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Genetic variants in Selenoprotein P plasma 1 gene (*SEPP1*) are associated with fasting insulin and first phase insulin response in Hispanics

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Abstract

Context—Insulin resistance is not fully explained on a molecular level, though several genes and proteins have been tied to this defect. Knockdowns of the *SEPP1* gene, which encodes the Selenoprotein P (SeP) protein, have been shown to increase insulin sensitivity in mice. SeP is a liver-derived plasma protein and a major supplier of selenium, which is a proposed insulin mimetic and antidiabetic agent.

Objective—*SEPP1* single nucleotide polymorphisms (SNPs) were selected for analysis with glucometabolic measures.

Participants and Measures—1424 Hispanics from families in the Insulin Resistance Atherosclerosis Family Study (IRASFS). Additionally, the multi-ethnic Insulin Resistance Atherosclerosis Study was used. A frequently sampled intravenous glucose tolerance test was used to obtain precise measures of acute insulin response (AIR) and the insulin sensitivity index (S_I).

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Design—21 *SEPP1* SNPs (tagging SNPs (n=12) from HapMap, 4 coding variants and 6 SNPs in the promoter region) were genotyped and analyzed for association.

Results—Two highly correlated ($r^2=1$) SNPs showed association with AIR (rs28919926; Cys368Arg; $p=0.0028$ and rs146125471; Ile293Met; $p=0.0026$) while rs16872779 (intronic) was associated with fasting insulin levels ($p=0.0097$). In the smaller IRAS Hispanic cohort, few of the associations seen in the IRASFS were replicated, but meta-analysis of IRASFS and all 3 IRAS cohorts (N= 2446) supported association of rs28919926 and rs146125471 with AIR ($p=0.013$ and 0.0047 , respectively) as well as rs7579 with S_1 ($p=0.047$).

Conclusions—Overall, these results in a human sample are consistent with the literature suggesting a role for *SEPP1* in insulin resistance.

Keywords

Acute Insulin Response (AIR); Selenium; Selenoproteins; Insulin Resistance; Fibrinogen; Hispanic Americans

Introduction

Selenoproteins are the primary sequestering unit of selenium obtained from the diet. Most selenoproteins contain only one selenocysteine residue at the enzyme active site, though selenoprotein P (SeP) in humans and rodents harbors 10 of these (Hill et al., 1993). Selenium is a nutrient essential for human health, and has been shown to play a role in proper immune function, thyroid function, and fertility (Schwarz and Foltz, 1958; Hill et al., 2003; Beckett and Arthur, 2005; Ferguson et al., 2012). Serum selenium levels have been shown to correlate with serum lipids in several studies (Stranges et al., 2010; Stranges et al., 2011). Selenium is also an insulin mimetic and has anti-diabetic properties in mouse models (Stapleton, 2000).

Selenoprotein P, encoded by the *SEPP1* gene, is present in the plasma at high concentrations (5–6 mg/liter) in humans (Burk et al., 2001). Two isoforms of this protein in humans have been well documented; one containing the full length transcript and all 10 selenocysteine residues, and the other is truncated prematurely, leaving only 1 selenocysteine residue (Mostert et al., 1998). Newer evidence suggests that there might be three isoforms, however, one of these did not contain any selenocysteine residues (Ballihaut et al., 2012). The liver produces most plasma SeP, contributing about 75% to the circulation, though almost all tissues express the protein, (Dreher et al., 1997). Serum levels of SeP in humans are considered a biomarker of selenium status in the entire body (Xia et al., 2005). SeP has been widely studied for association with metabolic health, including measures of insulin sensitivity, glucose control, body mass, C-reactive protein, serum lipids and carotid intima-media thickness (Yang et al., 2011). SeP levels and/or expression have been associated with insulin resistance (Misu et al., 2010; Zeng et al., 2012), obesity (Zhang and Chen, 2010; Choi et al., 2013) and nonalcoholic fatty liver disease (Choi et al., 2013) in humans and animal models.

Genetic variation in *SEPP1* has been reported to be associated with several metabolic phenotypes. Two single nucleotide polymorphisms (SNPs) in *SEPP1* were reported to have functional consequences on protein level and/or function. The coding SNP rs3877899 (Ala234Thr) has been shown to influence plasma selenium levels as well as plasma levels of SeP in both European Americans and South Asians (Meplan et al., 2007; Karunasinghe et al., 2012). A SNP in the 3' UTR (rs7579) has also been shown to affect the response of SeP to supplementation of selenium in the same populations (Meplan et al., 2007). Both of these variants also influenced the proportion of two SeP isoforms (~60 and ~50 kDa) in men and

women taking selenium (Se) supplements (Meplan et al., 2007). Genetic variants within *SEPP1* have not previously been evaluated for association with measures of insulin resistance, insulin response, or adiposity.

The roles of selenium and SeP with the pathophysiology of type 2 diabetes (T2D) have been recently reviewed, with conclusions that although there is significant evidence linking selenium and SeP to glucose metabolism and insulin resistance, the relationship between SeP and selenium, and T2D is complex and necessitates further study (Mao and Teng, 2013; Rayman and Stranges, 2013). The hypothesis for this study was that coding and non-coding variants in *SEPP1* would be associated with dynamic measures of glucose homeostasis and adiposity as well as related traits, such as inflammation, liver density and lipid levels.

