Brain, Behavior, and Immunity 49 (2015) 171–181

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Genetic and environmental determinants of population variation in interleukin-6, its soluble receptor and C-reactive protein: Insights from identical and fraternal twins

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ARTICLE INFO

Article history:
Received 18 December 2014
Received in revised form 19 May 2015
Accepted 25 May 2015
Available online 15 June 2015

Keywords:
Interleukin-6
Soluble interleukin-6 receptor
C-reactive protein
Twins
Obesity
Heritability

ABSTRACT

Interleukin-6 and C-reactive protein are commonly assessed biomarkers linked to illness, obesity, and stressful life events. However, relatively little is known about their heritability. By comparing Caucasian twins from the Midlife in the US project (MIDUS), we estimated the heritability of IL-6, its soluble receptor, and CRP. Based on the hypothesis that adiposity might contribute more to IL-6 than to sIL-6r, we fit heritability models quantifying the extent to which each reflected genetic and environmental factors shared with obesity. Genetic influences on IL-6 and its receptor proved to be distinct. Further, the appearance of a heritable basis for IL-6 was mediated largely via shared paths with obesity. Supporting this conclusion, we confirmed that when unrelated adult controls are carefully matched to twin participants on BMI, age, gender and socioeconomic indices, their IL-6 is similar to the corresponding twins. In contrast, the effect of BMI on CRP was split between shared genetics and environmental influences. In conclusion, IL-6 is strongly affected by factors associated with obesity accounting for its lability and responsiveness to diet, life style and contemporaneous events.

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1. Introduction

The pleiotropic cytokine, interleukin-6 (IL-6), is produced by many cells and tissues, and plays a major role in normal physiology and inflammatory responses. It has been widely employed in research on population health and aging because circulating levels of IL-6 tend to rise in old age, with obesity, and following stressful life events (Fried et al., 1998; Friedman et al., 2005; Kiecolt-Glaser et al., 2003). It has also been associated with chronic stress and vulnerability to depression (Bob et al., 2010; Lutgendorf et al., 1999; Miller et al., 2002). Although blood levels of IL-6 are often quantified in isolation, its biological actions are determined by two distinct membrane bound glycoproteins expressed on the surface of target cells: (1) a classical transmembrane IL-6 receptor (mIL-6r), and (2) a signal-transducing non-ligand binding subunit, gp130, which is activated by a complex formed by IL-6 and the soluble form of the IL-6 receptor (sIL-6r) (Kallen, 2002; Peters et al., 1998). Further, the sIL-6r has also been shown to moderate central actions of IL-6 within the brain (Schöbitz et al., 1995). Therefore, to more completely understand variation in IL-6 synthesis and responses across individuals, it is important to also quantify the soluble receptor, which was done in the following study.

IL-6 and sIL-6r are coded by different genes and controlled by distinct mechanisms of expression (Crichton et al., 1996; Jones et al., 2001; Lust et al., 1992). In addition, sIL-6r may be produced either by alternative mRNA splicing or by proteolytic cleavage and shedding from the surface of cells (Jones et al., 2001; Müllberg et al., 1993). Thus, it is of significance to determine the extent and similarity of the genetic constraints on IL-6 and its soluble receptor. It is known that the magnitude of the IL-6 response in inflammatory conditions can be affected by different single nucleotide polymorphisms (SNPs) associated with the IL-6 gene (Bruunsgaard et al., 2004; Sen et al., 2011; Walston et al., 2007). Accordingly, an examination of IL-6 responses to a strong inflammatory stimulus yielded high heritability estimates (de Craen et al., 2005). However, this general conclusion is often, and inappropriately, overgeneralized to all aspects of IL-6 synthesis and release. In the absence of inflammatory stimuli, SNPs have a weak or no association with baseline levels of IL-6 in the blood stream (Bagli et al., 2003; Bennermo et al., 2004; Brull et al., 2001; Burzotta et al., 2001; Herbert et al., 2006; Lieb et al., 2004; Nauck et al., 2002; Sen et al., 2011; Shah et al., 2013; van Oijen

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http://dx.doi.org/10.1016/j.bbi.2015.05.010
0889-1591/Published by Elsevier Inc.

et al., 2006; Wernstedt et al., 2004). On the other hand, SNPs affecting sIL-6r production appear to be more consistently associated with its levels in systemic circulation (Galicia et al., 2004; Rafiq et al., 2007; Sasayama et al., 2012; van Dongen et al., 2014).

