Exploring genome-wide – dietary heme iron intake interactions and the risk of type 2 diabetes

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Aims/hypothesis: Genome-wide association studies have identified over 50 new genetic loci for type 2 diabetes (T2D). Several studies conclude that higher dietary heme iron intake increases the risk of T2D. Therefore, we assessed whether the relation between genetic loci and T2D is modified by dietary heme iron intake.

Methods: We used Affymetrix Genome-Wide Human 6.0 array data (681,770 single nucleotide polymorphisms (SNPs)) and dietary information collected in the Health Professionals Follow-up Study (n = 725 cases; n = 1,273 controls) and the Nurses’ Health Study (n = 1,081 cases; n = 1,692 controls). We assessed whether genome-wide SNPs or iron metabolism SNPs interacted with dietary heme iron intake in relation to T2D, testing for associations in each cohort separately and then meta-analyzing to pool the results. Finally, we created 1,000 synthetic pathways matched to an iron metabolism pathway on number of genes, and number of SNPs in each gene. We compared the iron metabolic pathway SNPs with these synthetic SNP assemblies in their relation to T2D to assess if the pathway as a whole interacts with dietary heme iron intake.

Results: Using a genomic approach, we found no significant gene–environment interactions with dietary heme iron intake in relation to T2D at a Bonferroni corrected genome-wide significance level of 7.33 x 10^-8 (top SNP in pooled analysis: intergenic rs10980508; p = 1.03 x 10^-6). Furthermore, no SNP in the iron metabolic pathway significantly interacted with dietary heme iron intake at a Bonferroni corrected significance level of 2.10 x 10^-4 (top SNP in pooled analysis: rs11805313; p = 1.14 x 10^-3). Finally, neither the main genetic effects (pooled empirical p-value for the interactions = 0.72) were significant for the iron metabolic pathway as a whole.

Conclusions: We found no significant interactions between dietary heme iron intake and common SNPs in relation to T2D.

Keywords: type 2 diabetes, gene environment interactions, dietary heme iron, pathway analysis

INTRODUCTION

Type 2 diabetes (T2D) is a multifactorial condition whereby insulin resistance and beta-cell dysfunction produce glucose metabolism alterations, most notably hyperglycemia, resulting in microvascular and macrovascular complications. T2D affects over 25 million individuals (greater than 8% of the U.S. adult population; American Diabetes Association, 2011). Discovery of the combination of genetic and environmental factors contributing to T2D is essential so that more targeted preventive and management strategies can be devised.

Dietary heme iron intake is the iron derived from hemoglobin, the protein in the red blood cells found in animal foods such as red meat, fish, and poultry. A recent meta-analysis demonstrated that non-heme iron intake (such as iron derived from vegetables), total iron (iron from heme and non-heme sources) or iron supplements were not associated with increased risk of T2D (Bao et al., 2012). However, epidemiologic studies indicate that increased total body iron stores are associated with an increased risk of T2D (Salonen et al., 1998; Jiang et al., 2004b). While the relation between dietary heme iron intake and obesity has not been well-studied, diets with higher heme iron intake have consistently been associated with increased risk of T2D (Jiang et al., 2004a; Lee et al., 2004; Song et al., 2004; Rapapath et al., 2006). Iron is a catalyst in the formation of hydroxyl radicals, which are powerful pro-oxidants that attack cellular membrane lipids, proteins, and nucleic acids (Nelson, 1999). While heme iron intake is not directly linked to
Table 1 Characteristics of Health Professionals Follow-up Study (HPFS) and Nurses' Health Study (NHS) cohorts.

| Cohort          | HPFS Cases/Controls | Mean heme, SD (mg/day) | Mean body mass index, SD (kg/m²) | Mean age at baseline, SD (years) |
|-----------------|---------------------|------------------------|----------------------------------|----------------------------------|
| Cases/Controls  | 19987251273         | 1.29 (0.43139, 0.43123) (0.42) | 25.05 (3.462737, 3.821250.01) (2.74) | 54.19 (8.3652.93, 8.1654.91) (8.39) |
| NHS Cases/Controls | 277310811692     | 1.36 (0.45141, 0.44133) (0.45) | 26.08 (5.002866, 4.952443) (4.29) | 47.47 (8.764731, 7.734757) (6.76) |