Methods

Samples

The primary sample used for this study was the Hispanic cohort of the Insulin Resistance and Atherosclerosis Family Study (IRASFS) (Henkin et al., 2003). Briefly, subjects were ascertained on the basis of large family size in San Luis Valley, Colorado and San Antonio, Texas. Recruitment was based on family size rather than specific clinical characteristics. Sample phenotypes are summarized in Table 1. These subjects were extensively phenotyped, including a frequently sampled intravenous glucose test (FSIGT), measures of blood lipids and inflammatory markers, anthropomorphic measures, as well as fat deposition and liver density measures by computed tomography (CT) scan. Insulin sensitivity (S_I) was calculated from FSIGT data using mathematical modeling methods (MINMOD version 3.0; Harms Software, Los Angeles, CA). Acute insulin response (AIR) was calculated as the mean of 2- and 4-min insulin concentrations after glucose administration. Metabolic clearance rate of insulin (MCRI) was calculated as the ratio of the insulin dose over the incremental area under the curve of insulin from 20 min to infinity.

A second sample group was from the Insulin Resistance Atherosclerosis Study (IRAS), a predecessor of IRASFS. IRAS is a multi-ethnic epidemiological cohort of unrelated subjects consisting of 480 European Americans, 384 Hispanics and 288 African Americans (Wagenknecht et al., 1995). These samples were phenotyped in a manner similar to the IRASFS with FSIGT measures, blood lipids and a limited number of inflammatory markers. IRB approval was obtained at all clinical and analysis sites and all participants provided informed consent.

SNP Selection and Genotyping

SNPs were chosen to tag the *SEPP1* gene using HaploView ($r^2 = 0.8$) in the HapMap CEU (CEPH: Utah residents with ancestry from northern and western Europe) and MEX (Mexican ancestry in Los Angeles, California) populations, including SNPs from the promoter region. Additional coding SNPs from the NHLBI Exome Sequencing Project database (<http://snp.gs.washington.edu/EVS>) were also included for genotyping (Table 2). Genotyping of SNPs was performed using the Sequenom iPLEX Mass Array system (Sequenom; San Diego, CA) (Gabriel et al., 2009). Blind duplicates ($n=80$) were genotyped for quality control purposes and had a concordance rate of greater than 98.5%. SNP and plate call rate thresholds were set at $>90\%$. Only one SNP (rs28919923) failed to exceed this threshold in the initial IRASFS genotyping; none failed in the multi-ethnic IRAS cohort.

Statistical Analysis

PedCheck (O'Connell and Weeks, 1998) was used to identify Mendelian inconsistencies in the data ($N=72$) and they were resolved by removing conflicted genotypes ($n=118$). Hardy-

Weinberg equilibrium and linkage disequilibrium were assessed using a subset of unrelated individuals (n=229) and the Haploview program (Barrett et al., 2005). Hardy-Weinberg p-values ranged from 0.0045–1, with only 1 variant (rs6897301) having a p-value less than 0.01. Association analysis for IRAS-FS was performed using Sequential Oligogenic Linkage Analysis Routines (SOLAR) (Almasy and Blangero, 1998). The effective number of SNPs was computed using the Moskvina & Schmidt method in SOLAR as 16 independent SNPs based on LD correlations, and the required p-value for significance after correction was set at 0.0032. Although several phenotypes were analyzed with these SNPs, many of these are correlated with one another, for instance, BMI and S_I , and therefore they were not accounted for in the number of comparisons. Hardy-Weinberg equilibrium and association analysis for the IRAS samples were performed separately for each ethnic group using PLINK v1.07 (Purcell et al., 2007). One SNP (rs16872779) deviated from Hardy-Weinberg equilibrium in the IRAS HA sample ($p=4.27 \times 10^{-8}$) and was subsequently removed from analysis for this sample set. Trait variables were transformed to approximate normal distributions as follows: S_I , fasting insulin, HOMA-IR, MCRI, total cholesterol, HDL, BMI, VSR, waist circumference, WHR, fibrinogen, and PAI-1 were log transformed, ApoB, LDL, SAT and VAT were square root transformed, and DI and AIR were square root transformed but retained the mathematical sign of the original value. Fasting glucose and glucose effectiveness were not transformed. Covariates used in the analysis included age, sex, BMI, recruitment center, and admixture estimates (admixture estimates were used in IRASFS only; IRAS samples were analyzed within ethnic group). Admixture was estimated using a principal component analysis of 80 ancestry informative markers (AIMs) (Palmer et al., 2010). BMI was omitted as a covariate when assessing BMI, but was used for all other measures of adiposity and body composition. Meta-analysis was performed using the fixed effects model implemented in METAL (Willer et al., 2010).

Results

The initial analysis included 1424 Hispanic individuals from 90 families in the IRASFS. Demographic characteristics of these IRASFS subjects and, in addition, subjects from IRAS, are summarized in Table 1. The sample is representative of a cross section of the Hispanic population with both rural (San Luis Valley, CO) and urban (San Antonio, TX) areas represented. Twenty-one SNPs (Table 2, Figure 1) chosen to tag the *SEPP1* gene and to test known coding variants in the population were successfully genotyped in this Hispanic sample. The results of association analysis of the resulting data are shown in Table 3 which lists all nominal ($p < 0.01$) associations found with *SEPP1* SNPs and all available phenotypes. A complete listing of association analysis results is summarized in Supplementary Table 1. Two coding SNPs showed evidence of association with acute insulin response (AIR): rs28919926 (Cys368Arg) and rs146125471 (Ile293Met) with p-values of 0.0028 and 0.0026, respectively. These two SNPs were perfectly correlated ($r^2=1$) in this sample (Supplemental Figure 1). These two SNPs were also strongly associated with insulin clearance (MCRI; $p=0.0016$ and 0.0010). A different SNP (rs16872779) was nominally associated with fasting insulin levels ($p=0.0097$). Nominal associations were also seen with homeostatic model assessment of insulin resistance (HOMA-IR) (7 SNPs $p<0.05$, 6 additive model, 1 recessive) and S_I (3 SNPs $p<0.05$, 2 additive, 1 dominant). The cytokine IL6, a marker of inflammation, was also significant with two SNPs (rs72554691 and rs7719242; $p=0.00052$, additive, and 0.0083 , dominant respectively), and nominally associated with an additional two SNPs ($p<0.05$, 1 dominant, 1 recessive) while TNF-receptor 1 levels were significantly associated with two non-coding SNPs (rs12519443; $p=0.0040$ and rs7719242, $p=0.0048$, both recessive model). Measures of adiposity were also associated with SNPs in this gene: two SNPs, rs16872762 and rs28919891 were associated with visceral adipose tissue (VAT; $p=0.0086$, additive, and 0.00016 , recessive, respectively), one of which (rs16872762) was also associated with waist/hip ratio (WHR; $p=0.0091$,

