ORIGINAL ARTICLE

Endocrine Research

Plasma Levels of Soluble Interleukin 1 Receptor Accessory Protein Are Reduced in Obesity

Kiymet Bozaoglu,* Chantal Attard,* Hemant Kulkarni,* Nik Cummings, Vincent P. Diego, Melanie A. Carless, Katherine A. Shields, Matthew P. Johnson, Sudhir Kowlessur, Thomas D. Dyer, Anthony G. Comuzzie, Laura Almasy, Paul Zimmet, Eric K. Moses, Harald H. H. Göring, Joanne E. Curran, John Blangero,* and Jeremy B. M. Jowett*

Baker IDI Heart and Diabetes Institute (K.B., C.A., N.C., K.A.S., P.Z., J.B.M.J.), Melbourne, Victoria 3004, Australia; Texas Biomedical Research Institute (H.K., V.P.D., M.A.C., M.P.J., T.D.D., A.G.C., L.A., E.K.M., H.H.H.G., J.E.C., J.B.), San Antonio, Texas 78245–0549; and Ministry of Health and Quality of Life (S.K.), Port Louis, Mauritius

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019

Context: Adipokines actuate chronic, low-grade inflammation through a complex network of immune markers, but the current understanding of these networks is incomplete. The soluble isoform of the IL-1 receptor accessory protein (sIL1RAP) occupies an important position in the inflammatory pathways involved in obesity. The pathogenetic and clinical influences of sIL1RAP are unknown.

Objective: The objective of the study was to elucidate whether plasma levels of sIL1RAP are reduced in obesity, using affluent clinical, biochemical, and genetic data from two diverse cohorts.

Design, Setting, and Participants: The study was conducted in two cohorts: the San Antonio Family Heart Study (n = 1397 individuals from 42 families) and South Asians living in Mauritius, n = 230).

Main Outcome Measures: Plasma sIL1RAP levels were measured using an ELISA. The genetic basis of sIL1RAP levels were investigated using both a large-scale gene expression profiling study and a genome-wide association study.

Results: A significant decrease in plasma sIL1RAP levels were observed in obese subjects, even after adjustment for age and sex. The sIL1RAP levels demonstrated a strong inverse association with obesity measures in both populations. All associations were more significant in females. Plasma sIL1RAP levels were significantly heritable, correlated with IL1RAP transcript levels (NM_134470), showed evidence for shared genetic influences with obesity measures and were significantly associated with the rs2885373 single-nucleotide polymorphism ($P = 6.7 \times 10^{-23}$) within the *IL1RAP* gene.

Conclusions: Plasma sIL1RAP levels are reduced in obesity and can potentially act as biomarkers of obesity. Mechanistic studies are required to understand the exact contribution of sIL1RAP to the pathogenesis of obesity. **(*J Clin Endocrinol Metab* 99: 3435–3443, 2014)**

Chronic inflammation is a characteristic feature of obesity. Local accumulation of immunologically active cells in the adipose tissue (1, 2) combined with enhanced cytokine production (3, 4) and subsequent cellular responses leading to systemic inflammation (5) together characterize this process. Although the complete immu-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
Copyright © 2014 by the Endocrine Society
Received December 20, 2013. Accepted May 20, 2014.
First Published Online June 10, 2014

* K.B., C.A., H.K., J.B., and J.B.M.J. contributed equally to this work.

Abbreviations: AVI, abdominal volume index; BMI, body mass index; CoI, conicity index; CV, coefficient of variation; IL1RAP, IL-1 receptor accessory protein; mIL1RAP, membrane bound form of IL1RAP; SAFHS, San Antonio Family Heart Study; sIL1RAP soluble, circulating form of IL1RAP; SNP, single-nucleotide polymorphism; WC, waist circumference; WHR, waist to hip ratio; WHtR, waist to height ratio.

nological jigsaw of obesity is far from resolved, the roles of individual components of the immunological responses are better understood. The IL-1 signaling pathway is considered a critical component of the immunological ensemble involved in the pathogenesis of obesity (6, 7).

The IL-1 pathway is triggered by the IL-1 receptor and a coreceptor, the IL-1 receptor accessory protein (IL1RAP) (8, 9). IL1RAP also partakes in the IL-33/ST2 (suppression of tumorigenicity 2; the IL-33 receptor) signaling pathway involved in chronic allergies, atherosclerosis, and rheumatoid arthritis (10–12). Thus, IL1RAP assumes a theoretically crucial position in the immunological axis implicated in obesity development and complications. However, whether this molecule plays a clinically and epidemiologically significant role in obesity is unknown. Another feature of IL1RAP biology that beckons an investigation is that there are two isoforms of IL1RAP consequent to an alternative splicing event (13): a membrane bound form (mIL1RAP) that is involved in signaling pathways and a soluble, circulating form (sIL1RAP) (14–16) that acts as a decoy receptor. This circulating receptor can mop up excess IL-1β and IL-33 and thereby regulate the ambient levels of these circulating proinflammatory cytokines. Arguably, reduced levels of sIL1RAP would therefore be associated with an increased proinflammatory immune response that can enhance the risk or facilitate the progression to obesity and its complications. However, whether sIL1RAP levels in plasma correlate with the clinical features of obesity is currently unknown.

We hypothesized that reduced levels of sIL1RAP are associated with phenotypic traits characterizing obesity. We tested this conjecture in the large and extended families of Mexican Americans recruited in the San Antonio Family Heart Study (SAFHS) (17–19). This cohort has been extensively characterized with respect to obesity, has been genetically analyzed, and has full genome-wide gene expression profiles available on study participants (20). Therefore, we wanted to determine whether plasma sIL1RAP levels correlate with the phenotypic traits of obesity and whether there was a genetic basis for the association. In addition, we sought to replicate our findings in another large family cohort recruited in Mauritius. Here we show consistent epidemiological associations between plasma sIL1RAP and obesity features in two diverse cohorts and provide leads into the genetic basis of plasma sIL1RAP variability.