SD: standard deviation.
We identified 237 SNPs in genes coding for enzymes in this pathway. In men and women with higher dietary heme iron intake than controls in men and women (8 years) and women (4.8 years). As expected, cases had higher BMI and higher mean dietary heme iron intake than controls in men and women (Table 1). Dietary heme iron intake was adversely associated with T2D (OR = 1.36 (1.17, 1.58); pooled p = 7.51 × 10\(^{-11}\); Model 1). As expected, the top SNP associated with T2D was in TCF7L2 (rs7901695; pooled p-value = 1.88 × 10\(^{-14}\)) (Model 2).

Using the 1-df test (Model 3), no gene–environment interaction achieved genome-wide significance level. The most significant interaction with continuous dietary heme iron intake was rs10998508 (pooled p = 1.03 × 10\(^{-6}\); an intergenic SNP between muscle, skeletal, receptor tyrosine kinase (MUSK) and Sushi, von Willebrand factor type A, EGF, and pentraxin domains-containing 1 (SVEP1; Table 3). The 2-df test revealed that SNPs in TCF7L2 had genome-wide margin association with T2D but did not reveal new marginal gene effects of genome-wide significance; nor was there significant interaction between TCF7L2 and dietary heme iron intake in T2D (data not shown). When we generated models substituting dietary heme intake with red meat, processed meat, and total meat, we found similar results with top marginal genetic effects in TCF7L2; yet, Model 3 did not yield significant gene–environment interactions (data not shown).

No significant iron metabolism SNP – dietary heme iron intake interaction was detected with the 1-df test (Model 3) in relation to T2D (top SNP rs1805313; in ALAD (delta-aminolevulinic dehydratase); pooled p = 1.14 × 10\(^{-2}\); Bonferroni corrected significance level p = 2.10 × 10\(^{-14}\)). The 2-df test of gene and gene–environment interactions also did not reveal any significant interactions between dietary heme iron intake and SNPs in the iron metabolic pathway SNP in pooled analyses (data not shown).

Compared with synthetic pathways, the iron metabolic pathway was not associated with T2D when we performed the analyses by SNP (pooled empirical p by SNP = 0.41). Similar null results were obtained when interactions with dietary heme iron intake were considered (pooled empirical p-value for the interactions =0.72). Interactions between various forms of dietary meat intake and the iron metabolic pathway were also not significant.

**DISCUSSION**

Neither the 1-df test nor the 2-df test revealed any genome-wide significant interactions between dietary heme iron intake and genomic SNPs in T2D. Furthermore, the relation between an iron metabolic pathway SNP panel and T2D was not modified by dietary heme iron intake. Finally the iron metabolic pathway was not enriched with SNPs related to T2D.

There could be several possible reasons for the null results reported here. First, despite its large size (n = 4,771) our study could be underpowered to find modest interaction terms. In fact, only in a log additive model would we achieve 80% power to detect a genome-wide environmental interaction effect of 1.8 (assumes minor allele frequency = 0.4; genetic relative risk = 1.2, and relative risk of the highest tertile of dietary heme iron intake = 1.3). Nonetheless we had ~80% power to discover an interaction effect of 1.5 between iron metabolic SNPs and dietary heme iron intake in relation to T2D using similar assumptions in a dominant inheritance model. Second, power could be compromised due to inherent error in measuring dietary heme iron intake. Third, self-reported iron intake, while collected using a validated food frequency questionnaire, could be prone to recall bias. Finally, the
Table 2 | Genes and single nucleotide polymorphisms (SNPs) in the heme iron metabolic pathway on the Affymetrix 6.0 array that passed quality control.