Serum IL-6 and sIL-6r are both influenced by body adiposity, although IL-6 appears to be affected to a greater extent than the soluble receptor (Crichton et al., 1996; Mehra et al., 2006; Mohamed-Ali et al., 1999; Pini et al., 2012). Accordingly, sIL-6r can serve as an independent biomarker for certain pathological conditions. It does not always react to the same physiological stimuli as IL-6; it is not always responsive to changes in IL-6 levels, and it does not play a role in all IL-6 pathways (Hurst et al., 2001; Kallen, 2002; Lamas et al., 2013; Mehra et al., 2006; Montero-Julian, 2001; Peters et al., 1998). Adipocytes, as well as the macrophages embedded in fat tissue, are known to be a major source of the IL-6 found in blood, especially in the non-inflammatory, healthy state (Coppack, 2007; Fried et al., 1998; Khaodhiar et al., 2004; Suganami and Ogawa, 2010; Weisberg et al., 2003; Wisse, 2004). The adipokine leptin also stimulates the release of IL-6 from leukocytes and macrophages (Agrawal et al., 2011; Behrendt et al., 2010; Kredel et al., 2013). Although these biological pathways often interact in a bidirectional and reciprocal manner, there is considerable evidence to suggest that adiposity exerts a far greater influence on inflammatory physiology (Miller, 2003; Welsh et al., 2010). Given the strong association between obesity and IL-6, it is likely that heritable factors influencing weight gain would also have a parallel effect on IL-6, a hypothesized linkage specifically tested in our analyses.

Likewise, the acute phase reactant, C-reactive protein (CRP) has been consistently associated with adiposity and inflammatory conditions, including cardiovascular disease (Carroll et al., 2009; Gupta et al., 2012; Saijo et al., 2004). Obesity can increase production of CRP by the liver as well as in adipose tissue (Anty et al., 2006; Calabro et al., 2005). Although previous genetic and heritability studies have demonstrated direct and independent effects on CRP levels(de Maat et al., 2004; Dehghan et al., 2011; Pankow et al., 2001; Wörns et al., 2006), there is evidence suggesting a causal relationship between the genetics of obesity and circulating CRP levels. SNPs associated with body mass index (BMI) can influence blood levels of CRP, while the converse has not been demonstrated (Holmes et al., 2014; Welsh et al., 2010). Thus, to further probe the unique nature and strength of the association between adiposity and IL-6, we also considered the relationship between adiposity and CRP. It is known that both CRP and IL-6 are involved in inflammatory response pathways, but CRP production appears to be more readily stimulated by IL-6, even within adipose tissue (Anty et al., 2006; Calabro et al., 2005; Heinrich et al., 1990; Volanakis, 2001). In addition, circulating levels of CRP and IL-6 are regulated independently by different genes (Shah et al., 2013). By analyzing these associations in identical and fraternal twins, we were able to directly compare and contrast the relative influence of adiposity on both CRP and IL-6, and to consider the reciprocal relationship between CRP and IL-6.

Previous twin studies have determined that adiposity, measured as BMI, is highly heritable (Hjelmborg et al., 2008; Schousboe et al., 2003; Segal et al., 2008). Associations between a genetic score, consisting of 14 SNPs related to BMI, and multiple cardiovascular and inflammatory traits including both IL-6 and CRP, were recently assessed (Holmes et al., 2014). However, it is still not known whether the heritable influence of obesity on IL-6 and its soluble receptor are coordinated, and whether CRP is affected in a similarly linked manner. We probed these relationships by comparing IL-6, sIL-6r and CRP levels in identical and fraternal adult twins, who also varied in adiposity and anthropometric concordance.