Materials and Methods

Study subjects

Samples were from two independent studies; the SAFHS and the Mauritius Family Study. The SAFHS is a large, ongoing investigation of extended Mexican American families in San Antonio (17, 21). Details of enrolment procedures, inclusion and exclusion criteria, and phenotypic assessment of the study subjects have been detailed elsewhere (5, 6). Data and samples were collected during the first visit of the participants (from 1992 to 1996).

In the Mauritius cohort, individuals were drawn from a large longitudinal South Asian noncommunicable diseases survey in which participants were examined sequentially at either two or three time points over an 11-year period from 1987 to 1998 (22–24). sIL1RAP levels were measured in plasma drawn in 1992 when all individuals were normal glucose tolerant (n = 230). The clinical characteristics of the study participants from both cohorts are shown in Table 1.

Ethics statement

All participants provided written informed consent. The SAFHS was approved by the Institutional Review Board at the University of Texas Health Science Centre (San Antonio, Texas). The Mauritius Family Study was approved by ethics committees in Australia and Mauritius, according to the Council for International Organizations of Medical Sciences and World Health Organization guidelines for International Biomedical Research Involving Human Subjects (Geneva 1993).

sIL1RAP enzyme-linked immunosorbent assay

A sandwich ELISA was developed to measure sIL1RAP in the plasma of participants from both cohorts. The ELISA was highly sensitive with a lower limit of detection of 0.4 ng/mL and showed robust intra- and interassay reproducibility. The ELISA used commercially available unlabeled and biotinylated polyclonal antihuman IL1RAP antibodies (R&D Systems). A standard curve was established using recombinant human IL1RAP protein (R&D Systems). The interassay coefficient of variation (CV) was 5.6% and the within-assay CV was 3.9%. The linear working range of the ELISA assay was 0.4–50 ng/mL, and the midrange of the assay was 5 ng/mL. The mean CV among duplicates of measured human samples was 3.2%. The plasma sIL1RAP analyte was tested for stability across eight freeze-thaw cycles and found to be highly stable.

Obesity-related phenotypic traits

Data on waist circumference (WC), weight, height, body mass index (BMI), hip circumference, waist to hip ratio (WHR), waist to height ratio (WHtR), conicity index (CoI), abdominal volume index (AVI), and total body fat were collected as previously described (18, 25–28). Total body fat (percentage) was measured using bioimpedance as described previously (29, 30). Serum levels of leptin, adiponectin, and chemerin were also measured by an ELISA as previously described (31, 32). The association of plasma sIL1RAP levels with prevalent obesity was investigated. For these analyses, obesity was defined as a BMI of 30 kg/m^2 or greater.

Quantification of IL1RAP gene expression

Genome-wide quantitative transcriptional profiles previously obtained (20) from lymphocyte samples of 1240 participants were used to quantify the IL1RAP gene expression. A total of 19 648 autosomal transcripts were reliably detected, which contained two isoforms related to the *IL1RAP* gene: NM_002182 and NM_134470 (Entrez gene identification

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019

Table 1. Clinical Characteristics of the Study Subjects

Characteristic	SAFHS	Mauritius
Age, y, mean (SD)	39.18 (16.74)	46.52 (11.58)
Females, n, %	837 (59.9)	120 (52.2)
Fasting glucose, mmol/L, mean (SD)	5.57 (2.46)	5.41 (0.33)
Two-hour postglucose load glucose, mmol/L, mean (SD)	7.24 (4.96)	6.08 (1.05)
Fasting insulin, μU/mL, mean (SD)	15.97 (19.33)	13.29 (8.63)
Two-hour postglucose load insulin, μU/mL, mean (SD)	75.32 (73.88)	74.30 (50.06)
Homeostasis model of assessment-insulin resistance, mean (SD)	4.36 (7.13)	1.46 (0.27)
Participants with T2D, n, %	202 (14.46)	Not determined
Weight, kg, mean (SD)	76.55 (18.36)	64.80 (12.45)
Height, m, mean (SD) m]	1.62 (0.09)	1.57 (0.10)
BMI, kg/m^2, mean (SD)	29.25 (6.60)	26.33 (4.40)
WC, cm, mean (SD)	94.8 (171.7)	87.9 (9.0)
Hip circumference, cm, mean (SD)	105.8 (15.9)	98.5 (8.8)
WHR, mean (SD)	0.90 (0.09)	0.89 (0.06)
WHtR, mean (SD)	0.59 (0.11)	0.56 (0.06)
CoI, mean (SD)	1.27 (0.13)	1.26 (0.07)
AVI, mean (SD)	18.73 (7.07)	15.72 (3.12)
Systolic blood pressure, mm Hg, mean (SD)	120.35 (18.68)	124.66 (20.76)
Diastolic blood pressure, mm Hg, mean (SD)	70.67 (10.33)	76.36 (12.45)
Total serum cholesterol, mg/dL, mean (SD)	189.31 (39.60)	182.64 (40.91)
Serum triglycerides, mg/dL, mean (SD)	149.81 (128.04)	154.06 (102.74)
HDL cholesterol, mg/dL, mean (SD)	50.21 (12.83)	47.40 (11.04)
sIL1RAP, ng/dL, mean (SD)	131.24 (40.77)	54.57 (12.42)

Abbreviations: HDL, high-density lipoprotein; T2D, type 2 diabetes.

3556). Quality control measures, data normalization procedures, and analytical approaches for the transcript data have been previously described (20).

Single-nucleotide polymorphism (SNP) genotyping

The genotyping was performed previously using the Illumina HumanHap550 Genotyping BeadChip. in 1202 individuals, using the Infinium II assay and Illumina's standard protocols on a Tecan Freedom Evo Robot. After staining, each BeadChip was imaged on the Illumina BeadArray 500GX Reader using the Illumina BeadScan image data acquisition software (version 3.2.6). Genotype data were assessed using Illumina's BeadStudio software (version 2.0).

Statistical analysis

Heritability analysis was performed in the SAFHS participants under the classical approach decomposing the phenotypic variance into independent genetic and environmental components, assuming an additive model of gene action (narrow sense heritability) and expected kinship coefficients based on the observed intrafamilial relationships. Because of the assumption of multivariate normality, direct inverse Gaussian transformation was performed on both IL1RAP measures and obesity traits prior to all analyses.