additive). The SNP rs16872762 was also nominally associated ($p < 0.05$) with both visceral to subcutaneous ratio and waist circumference. One variant was also associated with apolipoprotein B (ApoB) levels under a dominant model: rs6413428 with a p-value of 0.0028.

To further analyze these SNPs in a variety of ethnic groups, the multi-ethnic IRAS sample was genotyped and association analysis performed. All 21 SNPs were successfully genotyped in this sample. Each of the three ethnic groups was analyzed separately for association. In the IRAS Hispanics ($n=384$; Supplemental Table 2a), notable associations included rs230819 with fibrinogen ($p=0.0061$, recessive), rs4987017 with ApoB ($p=0.0017$, dominant), rs6882786 with plasminogen activator inhibitor-1 (PAI-1; $p=0.0096$, additive), and rs7579 with fasting insulin and LDL ($p=0.0078$, additive, and 0.0044, dominant, respectively). The latter replicates the observation that increased LDL levels were nominally associated with rs7579 in the IRASFS cohort ($p=0.026$, dominant). None of the other variants replicated the direction of effect of associated traits from IRASFS. Several associations were found with alternative traits in the European Americans (Supplemental Table 2b) and African Americans (Supplemental Table 2c) in IRAS. Many of these results, e.g. rs6413428, rs10512806, and rs3877899 with ApoB ($p < 0.01$), support evidence of association seen in the IRASFS Hispanic sample.

Meta-analysis of the three ethnic groups in IRAS (Table 4) revealed several SNPs with evidence of association with glucose homeostasis traits ($p < 0.05$). The SNP rs6882786 was associated with S_I , AIR and fasting insulin with p-values < 0.05 . Nominal associations were seen with rs7579 and S_I , rs13154178 and fasting glucose, and rs1672762 and AIR.

Combining results from the larger IRASFS Hispanic cohort ($n=1424$) with the smaller IRAS Hispanic cohort ($n=384$) using meta-analysis revealed suggestive evidence for association with glucose homeostasis traits (Supplementary Table 3). Both rs146125471 and rs28919896 were associated with AIR ($p=0.0041$ and 0.0047, respectively), while four SNPs were associated with fasting insulin levels (rs230819, rs16872779, rs16872762, and rs7579; $p < 0.02$). One of these, rs230819, was also associated with S_I ($p=0.011$). Additionally, ApoB levels were associated with two SNPs (rs6413428 and rs4987017) which were also associated with BMI and total cholesterol levels, respectively. Another SNP, rs3877899 was also associated with BMI.

Meta-analysis of all cohorts (Table 5) identified several variants with consistent direction of effect across all ethnic groups. For the glucose homeostasis traits, the most significant result was rs146125471 with AIR (meta-analysis p-value of 0.0047, additive model). Additionally, rs16872762 was associated with fasting insulin with a p-value of 0.028 (additive), while rs7579 was associated with S_I ($p=0.047$, dominant) and rs28919926 was associated with AIR ($p=0.013$, additive). Inflammatory markers were also associated across cohorts, with four markers being associated with fibrinogen (rs6882786, $p=0.0042$, dominant; rs77199242, $p=0.0056$, dominant; rs7579, $p=0.0191$, additive; and rs230819, $p=0.0263$, dominant), as well as rs12519943 with plasminogen activator inhibitor-1 (PAI-1, $p=0.027$, dominant). One of the lipid trait associations seen in the Hispanic meta-analysis remained associated in the full meta-analysis (rs4987017 with ApoB, $p=0.031$, dominant), and another SNP (rs1052806, $p=0.039$, recessive) also was associated with this trait. Additionally, rs6882786 was associated with total cholesterol ($p=0.020$, dominant).

Discussion

While individual *SEPP1* SNPs do not yield striking evidence of association, overall there was a consistent pattern for association of *SEPP1* variants with multiple metabolic

measures. This was consistent with the functional role ascribed to the SeP protein, that is, regulation of metabolic processes. Many of these glucometabolic traits are correlated so a specific target for *SEPP1* is unclear, but these results are consistent with a global influence on metabolism. The prior literature suggested a role for SeP in insulin resistance and we hypothesized that we would see some evidence of association with measures of glucose homeostasis. Several such measures were in fact associated with polymorphisms in this gene (e.g. rs146125471 with AIR). The associations seen with ApoB suggest a further link to metabolic syndrome as well. Consistent with this is the striking associations with seen with fibrinogen, a thrombotic factor which has been shown to associate with increased risk of cardiovascular disease in diabetes patients (Ganda and Arkin, 1992; Bruno et al., 2001).

