidence that zinc exerts insulin-like effects by supporting the signal transduction of insulin and by reducing the production of cytokines, which lead to beta-cell death during the inflammatory process in the pancreas in the course of diabetes. Additionally, zinc might play a role in the development of diabetes, since genetic polymorphisms in the gene of zinc transporter 8 and in metallothionein (MT)-encoding genes could be demonstrated to be associated with type 2 diabetes mellitus (Jansen J., et al. J. Nutr. Biochem. 2009).

Therefore, it would be interesting to investigate the impact of dietary zinc intake on fasting glucose levels, in relation to genetic background. The aim of the proposed analysis is the evaluation of interactions between Dietary Zinc intake (mg/day – continuous variable) and 20 SNPs (applying an additive model), for the prediction of fasting glucose levels. Linear regression models will be used, including fasting glucose levels as the dependent variable (continuous), Zinc intake (separately for Dietary Zinc intake derived only from food sources and Total Zinc Intake derived from food sources and supplements), SNP and their interaction (Zinc intake x SNP) as predictors and adjustments for other potential cofounders. The analyses will be conducted in 12 cohorts, namely GHRAS, GENDAI, ARIC, FHS, ROTTERDAM, CHS, FENLAND, InCHIANTI, GLACIER, ULSAM, PIVUS and MALMÖ DIET AND CANCER. The cohort-specific results will be meta-analyzed.

5. Main Hypothesis/Study Questions:
The interaction between zinc intake and genotype (for selected SNPs, as listed below) does not have an impact on fasting glucose levels in non-diabetic subjects.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Exclusion criteria:

- Type 2 Diabetes, defined as:
  - ✓ Diagnosed and/or self-reported diabetes
  - ✓ Medication for diabetes
  - ✓ Fasting glucose ≥7 mmol/L
- Non-fasting status
- Implausible dietary data
- Non-white race

SNP list:

SNPs identified in MAGIC GWA as significant predictors of fasting glucose concentrations in GWA (1-16), plus some SNPs (17-20) in genes involved directly or through cross-talk pathways with zinc. We will use an additive model based on the estimated copies of the high-risk allele.

Chr	Nearest gene	Effect/other allele
2	*G6PC2*	C/T
11	*MTNR1B*	G/C
7	*GCK*	A/G
7	*DGKB/TMEM195*	T/G
2	*GCKR*	C/T
3	*ADCY5*	A/G
11	*MADD*	A/T
11	*CRY2*	A/C
10	*ADRA2A*	G/T
11	*FADS1*	T/C
1	*PROX1*	C/T
3	*SLC2A2*	T/A
9	*GLIS3*	A/C
8	*SLC30A8*	A/G
15	FAM148B	A/G
10	*TCF7L2*	T/A
1	*SEC63D1*	T/G
2	*IRS1*	T/A
5	*SAP30L*	T/G
12	*IGF1*	A/G

Linear Regression Models
Since supplemental zinc intake was not assessed at the baseline exam, all data for this analysis will be derived from exam 3 (FFQ and plasma glucose)

Model 1a: Dietary Zn intake (foods only) <u>without</u> BMI adjustment

Dependent variable: Fasting glucose levels (mmol/L, continuous, untransformed)

Predictors: SNP (additive model), Dietary Zinc Intake (mg/day, **derived only from food sources**, continuous, untransformed), Dietary Zinc Intake x SNP

Covariates: Sex, Age (years, continuous variable), Field center (if needed), Population substructure adjustment as needed

Model 1b: Dietary Zn intake (foods only) <u>with</u> BMI adjustment

Dependent variable: Fasting glucose levels (mmol/L, continuous, untransformed)

Predictors: SNP (additive model), Dietary Zinc Intake (mg/day, **derived only from food sources**, continuous, untransformed), Dietary Zinc Intake x SNP

Covariates: Sex, Age (years, continuous variable), **Body Mass Index (kg/m^2, continuous variable)**, Field center (if needed), Population substructure adjustment as needed

Model 2a: Total Zn intake (foods + supplements) <u>without</u> BMI adjustment

Dependent variable: Fasting glucose levels (mmol/L, continuous, untransformed)

Predictors: SNP (additive model), Total Zinc Intake (mg/day, **derived from food sources and supplements**, continuous, untransformed). Total Zinc Intake x SNP

Covariates: Sex, Age (years, continuous variable), Field center (if needed), Population substructure adjustment as needed

Model 2b: Total Zn intake (foods + supplements) <u>with</u> BMI adjustment

Dependent variable: Fasting glucose levels (mmol/L, continuous, untransformed)

Predictors: SNP (additive model), Total Zinc Intake (mg/day, **derived from food sources and supplements**, continuous, untransformed), Total Zinc Intake x SNP

Covariates: Sex, Age (years, continuous variable), **Body Mass Index (kg/m^2, continuous variable)**, Field center (if needed), Population substructure adjustment as needed

Data Sharing

- **Regression β coefficient, SE and p-value from models 1a and 1b for:**
  - ✓ Dietary Zinc intake (food sources) x SNP interaction term
  - ✓ SNP marginal effect term
  - ✓ Dietary Zinc intake (food sources) marginal effect term
  - ✓ Intercept term
- **Regression β coefficient, SE and p-value from models 2a and 2b for:**
  - ✓ Total Zinc intake (food sources and supplements) x SNP interaction term
  - ✓ SNP marginal effect term
  - ✓ Total Zinc intake (food sources and supplements) marginal effect term
  - ✓ Intercept term
- **Mean and SD, SE or % for:**
  - ✓ Sex distribution (% female)
  - ✓ Age (mean years ± SD, SE)
  - ✓ Body Mass Index (mean kg/m^2 ± SD, SE)
  - ✓ Fasting glucose levels (mean mmol/L ± SD, SE)
  - ✓ Dietary Zinc intake (mean mg/day ± SD, SE)
  - ✓ Total Zinc intake (mean mg/day ± SD, SE)

Meta-analysis

Meta-analysis will be conducted on the regression β coefficients for the Dietary Zinc intake x SNP interaction terms derived from all cohorts and for each SNP.

Significance: $p \leq 0.003$ for the interaction term in each meta-analysis, based on Bonferrroni correction for multiple testing (0.05/20 SNPs = 0.0025≈0.003).

7.a. Will the data be used for non-CVD analysis in this manuscript? __✓__ Yes ____ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? __✓__ Yes ____ No

(This file ICTDER03 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?
_✓_ Yes ____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"?
_✓_ Yes ____ No

9.The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

__✓__ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __✓__ Yes ____ No

GWAS via STAMPEDE & GENEVA, #2006.03
Interactions between Diet and Genes Related to Risk of Type II Diabetes, #2007.12

11.b. If yes—is the proposal a primarily the result of an ancillary study

(numbers 2007.12 and 2006.03)

ARIC is one of 12 cohort studies contributing data to the CHARGE/MAGIC-based meta-analysis.

Since this work is a product of CHARGE which utilizes GWA data, ancillaries related to STAMPEDE & GENVA are also acknowledged.

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.