Association analyses were done using polygenic regression models for SAFHS participants (to account for the kinship structure) and linear regression in the Mauritius cohort (because the study participants were not related to one another). In all regression analyses, potential confounding covariates including sex, age, and their interactions as well as the use of antidiabetic, antihypertension, and lipid-lowering medications were also included in all analyses. All hypothesis testing was performed using likelihood ratio statistics and used their expected asymptotic distributions to obtain P values. The SOLAR software package (version 7.2) (33) was used for all statistical analysis on related individuals using the variance component-based approach. Because we tested the association of sIL1RAP methods with multiple correlated phenotypic traits related to obesity, we adopted the method suggested by Suo et al (34) to avoid overinterpretation due to multiple testing. Using this procedure, we derived the first principal component based on the multiple phenotypes and generated each individual's principal component score. We then used this score as a phenotypic trait for association testing. We used Stata 12.0 (Stata Corp) to conduct the principal components analysis.

For the genome-wide association study, SOLAR was used to perform tests of Hardy-Weinberg equilibrium allowing for nonindependence among pedigree members. To eliminate missing genotypic data, we used likelihood-based imputation with the MERLIN computer package (35) to rapidly impute SNP data in pedigrees. Under this procedure, imputation is performed and the posterior genotypic probabilities for each missing genotype are stored for each pedigree. These posterior probabilities are used to construct an appropriately weighted covariate for each SNP that is then used in association analyses. Maximum likelihood marker allele frequencies were calculated using the modified genetic map developed by deCODE genetics (20).

We used measured genotype analysis (36) to test the association of SNPs with sIL1RAP gene and protein expression. The measured genotype model is embedded in a variance components-based linkage model, assuming an additive model of allelic effect (ie, the SNP genotypes AA, AB, and BB were coded as -1, 0, and 1, respectively, and used as a linear predictor of the phenotype) (37). To correct for the effect of multiple testing for a given phenotype, we estimated the effective number of SNPs using the method of Li and Ji (38). To eliminate the potential for hidden population stratification, we also utilized the Quantitative trait linkage disequilibrium test approach to association test-

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019

ing implemented in SOLAR (37). The Manhattan plot of genome wide association data was generated using HaploView (39). The localized view of the *IL1RAP* gene region showing SNP association data was generated using LocusZoom (40).

Lastly, we used the bivariate trait analysis methods to estimate the genetic correlation of the plasma sIL1RAP levels with obesity-related traits. The bivariate trait analyses permit the further partition of the phenotypic variance of a trait into genetic and environmental correlations. Specifically, the phenotypic covariance (ρ_p^2) is regarded as a function composed of the additive genetic (ρ_p^2) and environmental (ρ_p^2) correlations between two traits (denoted in the equation as i and j).

$$\rho_P(i,j) = \rho_G(i,j)\sqrt{h_i^2 h_j^2} + \rho_E(i,j)\sqrt{(1-h_i^2)(1-h_j^2)}$$

Significant genetic correlation (ρG(i,j)) between two phenotypes indicates the shared pleiotropic effect of causal genes. We used this approach to estimate the genetic correlation between plasma sIL1RAP levels and several obesity-related traits. The bivariate trait analyses were also conducted using the SOLAR software.

Results

sIL1RAP levels in Mexican American and Mauritius subjects

The SAFHS component of this study represents Mexican American families at a high risk of obesity. We measured sIL1RAP in 1397 participants of the SAFHS where levels ranged from 29.8 ng/mL to 356.6 ng/mL (mean 131.2 ng/mL; SD 40.8 ng/mL). In males (n = 560), the sIL1RAP levels ranged from 35.6 ng/mL to 356.6 ng/mL (mean 130.06 ng/mL; SD 40.80 ng/mL), whereas in females (n = 837), the sIL1RAP levels ranged from 29.8 ng/mL to 356.6 ng/mL (mean 132.03 ng/mL; SD 40.78 ng/mL). Interestingly, the estimated plasma sIL1RAP in subjects from the Mauritius cohort were strikingly low as compared with the SAFHS cohort (Table 1) and ranged from 30.22 ng/mL to 134.94 ng/mL, but the mean plasma sIL1RAP levels were marginally lower in males as compared with females (53.77 ng/mL vs 55.30 ng/mL), although not statistically significant (P = .3517). Detailed clinical characteristics of the study participants are described in Table 1.

Relationship of plasma sIL1RAP with age and sex

There is strong evidence for sex differentials with regard to the circulating levels as well as potential interactions of sIL1RAP with sex hormones. For example, IL1RAP is constitutively expressed in the uterus (41, 42) and can bind and interact with estradiol as well as T through different pathways. Although we did not have data on T and estradiol levels in the SAFHS subjects, there was a significant association between plasma levels of sIL1RAP and dehydroepiandrosterone sulfate in females [polygenic regression coefficient (ρ) = 0.0806 (0.0077)] but not in males [−0.0312 (0.2210)]. Considering these sex-based differences, we conducted all the ensuing analyses separately in males and females as well as in all subjects together.

When we studied the combined and interactive association of age and sex with circulating levels of sIL1RAP, the sIL1RAP levels were quadratically related to age and the age × sex interaction was also statistically significant (Figure 1A). Thus, younger males had substantially lower circulating levels of sIL1RAP than comparatively aged females, but this difference rapidly narrowed with increasing age. These observations together indicated that age, age^2, and sex could confound the association of sIL1RAP with metabolic syndrome risk because they can also act as risk factors of obesity.