Previous studies of variants in *SEPP1* have focused on plasma selenium levels and response to selenium supplementation (Meplan et al., 2007; Penney et al., 2012). Other studies have examined the link between *SEPP1* variants and SeP isoform concentrations (Meplan et al., 2009). Two variants (rs230813 and rs230819) in *SEPP1* were shown to be nominally associated ($p=0.03$ and 0.02) with plasma malondialdehyde, a marker of oxidative stress, but not with measures of SeP or other selenoprotein activities (Takata et al., 2012). Another *SEPP1* variant (rs3877899) was shown to be correlated with thioredoxin reductase activity ($p<0.003$, major allele homozygote and heterozygote genotypes), as well as with glutathione peroxidase 1 (GPX1) activity, but not with DNA damage after peroxide challenge (Karunasinghe et al., 2012). Additionally, the *SEPP1* SNP rs7579 was nominally associated ($p<0.05$) with reduced urine and hair mercury levels in a study of dental professionals (Goodrich et al., 2011). Other variants from a variety of glutathione s-transferases and glutathione synthesizing enzymes were also associated with one or both of those biomarkers.

Studies of genetic variation in other selenoproteins are often focused on the relationship of these genes and variants to colorectal cancer risk (Meplan et al., 2009; Méplan and Hesketh, 2012; Slattery et al., 2012). Variants in genes encoding glutathione peroxidases 1–4 were examined for association with markers of oxidative stress (plasma malondialdehyde and protein carbonyl content), GPX1 activity, GPX3 activity and SeP concentration (Takata et al., 2012). Only two SNPs in *GPX2* (rs4902346 and rs2071566) were shown to associate with SeP concentration; none of the other tests were significant.

SeP expression has been observed in macrophages which are suggested to have a role in protection against development of atherosclerosis (Brocheriou et al., 2011). Additionally, human SeP levels were found to be predictive of serum adiponectin levels (Misu et al., 2012), which are negatively correlated with type 2 diabetes and cardiovascular disease, though no SNP was associated with adiponectin at $P<0.05$ (Supplemental Table 1) in the present study. Additional studies have shown that adiponectin treatment protected from insulin resistance when induced with palmitate cells via the inhibition of SeP (Jung et al., 2013).

It has previously been shown that treatment of mice with purified human SeP resulted in glucose intolerance and insulin resistance (Misu et al., 2010). That group also demonstrated that knockdown of SeP using siRNA techniques improved both glucose tolerance and insulin resistance. In the same study, mice which were SeP-deficient showed reduced plasma insulin after feeding, while blood glucose levels remained the same. These knockout mice did show improved glucose tolerance, as well as an improved response of glucose to insulin injection. High fat, high sucrose diet in these mice did not result in significantly different weight gain between wild-type and knockout groups. This work extended the findings from a previous study in which SeP knockout mice had severely impacted motor control, loss of weight, and abnormal selenium distribution when presented with low

selenium intake, but glucose homeostasis was not measured in this set of experiments either (Hill et al., 2003).

Another study did examine the effect on selenium on glucose and body weight in streptozotocin-induced diabetic and non-diabetic rats, finding that selenite treatment resulted in a decrease in blood glucose in diabetic rats, but a slight increase in blood glucose in their non-diabetic counterparts (Can et al., 2005). Treatment also impacted liver tissue morphology, reversing the ultrastructural alterations seen in the untreated diabetic animals with little side effect. Comparison of several selenoprotein enzyme activity levels revealed that diabetic rats not treated with selenite had significantly lower activities than untreated control rats at 5 out of 6 selenoproteins measured ($p < 0.05$, glutathione reductase, glutathione peroxidase, glutathione-6-phosphate dehydrogenase, glutathione-S-transferase and 6-phosphogluconate dehydrogenase, but not acid soluble sulphhydryls), while treated diabetic rats had higher glutathione reductase activity and lower glutathione-6-phosphate dehydrogenase activity compared to their untreated counterparts, and higher acid soluble sulphhydryls activity when compared to the control animals. This study, however did not examine SeP activity.

This work is the first comprehensive analysis of *SEPP1* variants and glucometabolic traits. A limitation of the study is that we did not have selenium concentrations or selenoprotein enzyme activity levels available for these subjects. That data would be extremely valuable to elucidate the molecular and biochemical mechanisms connecting *SEPP1* genetic variants with the variety of glucometabolic traits, given the association data generated by this work. Despite this limitation, these genetic results are consistent with the literature suggesting an important role for *SEPP1* in metabolic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SeP	Selenoprotein P
IRAS	Insulin Resistance Atherosclerosis Study
IRASFS	Insulin Resistance Atherosclerosis Family Study
AIR	Acute Insulin Response
S_I	Insulin Sensitivity Index
DI	Disposition Index
ApoB	Apolipoprotein B
LDL	Low-density Lipoprotein
HDL	High-density Lipoprotein

HOMA-IR	Homeostasis model assessment of insulin resistance
MCRI	Metabolic Clearance Rate of Insulin
VAT	Visceral Adipose Tissue
SAT	Subcutaneous Adipose Tissue
WHR	Waist:Hip ratio
SNP	Single Nucleotide Polymorphism
PAI-1	Plasminogen Activator Inhibitor-1
SOLAR	Sequential Oligogenic Linkage Analysis Routines
FSIGT	Frequently Sampled Intravenous Glucose Tolerance Test
UTR	untranslated region
IL6	Interleukin 6
TNF	Tumor Necrosis Factor
BMI	Body Mass Index
T2D	Type 2 Diabetes Mellitus
MAF	Minor Allele Frequency
HA	Hispanic American
EA	European American
AA	African American