Twin studies take advantage of the different degree of genetic relatedness between monozygotic (MZ) and dizygotic (DZ) twins to estimate the relative contribution of genetic and environmental effects contributing to the phenotypic variance of a trait, as well as to the covariance between traits. Typical twin studies rely on the assumption that MZ twins share 100% and DZ twins share 50% of their genes, while both types of twins, regardless of zygosity, share a common rearing environment (Hall, 2003). In addition to the shared environment, it is also possible to discern effects of the non-shared environment, reflecting individual experiences not shared in common. Greater phenotypic similarity for MZ twins than found in DZ twins would be indicative of higher heritable contributions. On the other hand, when MZ and DZ twins present a similar phenotype, more variance attributable to life style and common environmental processes is assumed. Likewise, when monozygotic twins are discordant, it is usually attributed to unshared environmental influences (Boomsma et al., 2002; Christensen et al., 2001; Duffy et al., 1998; Hjelmborg et al., 2008).

We utilized bivariate ACE heritability models based on structural equation modeling (SEM) to estimate the proportion of IL-6, sIL-6r and CRP variation accounted for by genetic, shared and unshared environmental factors, as well as the heritable influences shared with adiposity. Our *a priori* hypothesis was that obesity would have a larger effect on IL-6 than on sIL-6r. Secondarily, the *a posteriori* hypothesis was tested: obesity would exert a, heritable influence on CRP, but one that was only moderately associated with the genetics of IL-6. We also compared IL-6 and sIL-6r intra-class correlations (ICCs) between co-twins in order to confirm the greater similarity between MZ co-twins than between DZ co-twins, indicative of heritable influences. To further test this hypothesis, we compared the ICC for MZ co-twins with that of genetically unrelated control participants matched to each MZ twin case. Assuming that environmental and lifestyle factors, BMI in particular, play a greater role in accounting for IL-6, we predicted that ICCs between the actual MZ co-twins would be no higher than for control adults who were closely matched on age, gender, BMI and a socio-economic index (SEI). By matching control subjects to the twin cases on these attributes, we simulated 4 of the major environmental and host factors known to influence IL-6 (Ershler and Keller, 2000; Friedman et al., 2005; Hjelmborg et al., 2008; Johnson and Krueger, 2005; O'Connor et al., 2007; Wisse, 2004). Hence, we could infer whether BMI and demographics (i.e., age, gender and SEI) accounted for the concordance between matched controls and co-twins. Given the likelihood of strong genetic constraints on sIL-6r, we anticipated small or negligible ICCs for sIL-6r with the unrelated, matched controls.

These analyses were possible because the recruitment strategy of a large survey of health and aging in the United States, Midlife Development in the United States (MIDUS), which included an over-sampling of twin siblings. It provided the unique opportunity to determine the heritability of circulating IL-6, sIL-6r and CRP, as well as the heritable contribution of obesity.

2. Methods

2.1. Participants

Participants were drawn from the MIDUS II Biomarker project, 2004–2009, a continuation of an earlier MIDUS 1 survey supported by the MacArthur Foundation in 1995–96. In addition to a representative probability sample, MIDUS 1 recruited a national sample of twin pairs, from which the current cohort was selected. Between 2003 and 2005, blood specimens were obtained, enabling the determination of cytokines and other biomarkers for each twin

pair (Love et al., 2010). The twin sample was comprised of 73 monozygotic and 32 dizygotic same-sex twin pairs, as well as 37 matched controls. In addition, we took advantage of BMI and IL-6/sIL-6r data on 830 unrelated participants. The same dataset was utilized for the CRP analyses, with information available on 72 monozygotic twins, 31 dizygotic twins, and 826 unrelated participants.