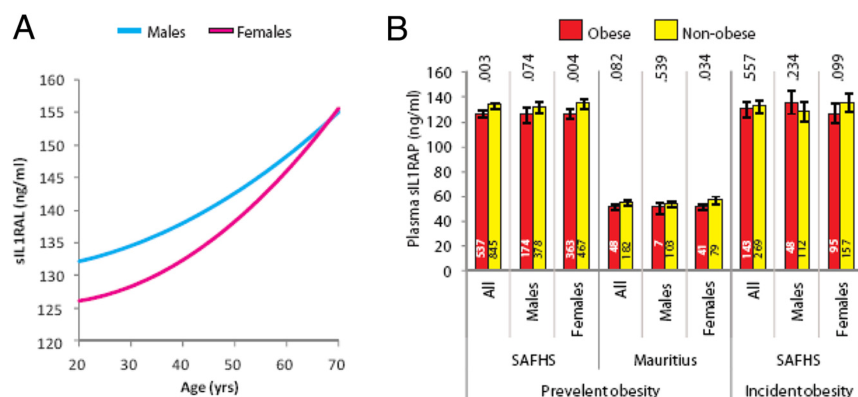

Figure 1. Relationship of plasma sIL1RAP levels with age, sex, and obesity. A, Predicted relationship with age and sex. Results are predictions based on the best fitting polygenic regression model that accounted for the kinship structure. Blue curve is for females and red curve is for males. Values estimated from a polygenic regression model were P = .0080 for age, P = .0177 for age^2, and P = .0572 for age × sex interaction. B, Mean plasma sIL1RAP levels in obese (red bars) and nonobese (yellow bars) subjects. Mean levels in the SAFHS participants were estimated using polygenic regression accounting for the kinship structure. Error bars indicate 95% confidence intervals, and the rotated numbers at the top show the statistical significance of the difference of means using Student's *t* test. Numbers of subjects included in the analyses are shown inside the bars.

Plasma sIL1RAP levels are reduced in obesity

In the SAFHS participants, the mean plasma sIL1RAP levels were significantly lower in obese subjects compared with nonobese subjects whether examined separately in males (125.7 ng/mL vs 132.4 ng/mL, P = .074), females (127.2 ng/mL vs 135.2 ng/mL, P = .004), or combined together (127.0 ng/mL vs 133.6 ng/mL, P = .003; (Figure 1B). These differences remained signifi-

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019

cant, even after adjustment for serum dehydroepiandrosterone sulfate levels ($P = .0985$ for males, $P = .0451$ for females, and $P = .0376$ for all subjects). In the Mauritius cohort, obese subjects had reduced mean plasma sIL1RAP levels as compared with nonobese subjects: males (51.0 ng/mL vs 54.0 ng/mL, $P = .539$), females (51.9 ng/mL vs 57.1 ng/mL, $P = .034$), or all combined (51.8 ng/mL vs 55.3 ng/mL, $P = .082$). In both cohorts the statistical significance of the studied association was greater for females than for males.

Association of plasma sIL1RAP with obesity-related traits

We next conducted a series of regression analyses in both the SAFHS and Mauritius cohorts with an aim to uncover potential consistency of the association of the plasma sIL1RAP levels, with several traits characterizing obesity (Table 2). In the SAFHS participants, we observed the strongest associations of inverse-normalized plasma sIL1RAP levels with WC, BMI, WHtR, AVI, and total body fat but not with WHR and CoI. The associations were also more significant in females than males. Furthermore, we were able to replicate these findings in the Mauritius cohort such that WC, BMI, WHtR, and AVI again showed statistical significance or near significance, and these observations were also more significant in females compared with males (Table 2).

To investigate the potential role of sIL1RAP in the immune component of obesity, we studied the association of inverse-normalized plasma sIL1RAP levels with three well-recognized adipokines: adiponectin and leptin in the SAFHS participants and chemerin in the Mauritius cohort. In the SAFHS cohort, plasma sIL1RAP levels were associated inversely with serum leptin but not with serum adiponectin (Table 2), in which the significance was higher in females compared with males. Notably, although an overall negative association of plasma sIL1RAP levels with serum chemerin in the Mauritius cohort was observed, this observation was more significant in males rather than females. Together these findings indicated that sIL1RAP levels associate somewhat specifically with serum leptin levels in females.

Lastly, to avoid overinterpretation due to multiple testing, we tested the association of plasma sIL1RAP levels with the first component derived from obesity-related traits listed in Table 2. We found that this first principal component explained 76.35% and 66.69% of the intersubject variability in the SAFHS and Mauritius cohorts, respectively. This principal component (labeled as obesity principal component in Table 2) was significantly associated with reduced plasma sIL1RAP levels more strongly in females as compared with males in the SAFHS cohort. In the Mauritius cohort, however, statistical significance for association of plasma sIL1RAP and obesity principal component was observed in females only.

sIL1RAP levels are heritable and share genetic influences with obesity traits

Given that plasma sIL1RAP levels were measured in the SAFHS family-based cohort, we were able to estimate the

Table 2. Association of sIL1RAP Levels With Obesity-Related Traits*

	All		Males		Females	
Trait (Abbreviation)	β	*P* Value	β	*P* Value	β	*P* Value
SAFHS participants						
WC	−.0986	**2.0×10^{-5}**	−.0715	**0.0172**	−.1263	**5.3×10^{-5}**
BMI	−.1071	**5.6×10^{-6}**	−.0707	**0.0252**	−.1408	**7.5×10^{-6}**
WHR	−.0443	**.0240**	−.0474	0.0505	−.0517	.0527
WHtR	−.0875	**.0001**	−.0728	**0.0248**	−.1058	**.0007**
CoI	−.0474	**.0431**	−.0468	0.0954	−.0514	.1318
AVI	−.0904	**.0002**	−.0774	**0.0226**	−.1022	**.0020**
Total body fat	−.0699	**.0014**	−.0644	0.0716	−.0776	**.0044**
Serum adiponectin	.0112	.7454	.0242	0.6572	.0174	.6954
Serum leptin	−.0631	**.0034**	−.0272	0.4291	−.0878	**.0014**
Obesity principal component	−.1268	**.0005**	−.1029	0.0497	−.1927	**.0016**
Mauritius cohort						
WC	−.1415	**.0278**	−.0919	0.2184	−.2015	.0594
BMI	−.1051	.0849	−.0491	0.4801	−.1728	.0911
WHR	−.1161	**.0430**	−.1252	0.0581	−.1050	.2730
WHtR	−.1720	**.0018**	−.1480	**0.0305**	−.2010	**.0222**
CoI	−.1303	**.0196**	−.1283	**0.0382**	−.1327	.1625
AVI	−.1359	**.0357**	−.0831	0.2664	−.1996	.0638
Total body fat	−.0921	.1292	−.0501	0.5467	−.1589	.0781
Serum chemerin	−.1175	**.0057**	−.1675	**0.0033**	−.0568	.3748
Obesity Principal Component	−.1734	**.0044**	−.1243	0.0855	−.2232	**.0189**

Abbreviation: β, regression coefficient. Bold values denote significance at $P < .05$.