| Gene   | Chromosome | SNPs                                                                 |
|--------|------------|----------------------------------------------------------------------|
| ACO1   | 24         | rs1028932, rs10345792, rs1031838, rs1393491, rs1293491, rs2006738, rs7032871, rs10738885, rs16918276, rs3780473, rs3780474, rs449514, rs13302577, rs7866419, rs7022554, rs4879631, rs10570961, rs7033149, rs10970972, rs10970974, rs7019520, rs1236250, rs1233941, rs3780474 |
| ALAD   | 7          | rs1677860, rs1677904, rs1688842, rs2283169, rs1376932, rs1685313, rs1685316 |
| ALAS1  | 6          | rs532766, rs327662, rs327669, rs1172164, rs392170, rs8943468 |
| ALVR4  | 8          | rs2324352, rs489161, rs699510, rs489162, rs489165, rs10233867, rs10268054, rs1317916 |
| ALVR5  | 11         | rs2136943 |
| HCP1   | 17         | rs1778738, rs3816893, rs3772006, rs6881598, rs6881634, rs16861580, rs4943389, rs10701755, rs362828, rs3017552, rs16681137, rs13075921, rs8633335, rs1879619, rs737050, rs16861679, rs11929061 |
| HMBS   | 7          | rs1799993, rs1784304, rs1006195, rs4940046, rs1144041, rs17075, rs649893 |
| HMOX2  | 16         | rs4786500, rs2160567, rs3002781, rs1706834, rs7386938, rs4786501, rs2270366, rs10500325, rs6036864, rs4786502, rs903475, rs7180551, rs8098352, rs844958, rs6055519 |
| IL6R   | 16         | rs11265618, rs4849628, rs4337456, rs10702641, rs4246082, rs11260616, rs6683618, rs1260250, rs1425941, rs4616618, rs10150236, rs6683993, rs1206935, rs2059267, rs2285942, rs1474476 |
| IRF5   | 18         | rs13180, rs268491, rs903421, rs268490, rs1478929, rs1698699, rs2660711, rs1698698, rs1746271, rs903472, rs6403272, rs10510198, rs7180418, rs5864843, rs2666707, rs8041628, rs11636431, rs2584942 |
| SLC2A3  | 18         | rs1712512, rs2286983, rs224672, rs224672, rs224679 |
| SLC5A1  | 33         | rs762624, rs2165716, rs1789832, rs2429419, rs1789834, rs6035754, rs7643586, rs17088935, rs1776719, rs2506872, rs762778, rs7946625, rs736032, rs7834883, rs783019, rs2026661, rs816624, rs10503752, rs7895881, rs2042204, rs2787471, rs8059352, rs7890532, rs7890532, rs7899332, rs1718222, rs7015188, rs12544753, rs7629004, rs13266950, rs17089358, rs2046444, rs4781881, rs4781880, rs7634536 |
| SLC4A1  | 10         | rs4676272, rs3047043, rs1439816, rs3003733, rs1231110, rs3790297, rs11569350, rs13431938, rs1121309, rs13440417 |
| SLC5A2  | 6          | rs7344486, rs10592313, rs16862796, rs12457546, rs946998 |
| SLC7A8  | 16         | rs8029582, rs2268090, rs4776646, rs17880202, rs6089845, rs3965988, rs7215104, rs494756, rs47899444, rs47899451, rs47899457, rs47899458, rs6036426, rs503698, rs1026919, rs211777, rs3816769, rs9112773 |
| SLC7A9  | 30         | rs580875, rs580765, rs7366935, rs89674, rs1271924, rs15427945, rs7065611, rs806083, rs706872, rs380722, rs7087670, rs665686, rs380065, rs12465926, rs1888609, rs381013, rs12990970, rs10182241, rs380890, rs1887856, rs10188946, rs803905, rs4509354, rs1204548, rs380897, rs6425417, rs838102, rs1867794, rs13401954, rs7220040 |
| TFR    | 37         | rs4854799, rs1772201, rs1358652, rs1772201, rs1773006, rs1049296, rs2719627, rs1780882, rs48314535, rs3033232, rs779311, rs18002272, rs17722241, rs17727227, rs17722250, rs1772232, rs7787632, rs17727222, rs12493968, rs2718796, rs115219, rs1772523, rs1772519, rs17722248, rs1256992, rs1772286, rs7645358, rs1771991, rs1772215, rs8181657 |
| TFR2   | 1          | rs4521695 |
| TNF    | 6          | rs3933, rs39304114, rs3804142, rs4672886, rs486786, rs12352245 |
| WDR20  | 7          | rs13938 |
| WDR55  | 12         | rs9519149, rs1571278, rs2281996, rs70394025, rs38146624, rs11244653, rs10061460, rs20275159, rs7340179, rs17075153, rs2149019, rs12531135 |