2.2. Twin recruitment

Twin pairs were recruited by asking randomly selected correspondents from about 50,000 households across 48 states whether there were twin pairs in the immediate family. With their permission, twin pairs were referred to MIDUS II recruiters. The recruited twin pairs were related to original correspondents, reared together but living apart as adults, ranging from 25 to 74 years in age, residing in the continental U.S., English speakers, possessing a residential telephone number, and were mentally and physically capable of participating in interviews and questionnaires. They had to be healthy enough to travel to one of 3 Institutes for Clinical and Translational Research (ICTR) for the Biomarker project, where they spent the night before sample collection on the following morning.

2.3. Socio-demographics, zygosity, clinical and biological measures

Zygosity was determined by self-report in MIDUS 1. Similarity of eye and hair colors, as well as the degree to which their identity was confused by others during childhood, were among the criteria for twin determination. This approach is more than 95% accurate when compared to blood tests (Nichols and Bilbro, 1966). Each sibling's Socioeconomic Index (SEI) was derived using income, educational attainment and occupation categories from the 1990 Census classification (Hauser and Warren, 1997) and incorporated into MIDUS II, 2004–2006.

Clinical and biological measures were assessed for a total of 1255 participants in the Biomarker project who consented to the overnight stay, either in Madison, WI, Los Angeles, CA, or Washington DC. Participants arrived on Day 1 at one of the three sites where they were admitted to the hospital research unit. They completed a medical history and physical exam, as well as a self-administered questionnaire. Smoking can affect IL-6 and could interact with genetic factors that influence transcription and release of IL-6 (Bruunsgaard et al., 2004; Semlali et al., 2012; Zhou et al., 2014). Therefore, we also examined the concordance of the smoking history between MZ and DZ co-twins.

Smoking history was assessed on the clinical questionnaire by asking: "Have you ever smoked cigarettes regularly?". Fasted blood samples were obtained between 0500 and 0700, and sera frozen until analyzed. All sample collections and analyses were approved by the Health Sciences Institutional Review Board at the University of Wisconsin-Madison, as well as by the IRBs at UCLA and Georgetown University. All participants provided informed consent. Nursing staff followed standardized procedures detailed in a general "Manual of Procedures", as well as specific "Guidelines for Collecting and Processing Biomarkers" in order to maintain consistency.

2.4. Cytokine assessment

Serum IL-6 levels were determined for all 1255 biomarker project participants by high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, Minneapolis, MN), with a lower sensitivity of detection at 0.16 pg/mL. All values were quantified in duplicate; any value over 10 pg/mL was re-run with sera diluted to fall on the standard curve. The laboratory intra-assay coefficient of variance (CV) was 4.1% and the inter-assay CV was 12.9% (generated by inclusion of a low and high IL-6 serum pool in each assay). Sandwich ELISA kits were also employed to quantify sIL-6r levels (Quantikine, R&D Systems). Sera were diluted 1:100 so values would fall on the standard reference curve from 7 to 2000 pg/mL. Thus, the effective assay range for sIL-6r was 0.7–200 ng/mL. The intra-assay and inter-assay CVs were 2.0% and 6.9%, respectively. Serum CRP levels were determined for all subjects via particle-enhanced immunonephelometric assay, and high values used as exclusion criteria.

2.5. Statistical analysis

Nine twin pairs were excluded when a sibling had CRP levels indicative of sickness (above 10 mg/L), or they were discrepant on smoking status or chronic illness. In addition, because there were insufficient numbers of African-Americans (only 2 twin pairs), and there are known race differences in cytokine and CRP levels (Carroll et al., 2009; Coe et al., 2011; Crawford et al., 2006), both pairs were excluded. Opposite-sex, dizygotic twin pairs were also excluded from the analysis because there were too few pairs to include in the model. In order to achieve normal distributions, IL-6, sIL-6r, CRP and BMI were natural log-transformed before statistical calculations.

