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Quantitative Trait Locus on Chromosome 19 for Circulating Levels of Inter cellular Adhesion Molecule-1 in Mexican Americans

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Abstract

Circulating soluble intercellular adhesion molecule-1 (sICAM-1) is a biochemical marker of inflammation. We performed variance-components-based quantitative genetic analyses in SOLAR of sICAM-1 in 1170 individuals from Mexican American families in the San Antonio Family Heart Study. The trait is heritable ($h^2 = 0.50 \pm 0.06$, $P < 10^{-6}$). Multipoint linkage analysis using a ~10-cM microsatellite map revealed a region on Chromosome 19p near marker D19S586 showing strong evidence of linkage for sICAM-1 (empirically adjusted univariate-equivalent LOD = 4.95), coincident with the structural gene *ICAM1*. This region has been identified previously as a QTL for inflammatory, autoimmune, and metabolic syndrome traits. There is significant evidence ($P = 0.0023$) of locus heterogeneity for sICAM-1 in this sample: a subset of pedigrees contributes most of the linkage signal for sICAM-1 on Chromosome 19, suggesting a logical focus for future genetic dissection of the trait.

Keywords

ICAM-1; inflammation; genetic heterogeneity; genome scan; quantitative trait locus; Mexican Americans

1. Introduction

The cell adhesion molecule intercellular adhesion molecule-1 (ICAM-1) is an agent and a marker of inflammation. Up-regulation of this molecule increases binding of monocytes and T-lymphocytes to the surface of endothelial cells and triggers a signal cascade that facilitates migration of immune cells into the arterial intima [1]. Elevated circulating levels of the soluble form (sICAM-1) is an indicator of the proinflammatory state [2].

A number of factors have been shown or postulated to contribute to increased sICAM-1 expression, including dyslipidemia and lipid oxidation [3] and hypertension [4]. The relationship between these factors and inflammation is complex, and knowledge of the underlying genetic mechanisms is still limited. Hyperglycemia and its associated oxidative stress are thought to induce an inflammatory response in the arterial wall, partly accounting

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for the elevated risk of cardiovascular disease (CVD) in patients with diabetes mellitus (DM) [5]. Conversely, several lines of evidence suggest that the development of insulin resistance in target tissues may itself be an inflammatory process [6,7]. Such inflammation may be stimulated by cytokines secreted by adipose tissue, especially visceral fat, providing one explanation for the correlation between obesity, type 2 diabetes (T2DM), and CVD [8].

In a recent study [9] we found that sICAM-1 levels are genetically correlated with several measures of obesity and insulin resistance in a Mexican American population with elevated prevalence of obesity and T2DM: the San Antonio Family Heart Study (SAFHS, [10]). This finding provides suggestive evidence for the existence of common genetic factors underlying development of obesity, T2DM, and inflammatory disease. As part of this ongoing research, we present evidence for a specific chromosomal location (quantitative trait locus, or QTL) linked to variation in circulating levels of sICAM-1 in the SAFHS. This is, to our knowledge, the first published report of a QTL for this trait.

2. Materials and methods

2.1 Study participants

The San Antonio Family Heart Study focuses on a Mexican American population with elevated prevalence of cardiovascular risk factors, including obesity, T2DM, and dyslipidemia of the high triglyceride/low HDL type. The study recruited large families without prior ascertainment of CVD, DM, or other disease status. All procedures in SAFHS were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio (UTHSCSA), and all participants gave informed consent.