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019

proportion of variance due to additive genetic factors. We observed a strong heritability ($h^2 = 0.40$) that was highly statistically significant ($P = 3.9 \times 10^{-23}$). However, the estimate of heritability was larger in females (0.50, $P = 1.7 \times 10^{-14}$) compared with males (0.45, $P = 1.7 \times 10^{-6}$). The potential correlation of *IL1RAP* gene expression and plasma levels of sIL1RAP was also investigated, and we found that the transcript NM_134470 was associated with the sIL1RAP protein differentially across sexes. In males, this transcript showed a negative nonsignificant association with sIL1RAP levels (polygenic regression coefficient −0.057, $P = .186$), but in females it showed a significant positive association (polygenic regression coefficient −0.087, $P = .013$).

Furthermore, bivariate trait analyses were conducted to estimate the genetic and environmental correlation between sIL1RAP levels and measures of obesity and we observed stronger correlations in females than males, even after adjusting for age, age^2, sex, and their interactions (Table 3). In females, the genetic correlation coefficients were statistically significant for BMI ($P = .0081$), AVI ($P = .0292$), and total body fat ($P = .0198$); and with an association nearing significance with WC ($P = .0780$). In contrast, BMI was the only trait that showed a trend toward a genetic correlation with plasma sIL1RAP in males ($P = .0818$). Interestingly, with the exception of CoI and WHR, there was a statistically significant inverse phenotypic correlation between plasma sIL1RAP and each of the remaining obesity-related traits in both males and females (Table 3).

In the Mauritius cohort, the total phenotypic correlation was estimated. We found that WHR and CoI were the only variables that failed to show a significant inverse association with plasma sIL1RAP levels. This pattern was not observed in males where the only significant phenotypic correlations of sIL1RAP levels were with WHR and WHtR. Considered in totality, these patterns of correlation demonstrated a consistency of direction and strength of evidence in females (in both cohorts). Thus, plasma sIL1RAP levels are not only significantly heritable but also likely share similar genetic influences as those of features of obesity, especially in females. Therefore, to gain insight into the biological mechanisms of control, we sought to identify genetic factors that may influence variation in plasma sIL1RAP levels.

Genome-wide association analysis identifies strongly associated genetic variation within sIL1RAP

We further evaluated genetic factors influencing sIL1RAP by performing genome-wide association using

Table 3. Bivariate Trait Analyses of sIL1RAP Levels in Plasma and Obesity-Related Traits

	Phenotypic Correlation				Genetic and Environmental Correlations			
	Mauritius		SAFHS					
Trait	ρ_P	*P* Value	ρ_P	*P* Value	ρ_G	*P* Value	ρ_E	*P* Value
All participants								
WC, cm	−0.1782	**.0068**	−0.1262	**1.4×10^{-5}**	−0.2463	**.0107**	−0.0232	.7205
BMI, kg/m^2	−0.1303	.0484	−0.1445	**7.1×10^{-9}**	−0.2921	**.0021**	−0.0126	.8365
WHR	−0.1529	**.0203**	−0.0444	0.1153	−0.0404	.7409	−0.0471	.3833
WHtR	−0.1628	**.0134**	−0.1193	**4.3×10^{-5}**	−0.2205	**.0205**	−0.0298	.6509
CoI	−0.1189	.0718	−0.0554	0.0535	−0.0674	.5309	−0.0472	.4207
AVI	−0.1718	**.0090**	−0.1241	**2.0×10^{-5}**	−0.2651	**.0056**	−0.0009	.9889
TBF	−0.0817	.3121	−0.1092	**0.0004**	−0.3420	**.0122**	0.0423	.8445
Males only								
WC, cm)	−0.1812	.0582	−0.1107	**.0156**	−0.2300	.2061	0.0196	.9058
BMI, kg/m^2	−0.1501	.1176	−0.1100	**.0149**	−0.3065	.0818	0.0901	.5477
WHR	−0.1912	**.0454**	−0.0805	.0763	−0.0656	.7439	−0.0937	.5259
WHtR	−0.2324	**.0145**	−0.1049	**.0237**	−0.1850	.3130	−0.0108	.9527
CoI	−0.1452	.1302	−0.0595	.1835	0.1129	.6441	−0.1728	.2148
AVI	−0.1704	.0751	−0.1104	**.0158**	−0.2327	.2027	0.0211	.8976
TBF	−0.1151	.3064	−0.0909	.0582	−0.2498	.3098	0.0009	.9941
Females only								
WC, cm	−0.1713	.0614	−0.1321	**.0005**	−0.2051	.0780	−0.0310	.8092
BMI, kg/m^2	−0.1779	.0518	−0.1692	**7.3×10^{-6}**	−0.3059	**.0081**	0.0257	.8440
WHR	−0.0758	.4108	−0.0175	.6321	0.1819	.2079	−0.1749	.0640
WHtR	−0.1818	.0469	−0.1243	**.0010**	−0.1649	.1493	−0.0685	.6025
CoI	−0.0726	.4304	−0.0429	.1487	0.0216	.8619	−0.1141	.2872
AVI	−0.1706	.0628	−0.1298	**.0006**	−0.2510	**.0292**	0.0463	.7235
TBF	−0.2270	.0518	−0.1265	**.0014**	−0.3650	**.0198**	0.0670	.5382

Abbreviation: TBF, total body fat. Bold values denote significance at $P < .05$.