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Table 3 | Top 10 p-values from genome-wide SNP-heme interactions predicting type 2 diabetes adjusting for age, body mass index, dietary iron intake, and eigenvectors using a one degree of freedom test from meta-analysis of the Health Professionals Follow-up Study and Nurses’ Health Study.1

| Chromosome | SNP | Beta, p-value (heme) | Beta, p-value (non-heme) | p-Value, pooled (heme) | p-Value, pooled (non-heme) | Associated gene |
|------------|-----|---------------------|-------------------------|------------------------|---------------------------|----------------|
| 9          | rs10865588 | 0.96 | 0.64 | 1.81 × 10^{-4} | 1.23 × 10^{-3} | 1.03 × 10^{-6} | Upstream of MUSK, downstream of SVEP1 |
| 9          | rs1327374/4 | 0.96 | 0.61 | 1.77 × 10^{-4} | 1.78 × 10^{-3} | 1.51 × 10^{-6} | Upstream of MUSK, downstream of SVEP1 |
| 9          | rs18170949 | 0.92 | 0.61 | 3.09 × 10^{-4} | 1.61 × 10^{-3} | 2.16 × 10^{-6} | Upstream of MUSK, downstream of SVEP1 |
| 9          | rs7046110 | 0.96 | 0.58 | 1.80 × 10^{-4} | 2.75 × 10^{-3} | 2.53 × 10^{-6} | Upstream of MUSK, downstream of SVEP1 |
| 9          | rs18170752 | 0.90 | 0.60 | 4.43 × 10^{-4} | 1.93 × 10^{-3} | 3.53 × 10^{-6} | Upstream of MUSK, downstream of SVEP1 |
| 16         | rs17173708 | 1.48 | 0.83 | 1.50 × 10^{-4} | 5.66 × 10^{-3} | 5.06 × 10^{-6} | Intron in TPRCA6 |
| 9          | rs10960495 | 0.91 | 0.57 | 3.60 × 10^{-4} | 3.33 × 10^{-3} | 5.49 × 10^{-6} | Downstream of AGR2, upstream of IRF5 |
| 7          | rs10448267 | 0.94 | 0.64 | 2.58 × 10^{-4} | 4.95 × 10^{-3} | 6.58 × 10^{-6} | Upstream of MUSK, downstream of SVEP1 |
| 22         | rs407088 | –0.59 | –0.55 | 2.90 × 10^{-3} | 9.43 × 10^{-4} | 8.64 × 10^{-6} | Intron in SULT4A1 |

1Metacontrols for BMI (kg/m²) and age (years), both as continuous variables, continuous mg dietary heme intake per day, centered on the mean heme value for each cohort and eigenvectors 1–3 for NNS or eigenvectors 1–4 for HPFS. SNPp are coded as 0, 1, or 2 minor alleles.
2Betas and p-values are from the β (gene variant) × dietary heme iron intake term in Model 3: T2D = β1 (BMI) + β2 (age) + β3 (dietary heme iron intake) + β4 (gene variant) × β3 (dietary heme iron intake) + eigenvector.
3P for heterogeneity < 0.05 (lowest p-value > 0.4).
4HPS, Health Professionals Follow-up Study; NNS, Nurses’ Health Study; MUSK, muscle, skeletal, receptor tyrosine kinase; SVEP1, Sushi, von Willebrand factor type A, EGF, and pentraxin domains-containing 1; TPRCA6, triunucleotide repeat-containing gene 6A; AGR2, anterior gradient 2; SULT4A1, sulfotransferase family 4A, member 1.

The reason why a higher dietary heme iron dietary intake increases the risk of T2D could be solely related to environmental influences.1

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