References

- International HapMap Project. International HapMap Consortium.
- NHLBI Exome Sequencing Project (ESP).
- Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *American Journal of Human Genetics*. 1998; 62:1198–211. [PubMed: 9545414]
- Ballihaut G, Kilpatrick L, Kilpatrick E, Davis W. Multiple forms of selenoprotein P in a candidate human plasma standard reference material. *Metallomics: integrated biometal science*. 2012; 4:533–538. [PubMed: 22552441]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–5. [PubMed: 15297300]
- Beckett GJ, Arthur JR. Selenium and endocrine systems. *J Endocrinol*. 2005; 184:455–65. [PubMed: 15749805]
- Brocheriou I, Maouche S, Durand H, Braunersreuther V, Le Naour G, Gratchev A, Koskas F, Mach F, Kzhyshkowska J, Ninio E. Antagonistic regulation of macrophage phenotype by M-CSF and GM-CSF: implication in atherosclerosis. *Atherosclerosis*. 2011; 214:316–24. [PubMed: 21159337]
- Bruno G, Cavallo-Perin P, Bargero G, Borra M, D’Errico N, Macchia G, Pagano G. Hyperfibrinogenemia and metabolic syndrome in type 2 diabetes: a population-based study. *Diabetes/metabolism research and reviews*. 2001; 17:124–30. [PubMed: 11307177]
- Burk RF, Hill KE, Motley AK. Plasma selenium in specific and non-specific forms. *Biofactors*. 2001; 14:107–14. [PubMed: 11568447]
- Can B, Ulusu NN, Kiliç K, Leyla Acan N, Saran Y, Turan B. Selenium treatment protects diabetes-induced biochemical and ultrastructural alterations in liver tissue. *Biol Trace Elem Res*. 2005; 105:135–50. [PubMed: 16034159]

- Choi HY, Hwang SY, Lee CH, Hong HC, Yang SJ, Yoo HJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Increased selenoprotein p levels in subjects with visceral obesity and nonalcoholic fatty liver disease. *Diabetes Metab J*. 2013; 37:63–71. [PubMed: 23439771]
- Dreher I, Jakobs TC, Kohrle J. Cloning and characterization of the human selenoprotein P promoter. Response of selenoprotein P expression to cytokines in liver cells. *The Journal of biological chemistry*. 1997; 272:29364–71. [PubMed: 9361018]
- Ferguson LR, Karunasinghe N, Zhu S, Wang AH. Selenium and its' role in the maintenance of genomic stability. *Mutat Res*. 2012; 733:100–10. [PubMed: 22234051]
- Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr Protoc Hum Genet*. 2009; Chapter 2(Unit 2):12. [PubMed: 19170031]
- Ganda OP, Arkin CF. Hyperfibrinogenemia. An important risk factor for vascular complications in diabetes. *Diabetes Care*. 1992; 15:1245–50. [PubMed: 1425083]
- Goodrich JM, Wang Y, Gillespie B, Werner R, Franzblau A, Basu N. Glutathione enzyme and selenoprotein polymorphisms associate with mercury biomarker levels in Michigan dental professionals. *Toxicol Appl Pharmacol*. 2011; 257:301–8. [PubMed: 21967774]
- Henkin L, Bergman RN, Bowden DW, Ellsworth DL, Haffner SM, Langefeld CD, Mitchell BD, Norris JM, Rewers M, Saad MF, Stamm E, Wagenknecht LE, Rich SS. Genetic epidemiology of insulin resistance and visceral adiposity. The IRAS Family Study design and methods. *Annals of Epidemiology*. 2003; 13:211–7. [PubMed: 12684185]
- Hill KE, Lloyd RS, Burk RF. Conserved nucleotide sequences in the open reading frame and 3' untranslated region of selenoprotein P mRNA. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90:537–41. [PubMed: 8421687]
- Hill KE, Zhou J, McMahan WJ, Motley AK, Atkins JF, Gesteland RF, Burk RF. Deletion of selenoprotein P alters distribution of selenium in the mouse. *The Journal of biological chemistry*. 2003; 278:13640–6. [PubMed: 12574155]
- Jung TW, Choi HY, Lee SY, Hong HC, Yang SJ, Yoo HJ, Youn BS, Baik SH, Choi KM. Salsalate and Adiponectin Improve Palmitate-Induced Insulin Resistance via Inhibition of Selenoprotein P through the AMPK-FOXO1alpha Pathway. *PLoS One*. 2013; 8:e66529. [PubMed: 23825542]
- Karunasinghe N, Han DY, Zhu S, Yu J, Lange K, Duan H, Medhora R, Singh N, Kan J, Alzahrer W, Chen B, Ko S, Triggs CM, Ferguson LR. Serum selenium and single-nucleotide polymorphisms in genes for selenoproteins: relationship to markers of oxidative stress in men from Auckland, New Zealand. *Genes Nutr*. 2012; 7:179–90. [PubMed: 22139612]
- Mao J, Teng W. The Relationship between Selenoprotein P and Glucose Metabolism in Experimental Studies. *Nutrients*. 2013; 5:1937–48. [PubMed: 23760059]
- Meplan C, Crosley LK, Nicol F, Beckett GJ, Howie AF, Hill KE, Horgan G, Mathers JC, Arthur JR, Hesketh JE. Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner (the SELGEN study). *FASEB J*. 2007; 21:3063–74. [PubMed: 17536041]
- Meplan C, Nicol F, Burtle BT, Crosley LK, Arthur JR, Mathers JC, Hesketh JE. Relative abundance of selenoprotein P isoforms in human plasma depends on genotype, se intake, and cancer status. *Antioxid Redox Signal*. 2009; 11:2631–40. [PubMed: 19453253]
- Misu H, Ishikura K, Kurita S, Takeshita Y, Ota T, Saito Y, Takahashi K, Kaneko S, Takamura T. Inverse correlation between serum levels of selenoprotein P and adiponectin in patients with type 2 diabetes. *PLoS One*. 2012; 7:e34952. [PubMed: 22496878]
- Misu H, Takamura T, Takayama H, Hayashi H, Matsuzawa-Nagata N, Kurita S, Ishikura K, Ando H, Takeshita Y, Ota T, Sakurai M, Yamashita T, Mizukoshi E, Honda M, Miyamoto K, Kubota T, Kubota N, Kadowaki T, Kim HJ, Lee IK, Minokoshi Y, Saito Y, Takahashi K, Yamada Y, Takakura N, Kaneko S. A liver-derived secretory protein, selenoprotein P, causes insulin resistance. *Cell Metab*. 2010; 12:483–95. [PubMed: 21035759]
- Mostert V, Lombeck I, Abel J. A novel method for the purification of selenoprotein P from human plasma. *Archives of biochemistry and biophysics*. 1998; 357:326–330. [PubMed: 9735174]
- Méplan C, Hesketh J. The influence of selenium and selenoprotein gene variants on colorectal cancer risk. *Mutagenesis*. 2012; 27:177–86. [PubMed: 22294765]