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019

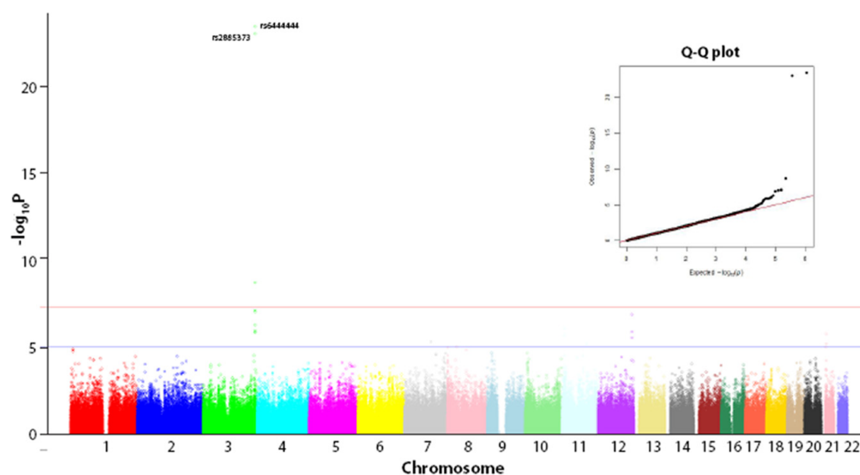

Figure 2. Genome-wide association study of sIL1RAP. Manhattan plot of genome wide association analysis results for plasma sIL1RAP levels. Chromosome position is on the x-axis, and the $-\log P$ value of the association result is shown on the Y-axis. The horizontal line at $-\log P = 7.0$ indicates the Bonferroni adjusted *P* value for multiple testing ($P = 9.2 \times 10^{-8}$) above which association is significant on a genome-wide scale. Black arrow indicates the two most significant associations. Inset, Genome-wide association results of regions containing IL1RAP in which the strongest association signals were observed. The expected $-\log P$ value of the association result is shown on the x-axis and the observed $-\log P$ value of the association result is shown on the y-axis.

542 944 SNPs previously genotyped in 1222 participants in the SAFHS cohort. After allowing for nonindependence, population admixture, and adjustment for sex and age, association analysis revealed two very strongly associated SNPs within intron 9 of the *IL1RAP* gene on chromosome 3q28 (Figure 2). These were rs6444444 ($P = 2.6 \times 10^{-23}$) and rs2885373 ($P = 6.7 \times 10^{-23}$). Because the two SNPs were in high linkage disequilibrium ($D' = 0.90$), we believe that they do not represent independent findings (Figure 2). The association result far exceeded the conservative Bonferroni-corrected threshold of $P = 9.2 \times 10^{-8}$ after an adjustment for multiple testing. This result was consistently observed in both males and females. In males, the most significantly associated SNP was rs2885373 ($\beta = .90$, $P = 2.9 \times 10^{-13}$) followed by rs6444444 ($\beta = .80$, $P = 2.4 \times 10^{-11}$), whereas in females the most significantly associated SNP was rs6444444 ($\beta = .89$, $P = 2.2 \times 10^{-23}$) followed closely by that for rs2885373 ($\beta = .87$, $P = 1.1 \times 10^{-22}$; Figure 2). Supplemental Figure 1 shows the position of rs2885373 relative to the alternatively spliced isoforms of *IL1RAP*. Interestingly, when we investigated the association between rs2885373 and *IL1RAP* gene expression, we observed that this variant was significantly associated with the transcript NM_134470 in females ($\beta = .22$, $P = .0269$) but not in males ($\beta = -.01$, $P = .9308$).

Discussion

Using data and samples from individuals who were clinically evaluated, we demonstrated that sIL1RAP levels were lower in plasma of obese compared with nonobese subjects. Our study has identified a novel, easy-to-measure, and consistent biomarker of obesity. The consistency of our observations across geoepidemiologically diverse cohorts further highlights the novelty of our findings. Moreover, demonstration of a strong genetic involvement and identification of novel polymorphisms through a genome-wide approach lends further credence to the candidature of plasma sIL1RAP levels as a marker of inflammation in obesity.

The consistent pattern of all associations in females across both cohorts is an important observation that demands a closer look. Our results indicate that the direction of association of *IL1RAP* gene expression and the sIL1RAP protein was inverted in males and females, but these associations and the genetic correlation coefficients were stronger in females. Even if one considers that the larger number of females in the SAFHS may work in favor of a better statistical significance, these observations raise the possibility of differential post-translational modifications, protein-protein interactions, and/or epigenetic regulatory mechanisms of sIL1RAP regulation across sexes. Despite these differentials, however, the association of the circulating levels of sIL1RAP with disease outcomes was directionally comparable in males as well as in females. Therefore, we believe that the potential disparity in mechanisms of genetic control of sIL1RAP levels will not impact the use of sIL1RAP protein as a biomarker of obesity-induced inflammation.

The concept that sIL1RAP is likely involved in the obesity-related inflammation is supported by its specific association with leptin but not with adiponectin in SAFHS participants. Leptin structurally resembles IL-6 and is known to be engaged in a cross talk with IL-1 family of cytokines in the context of breast cancer (43, 44). It is conceivable that the decoy activity of sIL1RAP may also be providing a sink effect for circulating the leptin levels also through unknown cross talk. Although data in support of this hypothesis are currently not available, our study provides a lead in this direction and warrants further investigation.

Our genetic analysis revealed that sIL1RAP was significantly heritable ($h^2 = 0.40$). Genome-wide association analysis revealed a single, very strongly associated locus

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019

directly within the *IL1RAP* gene near the boundary of intron 8 and exon 9 (rs6444444 and rs2885373). The primary splicing event determining membrane and soluble forms occurs at exon 9 and may be influenced directly by either rs6444444 or rs2885373 lying juxtaposed in intron 8, only 908 or 671 bp, respectively, from the start of exon 9. The 356-amino acid sIL1RAP protein is encoded by the first 9 exons of *IL1RAP* and lacks the transmembrane and intracellular domains of the longer 570-amino acid membrane bound isoform (mIL1RAP). Although mIL1RAP is recruited after binding of IL-1α and IL-1β to the IL-1R type I receptor and enhances IL-1 signaling, sIL1RAP has the opposite effect and is thought to inhibit IL-1α- and IL-1β-mediated signaling (45). Therefore, it is conceivable that the rs2885373 SNP may affect splicing directly (or is in linkage disequilibrium with another causative variant) and influences relative proportions of mIL1RAP to sIL1RAP that in turn could impact upon the body's response to inflammatory signals.