Linear regressions were employed to examine the effect of BMI on IL-6, sIL-6r, and CRP for singleton birth participants in the main Biomarker sample, after excluding the twin participants. Including the twins in these regression analyses would have violated assumption of independent observations. Similarly, these regression analyses were run on only the Caucasian participants of European descent, because of the known differences in African-Americans (Coe et al., 2011).

In order to estimate the additive genetic and environmental effects contributing to the IL-6, sIL-6r and CRP variance, including the covariance with factors affecting BMI, we fitted bivariate ACE models to these data by using Cholesky's decomposition approaches in SEM (Karmakar et al., 2012). SEM allows for testing whether covariance matrices of hypothetical models fit the covariance matrix of the actual data. They further allow for distinguishing the submodels that fit the data most parsimoniously. Classical ACE models decompose the phenotypic variance into three categories, A (Additive Genetic Effects), C (Common Environmental Effects) and E (Unshared Environmental Effects plus residual/error variance). Bivariate ACE models estimate A, C and E effects for two phenotypic traits and, in addition, estimate the covariance between each effect across phenotypes. We defined a saturated ACE covariance model as well as submodels for AE covariance (negligible sharing of common environmental effects) and CE covariance (negligible sharing of additive genetic effects) which were tested against the data covariance matrices, providing the bases upon which one may identify the models that best fits the data. The Cholesky's decomposition of phenotypic effects allowed us to parse out the additive genetic effects unique to IL-6, sIL-6r, or CRP from those shared with BMI by comparing the various covariance submodels, while allowing univariate variance components to vary freely. This same approach was used in order to test the genetic covariance shared between CRP and IL-6.

These biometric models were run in MX using a script adapted from file rawVC4a.mx, provided with the MX software (Neale et al., 2003; Posthuma and Boomsma, 2005). Because MZ and DZ twins share 100% and 50% of their independently segregating genes, respectively, SEM covariance coefficients for additive genetic effects were set to 1 for MZ twin pairs and to ½ for DZ pairs. Covariance coefficients for common environment effects were set to 1 and those attributed to unshared environment effects were set to 0. Age and Gender were included in Cholesky's

decomposition models as covariates in order to prevent indirect inflation of ICCs that could potentially confound heritability estimates (McGue and Bouchard, 1984). Saturated and nested covariance submodels (ACE, AE and CE) were fit to the data and submodels were tested against the saturated ACE models. Non-significant differences in Chi-square probabilities allowed us to discern nested submodels that provided fit of the data. The models that best described our data were identified on the basis of maximum likelihood estimates, −2LL, (−2 * log-likelihood = $-2 * \log(C - \chi^2/2)$), and relative fit indices, Akaike's Information Criterion, AIC, (AIC = $\chi^2 - 2 *$ d.f.), and Bayesian Information Criterion, BIC (BIC = $\chi^2 -$ d.f. * $\ln(n)$). Lower AIC and BIC values indicate models that fit the observed data most parsimoniously.

Intra-class correlations for IL-6, sIL-6r, and smoking history were calculated for the MZ and DZ co-twins (Hawkins, 1989; Hotelling, 1953; Sedgwick, 2013). One-tailed Fisher's r-to-z tests assessed whether IL-6 and sIL-6r ICCs for MZ twins were significantly greater than for DZ twins. To further evaluate the influence of obesity on IL-6 and sIL-6r, one-tailed Fisher's r-to-z tests were used to compare the ICCs between MZ co-twins to the ICCs between 37 MZ twins and unrelated controls matched for age, gender, BMI and SEI. Smoking history was assessed using a two-tailed Fisher's r-to-z test to examine the presence of possibly confounding differences between MZ and DZ ICCs. Except for the biometrical models, analyses were determined with SPSS 19.