- O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *American Journal of Human Genetics*. 1998; 63:259–66. [PubMed: 9634505]
- Palmer ND, Langefeld CD, Ziegler JT, Hsu F, Haffner SM, Fingerlin T, Norris JM, Chen YI, Rich SS, Haritunians T, Taylor KD, Bergman RN, Rotter JI, Bowden DW. Candidate loci for insulin sensitivity and disposition index from a genome-wide association analysis of Hispanic participants in the Insulin Resistance Atherosclerosis (IRAS) Family Study. *Diabetologia*. 2010; 53:281–9. [PubMed: 19902172]
- Penney KL, Li H, Mucci LA, Loda M, Sesso HD, Stampfer MJ, Ma J. Selenoprotein P genetic variants and mRNA expression, circulating selenium, and prostate cancer risk and survival. *Prostate*. 2012
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*. 2007; 81:559–75. [PubMed: 17701901]
- Rayman MP, Stranges S. Epidemiology of selenium and type 2 diabetes: Can we make sense of it? *Free Radic Biol Med*. 2013
- Schwarz K, Foltz C. Factor 3 activity of selenium compounds. *The Journal of biological chemistry*. 1958; 233:245–251. [PubMed: 13563479]
- Slattery ML, Lundgreen A, Welbourn B, Corcoran C, Wolff RK. Genetic variation in selenoprotein genes, lifestyle, and risk of colon and rectal cancer. *PLoS One*. 2012; 7:e37312. [PubMed: 22615972]
- Stapleton SR. Selenium: an insulin-mimetic. *Cellular and molecular life sciences: CMLS*. 2000; 57:1874–9. [PubMed: 11215514]
- Stranges S, Galletti F, Farinara E, D'Elia L, Russo O, Iacone R, Capasso C, Carginale V, De Luca V, Della Valle E, Cappuccio FP, Strazzullo P. Associations of selenium status with cardiometabolic risk factors: an 8-year follow-up analysis of the Olivetti Heart study. *Atherosclerosis*. 2011; 217:274–8. [PubMed: 21497349]
- Stranges S, Tabak AG, Guallar E, Rayman MP, Akbaraly TN, Laclaustra M, Alftan G, Mussalo-Rauhamaa H, Viikari JS, Raitakari OT, Kivimaki M. Selenium status and blood lipids: the cardiovascular risk in Young Finns study. *J Intern Med*. 2010; 270:469–77. [PubMed: 21554435]
- Takata Y, King IB, Lampe JW, Burk RF, Hill KE, Santella RM, Kristal AR, Duggan DJ, Vaughan TL, Peters U. Genetic variation in GPX1 is associated with GPX1 activity in a comprehensive analysis of genetic variations in selenoenzyme genes and their activity and oxidative stress in humans. *J Nutr*. 2012; 142:419–26. [PubMed: 22259188]
- Wagenknecht LE, Mayer EJ, Rewers M, Haffner S, Selby J, Borok GM, Henkin L, Howard G, Savage PJ, Saad MF, Bergman RN, Hamman R. The insulin resistance atherosclerosis study (IRAS) objectives, design, and recruitment results. *Annals of Epidemiology*. 1995; 5:464–72. [PubMed: 8680609]
- Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010; 26:2190–1. [PubMed: 20616382]
- Xia Y, Hill K, Byrne D, Xu J, Burk R. Effectiveness of selenium supplements in a low-selenium area of China. *The American journal of clinical nutrition*. 2005; 81:829–834. [PubMed: 15817859]
- Yang SJ, Hwang SY, Choi HY, Yoo HJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Serum selenoprotein P levels in patients with type 2 diabetes and prediabetes: implications for insulin resistance, inflammation, and atherosclerosis. *The Journal of clinical endocrinology and metabolism*. 2011; 96:E1325–9. [PubMed: 21677040]
- Zeng MS, Li X, Liu Y, Zhao H, Zhou JC, Li K, Huang JQ, Sun LH, Tang JY, Xia XJ, Wang KN, Lei XG. A high-selenium diet induces insulin resistance in gestating rats and their offspring. *Free Radic Biol Med*. 2012; 52:1335–42. [PubMed: 22342560]
- Zhang Y, Chen X. Reducing selenoprotein P expression suppresses adipocyte differentiation as a result of increased preadipocyte inflammation. *Am J Physiol Endocrinol Metab*. 2010; 300:E77–85. [PubMed: 20959537]

Highlights

- Literature suggests link between selenium and insulin resistance mediated by *SEPP1*
- Evaluation of 22 polymorphisms in *SEPP1* region with measures of glucose homeostasis
- Several *SEPP1* SNPs show evidence of association with various metabolic measures
- Many of these traits are correlated, so a specific target for *SEPP1* remains unclear
- Some associations were found to be of consistent direction across ethnic groups

Genotyped SEPP1 SNPs

Figure 1.