Two other issues from this study deserve closer scrutiny. First, our results show that sIL1RAP levels are significantly influenced by age. However, it is conceivable that population-specific differences in the relationship of age with sIL1RAP levels may exist. Therefore, future longitudinal studies are required to establish whether there are individual-level sIL1RAP trajectories with respect to age such that a deviation from the expected trajectory of sIL1RAP levels can signal the impending risk of obesity. Second, we observed overall lower levels of plasma sIL1RAP in the Mauritius cohort than in the Mexican American cohort. Factors contributing to this difference may include natural genetic and environmental variation in the cohorts with differing ancestries. However, the sample-specific z-scores in the obese subjects from both cohorts were highly comparable, indicating that a similar degree of relative reduction in sIL1RAP across populations may be associated with an increased risk of obesity (data not shown).

In summary, this study demonstrates the validity of combined genome wide genetic variation and gene expression data in a large human cohort for the discovery of biomarkers of obesity. The observations reported here also raise the possibility that a recombinant sIL1RAP protein or related therapeutic agent may enhance existing anti-IL-1β therapies and provide clinical utility in modulation or reversal of inflammation induced by the adipose tissue and complications thereof. Future studies need to investigate these possibilities in more controlled and direct research settings.

Acknowledgments

We are grateful to the participants in the San Antonio Family Heart Study. We further extend our gratitude to the participants of the Mauritius cohort and the research staff and clinical team that assisted during sample collection and phenotype ascertainment.

Address all correspondence and requests for reprints to: Dr Kiymet Bozaoglu, Baker IDI Heart and Diabetes Institute, 75 Commercial Road, Melbourne, Victoria 3004, Australia. E-mail: kiymet.bozaoglu@bakeridi.edu.au.

Data collection for the San Antonio Family Heart study samples was supported by Grant HL045522 from the National Heart, Lung, and Blood Institute, National Institutes of Health. The high-density SNP genotyping data and transcriptional profiles were generated with generous support from the Azar and Shepperd Families of San Antonio. Additional funds for transcriptional profiling, sequencing, genotyping, and statistical analysis were provided by ChemGenex Pharmaceuticals Ltd, Australia. The development of the analytical methods and software used in this study was supported by National Institutes of Health Grant R37 MH059490. The AT&T Genomics Computing Centre supercomputing facilities used for this work were supported in part by a gift from the AT&T Foundation and with support from the National Centre for Research Resources Grant S10 RR029392. Parts of this investigation were conducted in facilities constructed with support from Research Facilities Improvement Program Grants C06 RR013556 and C06 RR017515 from the National Centre for Research Resources, National Institutes of Health.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Chmelar J, Chung KJ, Chavakis T.** The role of innate immune cells in obese adipose tissue inflammation and development of insulin resistance. *Thromb Haemost*. 2013;109:399–406.
2. **Suganami T, Tanaka M, Ogawa, Y.** Adipose tissue inflammation and ectopic lipid accumulation. *Endocr J*. 2012;59:849–857.
3. **Piya MK, McTernan PG, Kumar S.** Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. *J Endocrinol*. 2013;216:T1–T15.
4. **Smorlesi A, Frontini A, Giordano A, Cinti S.** The adipose organ: white-brown adipocyte plasticity and metabolic inflammation. *Obes Rev*. 2012;13(suppl 2):83–96.
5. **Murray KN, Buggey HF, Denes A, Allan SM.** Systemic immune activation shapes stroke outcome. *Mol Cell Neurosci*. 2013;53:14–25.
6. **Meshkani R, Adeli K.** Hepatic insulin resistance, metabolic syndrome and cardiovascular disease. *Clin Biochem*. 2009;42:1331–1346.
7. **Tack CJ, Stienstra R, Joosten LA, Netea MG.** Inflammation links excess fat to insulin resistance: the role of the interleukin-1 family. *Immunol Rev*. 2012;249:239–252.
8. **Banerjee M, Saxena M.** Interleukin-1 (IL-1) family of cytokines: role in type 2 diabetes. *Clin Chim Acta*. 2012;413:1163–1170.
9. **Humphreys NE, Grencis RK.** IL-1-dependent, IL-1R1-independent resistance to gastrointestinal nematodes. *Eur J Immunol*. 2009;39:1036–1045.
10. **Ali S, Huber M, Kollewe C, Bischoff SC, Falk W, Martin MU.** IL-1 receptor accessory protein is essential for IL-33-induced activation