2.2 Phenotypic assessments

The collection of demographic, pedigree, and morphometric data in SAFHS has been described elsewhere [10]. Plasma glucose levels were measured at fasting and 2h after administration of 75g oral glucose. For the present study, participants were classified as having DM if they exhibited fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl), plasma glucose ≥ 11.1 mmol/l (200 mg/dl) at 2h after oral glucose challenge, or if they reported using antidiabetic medication. SAFHS does not classify participants by type of DM based on medical histories or clinical observations. Although 39 (21%) of the 186 participants with DM reported using insulin, most diabetes cases in the SAFHS cohort are mature-onset and associated with obesity and are thus presumed to be Type 2 (see Results).

sICAM-1 levels in serum from fasting blood samples were measured by ELISA (R&D Systems, Inc., Minneapolis, MN). Serum samples were diluted 1:20 and incubated in plate according to the manufacturer's instructions. Plates were read at 450 nm, and results were analyzed with a four parameter logistic fit. Samples were assigned randomly to each plate, and ~10% of these were randomly chosen to be run in duplicate in the same plate. (The average of duplicate measures was used as the phenotypic value for these individuals.) A standards curve was generated for each plate from the manufacturer's standards run in duplicate without dilution.

2.3 Marker genotypes and map

Lymphocyte DNA was used as template for polymerase chain reactions with fluorescently labeled primers for 432 microsatellite markers spaced at ~10 cM intervals across the 22 autosomes (MapPairs Human Screening set v6/v8; Research Genetics, Huntsville, AL). Reactions were performed separately as specified by the manufacturer and products were pooled into multiplexed panels for genotyping on an automated DNA sequencer (ABI PRISM model 377 or model 3100 with Genescan and Genotyper programs; Applied Biosystems, Foster City, CA).

2.4 Statistical genetic analyses

Genotype data were cleaned for both Mendelian and spurious double-recombinant errors using Simwalk2 [11]. Allele frequencies were estimated by maximum likelihood methods implemented in SOLAR [12] and multipoint identity-by-descent matrices were estimated using Markov chain Monte Carlo methods implemented in LOKI [13].

Procedures for handling outlying data and other deviations from the assumption of multivariate normal distribution are explained in Results. A Bayesian model selection routine implemented in SOLAR [14] was used to screen the effects of environmental and physiological covariates. This method computes for each covariate model an approximate Bayes factor, the Bayesian Information Criterion (BIC) [15], defined as:

$$\text{BIC}_k = -\Lambda_{k0} + \text{df}_k \ln N_e,$$

where Λ_{k0} is the likelihood ratio statistic comparing model k to a null model (random environmental effect with no covariates), df_k is the number of additional degrees of freedom in k , and N_e is the effective sample size [14]. Thus, BIC_k represents the likelihood ratio test for k with a penalty for overparameterization. The covariate model with the lowest value of BIC was selected as the best fit to the data and became the basis for quantitative genetic analysis (see Results).

Quantitative genetic parameter estimation and linkage analysis were performed using variance-components decomposition methods implemented in SOLAR. Parameters of interest include the heritability (h^2) of sICAM-1 and evidence of linkage from multipoint scans of the autosomal genome. Where appropriate, linkage evidence (logarithm of odds, or LOD scores) was adjusted downward by comparison with the empirical distribution of LOD scores in the absence of linkage (using the “LOD adjust” routine in SOLAR [16]).

Once significant evidence of linkage is obtained for a particular locus, it is possible to test the hypothesis that the linkage signal is due to a subset of pedigrees – in other words, that there is locus heterogeneity in the sample. SOLAR's ‘hlod’ routine [17] performs a classical homogeneity test under a model allowing for two classes of families, with only one class segregating the locus of interest. The distribution of the test statistic for homogeneity conditional on linkage is taken to be a 50:50 mixture of a chi-squared distribution with one degree of freedom and a point mass at zero [17].

3. Results

3.1 Characteristics of the study participants

Circulating sICAM-1 levels were measured in 1269 participants from the first clinic visit of SAFHS. As noted in the Methods, 10% of samples were run in duplicate; the in-plate coefficient of variation (CV) among these samples was 2.76%. The cross-plate CV among standards was 5.60%. Seven individuals were excluded from further analysis because their sICAM-1 measures were outside the detection limits of the plate reader.