Genotyped *SEPP1* SNPs with respect to the coding region. Black diamonds represent SNP location and items in bold are coding variants.

Table 1

Demographic and phenotypic characteristics of the IRASFS and IRAS cohorts, by ethnicity. Data presented as mean (SD). NA indicates a phenotype not used in IRAS samples.

Trait	Cohort			
	IRASFS	IRAS HA	IRAS EA	IRAS AA
Ethnicity	Hispanic	Hispanic	European American	African American
N (genotyped)	1424	384	480	288
Age (yrs)	42.81 (14.60)	55.14 (8.57)	56.17 (8.19)	55.35 (8.29)
Gender (%F)	51.9%	58.6%	51.9%	49.3%
BMI (kg/m²)	28.89 (6.71)	28.94 (5.35)	28.74 (5.68)	30.38 (6.19)
S_I (x 10⁻⁵ min⁻¹/[pmol/L])	2.14 (1.86)	1.61 (1.93)	1.98 (2.12)	1.47 (1.71)
AIR (pmol · ml⁻¹ · min⁻¹)	760.80 (648.87)	321.52(376.60)	209.48(244.48)	251.02(334.01)
DI (S_I · AIR)	1315.79(1234.31)	517.58(699.75)	416.53(545.52)	479.15(842.22)
Fasting Insulin (μU/ml)	14.93 (10.99)	18.72 (13.27)	16.48 (15.24)	18.21 (15.08)
Fasting Glucose (mg/dl)	93.38 (9.51)	123.98(54.45)	118.11 (40.62)	129.96 (53.45)
HOMA-IR	63.94 (52.09)	NA	NA	NA
Glucose Effectiveness (·100 min⁻¹)	0.021 (0.0089)	0.019 (0.0079)	0.019 (0.013)	0.017 (0.0084)
MCRI (mL/kg/min)	5.51 (2.58)	NA	NA	NA
Total Cholesterol (mg/dl)	178.05 (37.58)	209.26 (50.92)	212.03 (36.26)	214.07 (39.94)
HDL (mg/dl)	43.80 (13.01)	41.16 (13.31)	44.75 (14.66)	50.10 (14.51)
LDL (mg/dl)	109.26 (30.98)	136.07 (35.82)	140.56 (33.86)	145.92 (34.90)
ApoB (mg/dl)	89.24 (22.85)	109.77 (26.68)	105.13 (24.60)	104.36 (23.72)
Triglycerides (mg/dl)	124.78 (85.42)	156.94 (93.76)	143.21 (98.27)	101.96 (63.61)
Fibrinogen (mg/dl)	266.01 (62.39)	282.29 (57.49)	274.98 (57.13)	282.08 (62.70)
PAI-1 (ng/ml)	43.54 (39.31)	30.49 (26.86)	24.46 (18.90)	23.91 (22.99)

Table 2

SEPP1 SNPs genotyped with position, function, and minor allele frequencies by cohort.

SNP	Position ¹	Annotation	Amino Acid Change	IRASFS	MAF and Genotype Counts			
					IRAS HA	IRAS EA	IRAS AA	
rs16872762	42799925	utr-3		0.016 0/24/1194	0.017 0/12/337	0.014 0/13/437	0.009 0/5/276	
rs4987017	42800004	utr-3		0.012 1/21/1156	0.014 1/8/359	0 0/0/450	0.083 1/45/236	
rs6413428	42800724	utr-3		0.194 55/420/746	0.186 13/103/231	0.249 26/169/248	0.342 30/131/118	
rs7579	42800808	utr-3		0.398 424/578/221	0.395 55/181/132	0.287 31/194/221	0.166 8/77/196	
rs28919926	42800866	missense	Arg368Cys	0.009 0/15/1216	0.004 0/3/354	0.002 0/2/451	0.002 0/1/282	
rs28919923	42801135	missense	Arg308Glu	Failed	0 0/0/351	0 0/0/449	0 0/0/281	
rs146125471	42801179	missense	Met293Ile	0.009 0/14/1228	0.004 0/3/367	0.002 0/2/451	0 0/0/282	
rs3877899	42801268	missense	Ala234Thr	0.169 61/382/782	0.181 12/104/237	0.248 25/173/252	0.274 23/109/151	
rs13168440	42804115	intron		0.152 22/323/873	0.113 6/67/278	0.183 17/126/294	0.108 6/48/224	
rs16872779	42805037	intron		0.009 2/3/1204	HWE	0.000 0/0/445	0.005 0/3/278	
rs230819	42805965	intron		0.421 192/463/451	0.453 71/149/10	0.483 106/222/121	0.451 47/125/71	
rs28919895	42807080	missense	Pro112Ser	0.0004 0/1/1219	0	0 0/0/448	0 0/0/280	
rs72554691	42808371	missense	Lys29Glu	0.0004 0/1/1245	0 0/0/370	0 0/0/449	0.042 1/22/261	
rs28919893	42808532	intron		0.002 0/4/1229	0.003 0/2/350	0 0/0/450	0.053 2/26/253	
rs28919891	42809719	intron		0.046 1/114/1105	0.031 0/22/335	0.001 0/1/452	0.002 0/1/282	
rs28919886	42811450	intron		0.011 0/18/1227	0.004 0/3/351	0.006 0/5/449	0.042 0/24/261	
rs12519443	42819921	5'		0.394 203/559/443	0.375 47/171/135	0.258 30/171/247	0.067 3/32/248	