3. Results

3.1. Twin sample

The MIDUS Biomarker project has been shown previously to be comparable to the larger MIDUS participants for most socio-demographic, health status, and health behavior indicators, but not race (Love et al., 2010). The latter was not an issue for the current analyses because the 2 African-American twin pairs were excluded. Table 1 presents the socio-demographic and clinical information, and mean cytokine values for MZ and DZ twins and the unrelated controls who were matched with the cases, as well as for the remaining Biomarker participants from which the controls were selected.

3.2. Effect of BMI on IL-6, sIL-6r and CRP

After adjusting the regression models for age and gender by including them as covariates, BMI accounted for 7.8% of the variance in IL-6 levels ($\beta = 0.28$, $F[1,829] = 42.4$, $p < 0.001$), and 18.0% of the variance in CRP levels ($\beta = 0.43$, $F[1,826] = 70.0$, $p < 0.001$), but predicted only 0.3% of the variance in sIL-6r ($\beta = 0.07$, $F[1,829] = 3.2$, $p = 0.02$). In keeping with our predictions, BMI exerted a stronger effect on IL-6 than on the soluble IL-6 receptor among unrelated MIDUS participants, as well as had a substantial effect on CRP.

3.3. Heritability estimates

Non-significant chi-square difference tests indicated that CE and AE covariance submodels for BMI X IL-6, BMI X sIL-6r, BMI X CRP and CRP X IL-6 presented an equivalent fit for the data as did the saturated ACE covariance models. In addition, lower relative fit indices (AIC and BIC) identified the AE submodels as most parsimoniously fitting all three sets of BMI covariance data, and the CE submodel as a better fit for the CRP X IL-6 data (Table 3). In the three bivariate BMI models, additive genetic effects and unshared environmental effects comprised almost all of the influences acting independently on BMI, thus providing two available pathways for covariance with IL-6, sIL-6r, and CRP (Table 4). Covariance was notably high in accounting for the heritable associations between BMI and IL-6 (83.0%). The proportions presented in Table 4 were calculated based on the SEM path coefficient estimates.

These effects are also portrayed in Fig. 1 for IL-6 and in Fig. 2 for its soluble receptor, which show the discrete effects parsed by specific sources of variation. These estimations indicated further that the suggestion of a genetic constraint on IL-6 levels was not attributable specifically to the genetics of IL-6, but rather was driven more indirectly by factors shared with the predisposition for BMI (Fig. 1). Even when inflated by considering the high covariance with BMI (Table 4), the total genetic effect estimated for IL-6 was still small (averaging 26.2%). In contrast, the heritable influences on the soluble receptor for IL-6 were significantly stronger (averaging 42.2%). Although sIL-6r also showed some covariance with genetic factors associated with BMI (33.7%, Table 4), path coefficients indicated the effects attributable to BMI were minimal (Fig. 2). In addition, a much larger portion of the total phenotypic variance was unique to sIL-6r (Fig. 2). CRP also exhibited a high genetic covariance with BMI (54.5%, Table 4); however, not to the same extent as the IL-6 covariance (83.0%). Further, the additive genetic effects on CRP were almost evenly divided between those shared with BMI and factors unique to CRP (Fig. 3). Moreover, our models did not reveal shared genetic effects between CRP and IL-6 (Fig. 4). The covariance between CRP and IL-6 phenotype was split between shared and unshared environmental effects (Table 4).

3.4. Intra-class correlations

Stronger genetic constraints on sIL-6r were also clearly evident when comparing the trait ICCs between the twins and unrelated adult controls who had been matched on BMI, gender, age, and

Table 1
Sample descriptives for primary variables used in the twin analyses.