Circulating sICAM-1 measures were $\log_e$ transformed to achieve kurtosis (κ) < 2 . (Simulation experiments have shown that genetic linkage analyses of traits with leptokurtic distributions (especially $\kappa > 2$) yield unacceptably high false-positive rates [16]). Some individuals were excluded due to lack of marker genotype data, leaving an analysis sample with $N = 1231$ for sICAM-1 ($\kappa = 0.8609$ after $\log_e$ transformation).

Table 1 presents descriptive statistics for the sample, including measurements of several phenotypes related to CVD risk (glucose metabolism, blood pressure, and adiposity). As these data indicate, the SAFHS cohort exhibits elevated values of key CVD risk factors even though the cohort was recruited without ascertainment for disease status.

In particular, diabetes is relatively prevalent in this sample (15.9% of females, 13.9% of males), consistent with the elevated risk for DM in the Mexican American population [18]. As noted in the Methods, most DM cases in the SAFHS are presumed Type 2 based on age of onset and correlation with obesity; however, the data collected for the study participants do not include a formal diagnosis of type of DM. For the present study, we used an algorithm (participant used insulin, had DM onset by age 20y, and had BMI < 30 kg/m²) that identified 3 of the 186 participants with DM as “possible Type 1”. Exclusion of these 3 individuals did not materially affect the outcome of preliminary statistical or genetic analyses (data not shown), and consequently they were not excluded from the final analyses.

3.2 Bayesian model selection

We examined the proportion of total variance of sICAM-1 attributable to 20 environmental and physiological covariates (Table 2). This panel of covariates yields a very large number (>10⁶) of possible models; to reduce the computational burden, we limited our analysis to models comprising no more than 5 covariates. We employed a Bayesian model selection algorithm (see Methods) to identify those models that provided the best fit to the data. For each degree (=number of covariates included), the model with the lowest BIC (i.e., with the best fit to the data) always included waist circumference as covariate; the final model includes waist circumference, smoking, age, and diabetes status (Table 2). Because not all covariates were available for all participants, the final sample size for this model is 1170.

3.3 Heritability and linkage analysis

Circulating sICAM-1 level, corrected for the covariates in the ‘best-fit’ model described above, is a significantly heritable trait: $h^2=0.50\pm0.06$, $P<10^{-6}$.

Fig. 1 shows results from a genome-wide linkage scan for sICAM-1. One locus on Chr. 19 shows genome-wide-significant evidence of linkage: adjusted LOD = 4.95 at 33 cM from p-terminus (detail in Fig. 2).

The genome scan shows a subsidiary linkage peak at ~43 cM, but support for this as a separate QTL is weak. Support for the subsidiary peak declined to LOD = 0.43 when a second multipoint linkage scan was conditioned on the primary linkage (Fig. 2), and a model that estimated locus-specific heritability (h^2_q) at both loci was not significantly more likely than the primary linkage model (Table 3).

3.4 Heterogeneity test

For the maximum linkage signal on Chr. 19, the test of homogeneity given linkage was highly significant ($P=0.0023$; Table 4), suggesting that, in this sample, there is heterogeneity among pedigrees in the segregation of this locus. (The test of homogeneity makes sense only at a locus showing evidence of linkage; consequently, the pointwise threshold $P<0.05$ is an appropriate level of significance for this test [17].) Three pedigrees contributed nearly all of the support for linkage assuming homogeneity: their combined per-pedigree adjusted LOD is 4.67 (the total adjusted LOD = 4.95 reflects some pedigrees providing negative support for linkage).

3.5 Positional candidate genes

We searched the public online databases of the National Center for Biotechnology Information (NCBI) [19] for known genes and QTLs within the Chr. 19 linkage region identified by our

genome scan. Table 5 aligns the linkage data with composite information from the public maps, and includes flanking regions. Alignment data include recombination map locations in centiMorgan (cM) from the SAFHS map and the public access Marshfield map; physical locations in millions of base pairs (Mbp) from NCBI human genome map build 35.1 and the Celera map; and cytogenetic position data.

The region flanked by D19S1034 and D19S714 (covering approximately 1.7 times the 1-LOD CI of the Chr. 19 peak) includes the ICAM-1 structural gene. This region also has been identified repeatedly in genome scans for inflammatory disease and autoimmunity, dyslipidemia, and diabetes (reviewed in [20]).