SNP	Position [/]	Annotation	Amino Acid Change	IRAFS	MAF and Genotype Counts			
					IRAS HA	IRAS EA	IRAS AA	
rs13154178	42828101	5'		0.224 54/477/676	0.228 22/125/223	0.296 37/191/220	0.406 48/133/101	
rs10512806	42838556	5'		0.054 13/174/1048	0.082 3/52/298	0.108 6/85/357	0.134 7/61/212	
rs6882786	42843103	5'		0.363 196/532/481	0.379 47/172/132	0.237 27/159/263	0.277 20/116/146	
rs6897301	42843255	5'		0.005 1/14/1203	0 0/0/356	0 0/0/451	0.104 5/49/229	
rs7719242	42844121	5'		0.363 186/524/484	0.378 47/169/132	0.237 26/159/261	0.171 8/80/192	

[/] Position from hg19.

Table 3

Selected ($P < 0.01$) association results of *SEPP1* SNPs in the IRASFS samples.

SNP	Trait	Additive Model			Dominant Model			Recessive Model		
		p-value	β	SE [/]	p-value	β	SE [/]	p-value	β	SE [/]
rs28919926	AIR	0.00279	9.934	3.313	0.00279	9.934	3.313	NA	NA	NA
rs146125471	AIR	0.00259	10.041	3.322	0.00259	10.041	3.322	NA	NA	NA
rs28919926	MCR1	0.00162	-0.292	0.092	0.00162	-0.292	0.092	NA	NA	NA
rs146125471	MCR1	0.00104	-0.303	0.092	0.00104	-0.303	0.092	NA	NA	NA
rs16872779	Fasting Insulin	0.00967	0.471	0.182	0.0126	0.715	0.286	0.0245	0.917	0.407
rs72554691	IL6	5.23E-04	2.010	0.577	5.23E-04	2.010	0.577	NA	NA	NA
rs7719242	IL6	0.212	0.038	0.031	0.00831	0.112	0.043	0.202	-0.076	0.060
rs12519443	TNF1	0.102	-0.023	0.014	0.882	-0.003	0.019	0.00401	-0.075	0.026
rs7719242	TNF1	0.198	-0.018	0.014	0.803	0.005	0.019	0.00476	-0.076	0.027
rs10512806	BMI	0.00446	-0.007	0.002	0.00561	-0.007	0.003	0.248	-0.010	0.009
rs16872762	VAT	0.00861	1.044	0.397	0.00861	1.044	0.397	NA	NA	NA
rs28919891	VAT	0.773	0.057	0.197	0.877	-0.031	0.200	1.61E-04	6.325	1.671
rs230819	SAT	0.0943	-0.331	0.198	0.827	-0.062	0.283	0.00645	-0.962	0.353
rs16872762	WHR	0.00912	0.033	0.012	0.00912	0.033	0.012	NA	NA	NA
rs6413428	ApoB	0.00956	-0.171	0.066	0.00276	-0.232	0.077	0.932	-0.015	0.171

P-values less than 0.05 are indicated in boldface. NA indicates that the model was unable to be calculated due to a lack of rare homozygotes for that SNP.

[/] Standard error.

Table 4

Meta Analysis of IRAS cohorts

SNP	Trait	Model	Sample Size	Z-score	p-value	Direction
rs13154178	Fasting Glucose		1152	-1.994	0.0461	---
rs6882786	S _I		1152	-2.657	0.00787	---
rs7579	S _I		1152	-2.447	0.0144	---
rs6882786	Fasting Insulin		1152	2.105	0.0352	+++
rs6882786	AIR		1152	-2.508	0.0121	---
rs16872762	AIR		1152	-1.961	0.0499	---

Table 5

Meta Analysis of IRASFS HA and all IRAS Cohorts

SNP	Trait	Model	Sample Size	Z-score	p-value	Direction ^I
rs146125471	AIR	ADD	2015	2.826	0.00471	+++0
rs28919926	AIR	ADD	1948	2.772	0.00557	+++
rs16872762	Fasting Insulin	ADD	2078	-2.2	0.0278	- - - - -
rs7579	Fasting Insulin	DOM	2095	2.067	0.0387	+++0
rs7579	S _I	DOM	1954	-1.984	0.0472	- - - - -
rs6897301	DI	REC	1948	1.976	0.0481	+00+
rs6882786	Fibrinogen	DOM	2209	2.865	0.00416	+++
rs7719242	Fibrinogen	DOM	2188	2.77	0.00560	+++
rs7579	Fibrinogen	DOM	2233	2.343	0.0191	+++
rs230819	Fibrinogen	ADD	2041	2.222	0.0263	+++
rs12519443	PAI-1	DOM	2207	-2.209	0.0271	- - - - -
rs4987017	ApoB	DOM	2097	-2.151	0.0314	- - 0 -
rs10512806	ApoB	REC	2128	2.058	0.0396	+++

^I0 indicates that the SNP of interest was monomorphic in the sample set indicated. Sample groups are presented in the following order: IRASFS HA, IRAS HA, IRAS CA, and IRAS AA.