	MZ		DZ		Control		Larger sample	
Age	52.8	(11.2)	50.2	(9.1)	52.0	(8.5)	56.0	(11.9)
BMI (Kg/m^2)	27.6	(5.4)	29.6	(5.7)	25.6	(3.4)	29.4	(6.1)
IL-6 (pg/mL)	2.1	(1.7)	2.7	(2.1)	2.5	(1.7)	2.9	(3.0)
sIL-6r (pg/mL)	37,401	(10,582)	33,611	(7826)	34,600	(9407)	36,412	(10,351)
CRP	2.10	(2.22)	2.54	(2.26)	2.12	(1.87)	3.02	(4.78)
SEI	44.6	(13.9)	44.8	(15.8)	44.2	(10.7)	43.0	(14.1)
Ever Smoked	36.0%		39.0%		43.3%		46.5%	
Males	62		16		20		394	
Females	84		48		17		436	
Total Participants	146		64		37		830	

Mean values and standard deviations, or counts for numbers of participants.
MZ, Monozygotic Twins; DZ, Dizygotic twins; the Larger Sample was comprised of all other subjects in Biomarker project, after excluding the twins and African-American participants.

Table 2
Intra-class correlations and statistical difference between coefficients demonstrating MZ and DZ twins had similar values for IL-6, but differed for sIL-6r.

	Intra-class correlation coefficients and 95% C.I.						Fisher's *r*-to-*z*, *z*-values			
	MZ		DZ		MC		MZ vs. DZ		MZ vs. MC	
IL-6	0.35 (.17–.50)	$P < 0.001$	0.32 (−.02 to .60)	$p = 0.03$	0.36 (.02 to .63)	$p = 0.02$	0.15	n.s.	0.05	n.s.
sIL−6r	0.73 (.62–.80)	$p < 0.001$	0.48 (.17 to .70)	$p = 0.002$	−0.20 (−.51 to .16)	n.s.	1.84	$p = .03$	5.41	$p < 0.001$

MZ, Monozygotic Twins; DZ, Dizygotic Twins; MC, Matched Controls.

Table 3
Comparison of nested model statistics.

Covariance Models		−2LL	D.F.	AIC	BIC	Difference χ^2	*p*-Value
BMI X IL-6	ACE	39.22	405	−770.77	−922.81		
	CE	41.52	406	−770.48	−924.00	2.29	0.13
	AE	**40.69**	**406**	**−771.31**	**−924.41**	**1.46**	**0.23**
BMI X sIL-6r	ACE	−216.23	405	−1026.23	−1050.54		
	CE	−215.63	406	−1027.63	−1052.57	0.60	0.44
	AE	**−215.99**	**406**	**−1027.99**	**−1052.75**	**0.25**	**0.62**
BMI X CRP	ACE	1396.80	401	594.80	−232.80		
	CE	1398.10	402	594.10	−234.47	1.31	0.25
	AE	**1396.81**	**402**	**592.81**	**−235.12**	**0.01**	**0.91**
CRP X IL-6	ACE	1415.15	401	613.15	−223.63		
	CE	**1415.46**	**402**	**611.46**	**−225.79**	**0.31**	**0.57**
	AE	1415.48	402	611.47	−225.78	0.33	0.57

−2LL (−2 * Log-Likelihood), maximum likelihood estimate; AIC, Akaike's Information Criterion; BIC, Bayesian Information Criterion; Lower −2LL, AIC and BIC indicate models that more parsimoniously fit the data; Non-significance in chi-square (χ^2) values between saturated and nested models indicate equivalent fit of data; Bold highlighted font indicates the model that most parsimoniously fit the data, based on AIC and BIC.

Table 4
Proportion of genetic and environmental effects for best-fit models.

Variance/Covariance components	Estimated effects (95% C.I.)					
	Additive genetic (A)		Common environment (C)		Unshared environment (E)	
BMI	68.8%	(41.4–78.5)	0.0%		31.2%	(21.5–44.8)
IL-6	26.1%	(12.5–51.8)	7.6%	(0–7.6)	66.3%	(48.2–84.3)
Covariance	83.0%				17.0%	
BMI	68.2%	(21.1–78.9)	1.3%	(1.1–44.7)	30.5%	(21.1–44.0)
sIL-6r	42.2%	(7.7–82.9)	35.6%	(0–67.9)	22.2%	(15.3–32.2)
Covariance	33.7%				66.3%	
BMI	70.6%	(27.2–79.8)	0.0%		29.4%	(20.2–42.7)
CRP	18.2%	(1.2–50.0)	13.7%	(0.0–36.6)	68.1%	(50.0–88.2)
Covariance	54.5%				45.5%	
CRP	15.2%	(0.0–31.7)	14.7%	(2.8–44.2)	70.1%	(52.8–90.0)
IL-6	0.0%	(0.0–35.0)	37.8%	(6.4–53.0)	62.2%	(45.4–79.9)
Covariance			51.3%		48.7%	