4. Discussion

We have performed a genetic analysis in the SAFHS of circulating soluble ICAM-1, a protein that participates in vascular endothelial activation and also serves as a clinical marker of inflammation. Our primary interest is to elucidate the genetic basis of normal variation in sICAM-1 level in our study population for two broad reasons: to clarify the physiological mechanisms of inflammation, and ultimately (once relevant genotypes are known) to be able to assess genetic risk for inflammation independent of other contributing factors. To control for possible effects of such factors, we first examined the mean effect on sICAM-1 levels of 20 plausible environmental and physiological factors. Bayesian model selection among the very large number of possible covariate models identified one model with optimal 'fit' to the sICAM-1 data; this model comprised 4 established risk factors for CVD (age, smoking, diabetes status, and waist circumference). The latter effect is especially noteworthy, given increasing attention to pro-inflammatory cytokine secretion by abdominal fat [21]. Interestingly, self-reported prior heart attack or heart surgery were not significant covariates of sICAM-1, which may be due to the imprecision of these measures (the length of time elapsed since the cardiac events was not recorded).

After correcting for the covariates, we found strong evidence of linkage on Chr. 19. This chromosomal region has been identified in other studies as a QTL for autoimmune diseases (irritable bowel disease and psoriasis), albuminuria, hypertension, dyslipidemia, and diabetes.

The obvious positional candidate gene in the linkage region is the ICAM-1 structural gene (*ICAM1*). Proximal to *ICAM1* are the structural genes for 3 other members of the ICAM gene family (*ICAM3*, 4, and 5).

The Chr. 19 QTL is flanked by genes for the insulin receptor and the LDL receptor; mutations in the former are implicated in rare familial forms of diabetes while mutations in the latter are associated with familial hypercholesterolemia (reviewed in [20]). Other genes in the region related to the metabolic syndrome are resistin and *UBL5*. The adipocyte-derived hormone resistin was originally identified in connection with allergic pulmonary inflammation in mice, but has received wider attention for its role in insulin resistance. Although not all human studies agree, there is evidence that resistin influences obesity, insulin sensitivity, and subclinical inflammation in our species (reviewed in [22]). *UBL5*, also known as *BEACON*, has drawn attention for its differential expression in lean and obese *Psammomys obesus*, an animal model of the metabolic syndrome. Polymorphisms in human *UBL5* are associated with variation in body composition; blood levels of total cholesterol, LDL, and triglycerides; and insulin response to glucose challenge [23]. Given the correlation between inflammation and the metabolic syndrome [6,9] it is interesting to note that the Chr. 19 QTL coincides with genes related to dyslipidemia, hypertension, and insulin resistance.

The Chr. 19 QTL shows significant evidence of linkage heterogeneity: 3 pedigrees provide most of the evidence for linkage of sICAM-1 to this locus. Cases where a limited number of

families are segregating alleles providing such strong genetic signal are especially promising targets for future genetic dissection [26]. In particular, we have initiated SNP analysis of positional candidate genes in the linkage region to determine whether the causative variant(s) in these families are indeed within the *ICAM1* gene. Identification of such cis-acting variant(s), if any, may illuminate the complex role of protein shedding in regulating circulating levels of sICAM-1. Recent evidence suggests that sICAM-1 levels may reflect not only increased synthesis of the protein at a localized inflammatory site, but also the degree of catalytic cleavage and shedding of the protein ectodomain; paradoxically, such cleavage may moderate inflammation at the primary site while the circulating protein may serve as a systemic proinflammatory signal [24]. Genetic variation in a regulatory region of *ICAM1* might alter its rate of expression, while variation in the coding region could alter protein function, perhaps including its susceptibility to cleavage. Any such information would help to clarify the physiological role of circulating sICAM-1 and consequently its clinical significance as a marker of inflammation.