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019

of T lymphocytes and mast cells. *Proc Natl Acad Sci USA*. 2007; 104:18660–18665.
11. **Kakkar R, Lee RT.** The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat Rev Drug Discov*. 2008;7:827–840.
12. **Palmer G, Lipsky BP, Smithgall MD, et al.** The IL-1 receptor accessory protein (AcP) is required for IL-33 signaling and soluble AcP enhances the ability of soluble ST2 to inhibit IL-33. *Cytokine*. 2008; 42:358–364.
13. **Lu HL, Yang CY, Chen HC, Hung CS, Chiang YC, Ting LP.** A novel alternatively spliced interleukin-1 receptor accessory protein mIL-1RAcP687. *Mol Immunol*. 2008;45:1374–1384.
14. **Libra M, Mangano K, Anzaldi M, et al.** Analysis of interleukin (IL)-1beta IL-1 receptor antagonist, soluble IL-1 receptor type II and IL-1 accessory protein in HCV-associated lymphoproliferative disorders. *Oncol Rep*. 2006;15:1305–1308.
15. **Smeets RL, Joosten LA, Arntz OJ, et al.** Soluble interleukin-1 receptor accessory protein ameliorates collagen-induced arthritis by a different mode of action from that of interleukin-1 receptor antagonist. *Arthritis Rheum*. 2005;52:2202–2211.
16. **Smith DE, Hanna R, Della F, et al.** The soluble form of IL-1 receptor accessory protein enhances the ability of soluble type II IL-1 receptor to inhibit IL-1 action. *Immunity*. 2003;18:87–96.
17. **MacCluer JW, Stern MP, Almasy L, et al.** Genetics of atherosclerosis risk factors in Mexican Americans. *Nutr Rev*. 1999;57:S59–S65.
18. **Mitchell BD, Kammerer CM, Blangero J, et al.** Genetic and environmental contributions to cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study. *Circulation*. 1996;94:2159–2170.
19. **MacCluer JW, Stern MP, Almasy L, et al.** Genetics of atherosclerosis risk factors in Mexican Americans. *Nutr Rev*. 1999;57:S59–65.
20. **Goring HH, Curran JE, Johnson MP, et al.** Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. *Nat Genet*. 2007;39:1208–1216.
21. **Mitchell BD, Almasy LA, Rainwater DL, et al.** Diabetes and hypertension in Mexican American families: relation to cardiovascular risk. *Am J Epidemiol*. 1999;149:1047–1056.
22. **Magliano DJ, Soderberg S, Zimmet PZ, et al.** Mortality, all-cause and cardiovascular disease, over 15 years in multiethnic Mauritius: impact of diabetes and intermediate forms of glucose tolerance. *Diabetes Care*. 2010;33:1983–1989.
23. **Soderberg S, Zimmet P, Tuomilehto J, et al.** Increasing prevalence of Type 2 diabetes mellitus in all ethnic groups in Mauritius. *Diabet Med*. 2005;22:61–68.
24. **Soderberg S, Zimmet P, Tuomilehto J, et al.** High incidence of type 2 diabetes and increasing conversion rates from impaired fasting glucose and impaired glucose tolerance to diabetes in Mauritius. *J Intern Med*. 2004;256:37–47.
25. **Voruganti VS, Lopez-Alvarenga JC, Nath SD, et al.** Genetics of variation in HOMA-IR and cardiovascular risk factors in Mexican-Americans. *J Mol Med (Berl)*. 2008;86:303–311.
26. **Chan DF, Li AM, Chan MH, et al.** Validation of prediction equations for estimating resting energy expenditure in obese Chinese children. *Asia Pac J Clin Nutr*. 2009;18:251–256.
27. **Mamtani MR, Kulkarni, HR.** Predictive performance of anthropometric indexes of central obesity for the risk of type 2 diabetes. *Arch Med Res*. 2005;36:581–589.
28. **Guerrero-Romero F, Rodriguez-Moran, M.** Abdominal volume index. An anthropometry-based index for estimation of obesity is strongly related to impaired glucose tolerance and type 2 diabetes mellitus. *Arch Med Res*. 2003;34:428–432.
29. **Cummings N, Shields KA, Curran JE, et al.** Genetic variation in SH3-domain GRB2-like (endophilin)-interacting protein 1 has a major impact on fat mass. *Int J Obes (Lond)*. 2012;36:201–206.
30. **Duggirala R, Stern MP, Mitchell BD, et al.** Quantitative variation in obesity-related traits and insulin precursors linked to the OB gene region on human chromosome 7. *Am J Hum Genet*. 1996;59:694–703.
31. **Bozaoglu K, Bolton K, McMillan J, et al.** Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology*. 2007;148:4687–4694.
32. **Comuzzie AG, Hixson JE, Almasy L, et al.** A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nat Genet*. 1997;15:273–276.
33. **Almasy L, Blangero J.** Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet*. 1998;62:1198–1211.
34. **Suo C, Toulopoulou T, Bramon E, et al** Analysis of multiple phenotypes in genome-wide genetic mapping studies. *BMC Bioinformatics*. 2013;14:151.
35. **Burdick JT, Chen WM, Abecasis GR, Cheung, VG.** In silico method for inferring genotypes in pedigrees. *Nat Genet*. 2006;38:1002–1004.
36. **Boerwinkle E, Chakraborty R, Sing, CF.** The use of measured genotype information in the analysis of quantitative phenotypes in man. I. Models and analytical methods. *Ann Hum Genet*. 1986;50: 181–194.
37. **Blangero J, Goring HH, Kent JW Jr, et al.** Quantitative trait nucleotide analysis using Bayesian model selection. *Hum Biol*. 2005;77: 541–559.
38. **Li J, Ji L.** Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity (Edinb)*. 2005;95: 221–227.
39. **Barrett JC, Fry B, Maller J, Daly, MJ.** Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21: 263–265.
40. **Pruim RJ, Welch RP, Sanna S, et al.** LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010;26:2336–2337.
41. **Bigonnesse F, Marois M, Maheux R, Akoum, A.** Interleukin-1 receptor accessory protein is constitutively expressed in human endometrium throughout the menstrual cycle. *Mol Hum Reprod*. 2001; 7:333–339.
42. **Seo H, Choi Y, Shim J, Ka H.** Regulatory mechanism for expression of IL1B receptors in the uterine endometrium and effects of IL1B on prostaglandin synthetic enzymes during the implantation period in pigs. *Biol Reprod*. 2012;87:31.
43. **Newman G, Gonzalez-Perez RR.** Leptin-cytokine crosstalk in breast cancer. *Mol Cell Endocrinol*. 201425;382(1):570–582.
44. **Perrier S, Caldefie-Chezet F, Vasson MP.** IL-1 family in breast cancer: potential interplay with leptin and other adipocytokines. *FEBS Lett*. 2009;583:259–265.
45. **Jensen LE, Whitehead AS.** The 3′ untranslated region of the membrane-bound IL-1R accessory protein mRNA confers tissue-specific destabilization. *J Immunol*. 2004;173:6248–6258.

Downloaded from https://academic.oup.com/jcem/article-abstract/99/9/3435/2538806 by Biomedical Library user on 25 September 2019