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Fig. 1.
Whole-genome multipoint linkage scan for sICAM-1 (empirically adjusted LOD scores).

Fig. 2. Detailed multipoint linkage plot for sICAM-1 on Chromosome 19 (empirically adjusted LOD scores) for the initial multipoint scan (solid trace) and a second scan conditioned on the maximum linkage signal at 33 cM (dotted trace).

Table 1**Characteristics of the Study Participants**

Selected clinical and biochemical measures for 1269 participants from SAFHS as measured at the first clinic visit. Except for diabetes, menopause status, and smoking, data are reported as median (interquartile range).

	Males	Females
<i>n</i>	500	769
Age (years)	36.0 (24.0-50.1)	38.0 (25.3-49.0)
sICAM1 (ng/ml)	308.0 (249.8-378.0)	297.0 (245.9-359.8)
BMI (kg/m ²)	27.4 (24.0-31.3)	29.0 (24.8-33.4)
Waist circumference (mm)	940 (850-1041)	916 (800-1044)
Systolic BP (mm Hg)	120 (112-129)	114 (106-128)
Diastolic BP (mm Hg)	72 (67-78)	69 (63-76)
Fasting glucose (mmol/l)	4.95 (4.56-5.46)	4.82 (4.41-5.32)
Fasting insulin (pmol/l)	63.42 (38.1-108.6)	68.10 (43.5-108.5)
Fasting cholesterol (mmol/l)	4.81 (4.16-5.51)	4.84 (4.27-5.49)
Fasting HDL (mmol/l)	1.14 (0.99-1.34)	1.32 (1.14-1.55)
Fasting triglycerides (mmol/l)	1.47 (0.99-2.12)	1.34 (0.95-1.88)
With diabetes (%)	13.9	15.9
Report smoking (%)	36.2	15.6
Post-menopause (%)	--	27.1

Table 2**Bayesian model selection for covariates of sICAM-1**

Covariates in *italics* are discrete (condition present/absent). Degree = number of covariates included in test models. BIC = Bayesian Information Criterion (see text for explanation). *Maximum-likelihood estimates of each covariate beta (β_i) tested against null hypothesis ($\beta_i = 0$).

Degree	Model	BIC		
Covariates tested: age, sex, <i>menopausal status</i> , <i>diabetes status</i> , <i>pregnancy</i> , <i>prior ovariectomy</i> , <i>prior hysterectomy</i> , <i>prior heart attack</i> , <i>prior heart surgery</i> , <i>oral contraceptive use</i> , <i>hormone replacement</i> , <i>diabetes medication</i> , <i>antihyperlipidemia medication</i> , <i>antihypertensive medication</i> , <i>smoking</i> , <i>alcohol use</i> , log _e (BMI), log _e (waist circumference), log _e (systolic BP), log _e (diastolic BP)				
0	[no covariates]	0.00		
1	log _e (waist circumference) (= WC)	-96.58		
2	WC, <i>smoking</i>	-145.10		
3	WC, <i>smoking</i> , age	-161.67		
4	WC, <i>smoking</i> , age, <i>diabetes</i>	-165.52		
5	WC, <i>smoking</i> , age, <i>diabetes</i> , <i>diabetes medication</i>	-163.12		
Multiple linear regression of log _e (sICAM-1) on covariates, Degree 4:				
	Covariate	Unit	β _i	P-value*
	Waist circumference	mm (log _e scale)	+0.390	10 ⁻¹³
	<i>Smoking</i>	1 = reports smoking	+0.153	10 ⁻¹⁶
	Age	years	+0.002	0.0004
	<i>Diabetes status</i>	1 = with DM	+0.086	0.0010

Table 3

Quantitative genetic models

*Constrained parameter value. Trait: log_e(sICAM-1). Covariates: age, diabetes status, smoking, log_e(waist circumference).
Abbreviations: h²_T, residual heritability; h²_{qi}, locus-specific heritability; SE, standard error; ns, not significant at *P*<0.05.

Model	h ² _T (SE)	h ² _{q1} (SE)	h ² _{q2} (SE)	Loglikelihood	Test	P-value
0. Sporadic	0*	0*	0*	843.7434	N/A	N/A
1. Polygenic	0.50(0.06)	0*	0*	900.8745	1 vs. 0	10 ⁻²⁷
2. Linkage, loc 33	0.17(0.10)	0.33(0.07)	0*	914.2067	2 vs. 1	10 ⁻⁷
3. Linkage, loc 43	0.20(0.10)	0*	0.29(0.07)	912.0262	3 vs. 1	10 ⁻⁶
4. Linkage, both loci	0.12(0.10)	0.23(0.10)	0.14(0.09)	915.3588	4 vs. 2	0.064 (ns)

Table 4**Locus heterogeneity test for linkage of sICAM-1 to Chromosome 19, locus 33**

Data shown are per-pedigree contributions to the total adjusted LOD scores for tests of linkage assuming homogeneity and heterogeneity given linkage. Pedigrees are numbered arbitrarily by rank for probability of linkage. *Significant at $P < 10^{-5}$ (genome-wide $P < 0.05$). †Significant at pointwise $P < 0.01$.

Pedigree(s)	Test of linkage under homogeneity, LOD scale	Test of homogeneity given linkage, LOD scale	Posterior probability of linkage
1	2.06	0.58	1.00
2	1.36	0.16	0.99
3	1.25	0.07	0.99
4 – 46	-1.46 – 0.41	-0.11 – 1.16	0.00 – 0.91
Total LOD = 4.95*		Total LOD = 1.72†	

Table 5
Map alignment for sICAM-1 linkage peak on Chr. 19

Abbreviations: *BP3*, blood pressure QTL 3; *UAE1*, urinary albumin excretion QTL 1; *IBD6*, inflammatory bowel disease locus 6; *INSR*, insulin receptor; *RETN*, resistin; *ANGPTL4*, angiotensin-like 4; *PSORS6*, psoriasis susceptibility locus 6; *CMTDII*, Charcot-Marie-Tooth disease dominant intermediate 1; *UBL5*, ubiquitin-like 5; *LDLR*, low-density lipoprotein receptor; (*BPN*) (not an OMIM designation), blood pressure QTL in Nigerians [25]; *OA8*, osteoarthritis QTL 8. Marker D19S916 was not genotyped in SAFHS. Map alignment data from [19]. Recombination map positions of ICAMs [in brackets] are interpolated from the physical distances of the loci to flanking markers on the NCBI map.

Adjusted LOD	Marker	Gene/QTL	cM (SAFHS)	cM (Marshfield)	Mbp (NCBI)	Mbp (Celera)	Cyto. Locus
0.0474	D19S591	<i>BP3, UAE1</i>	10.22	9.84	3.0	3.07	
0.9856	D19S1034	<i>BP3, IBD6</i>	20.37	20.75	6.1		19p13
		<i>INSR</i>			7.1		19p13.3-p13.2
		<i>RETN</i>			7.6		19p13.2
		<i>ANGPTL4</i>			8.3		19p13.3
	D19S916 (not typed)	<i>PSORS6</i>			9.2	8.98	19p13.2
	D19S586	<i>CMTDII</i>	30.54	32.94	9.7	9.87	19p13.2-p12
		<i>UBL5</i>			9.8		19p13.3
4.8687		<i>ICAM1</i>	[31.17]		10.2		19p13.3-p13.2
		<i>ICAM4</i>	[31.30]		10.3		19p13.2
		<i>ICAM5</i>	[31.30]		10.3		19p13.2
		<i>ICAM3</i>	[31.30]		10.3		19p13.3-p13.2
		<i>LDLR</i>	[32.30]		11.1		19p13.3
4.9471	(Peak LOD)		33.00				
4.6028	D19S840		35.55	37.94	13.7		
3.6772	D19S714	(<i>BPN</i>)	39.52				
1.3988	D19S433	<i>OA8</i>	52.20	51.99	35.1	35.32	19q12