Best-fit models were chosen on the basis of the AIC and BIC.

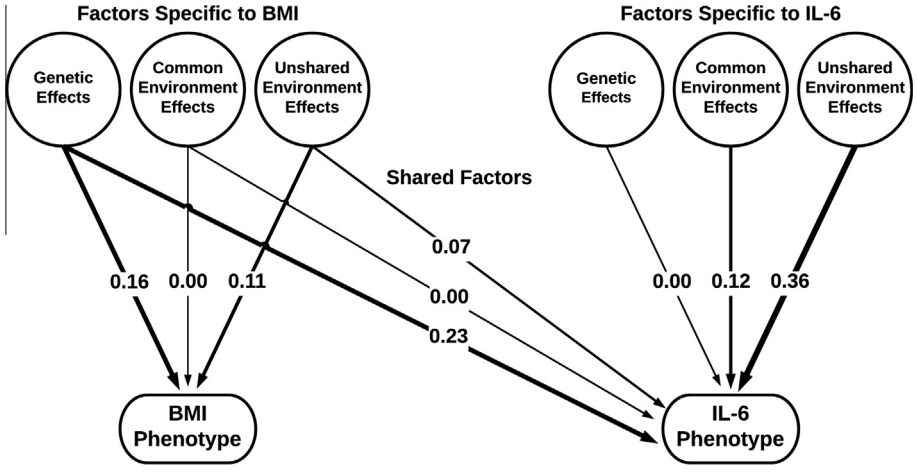

Fig. 1. Path coefficient estimates for bivariate BMI X IL-6 model, including the corresponding percent contributions of additive genetic effects, and common and unshared environmental effects affecting IL-6. The model also parses effects specific to IL-6 from those shared with BMI. The most parsimonious model indicated that BMI and IL-6 share both genetic and unshared environmental effects. However, model estimates further indicated that the additive genetic effect acting on IL-6 is mostly, shared with BMI.

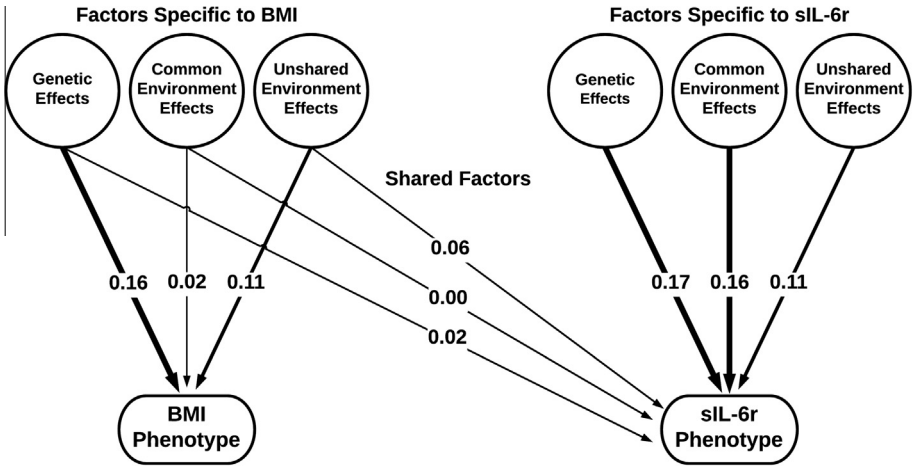

Fig. 2. The path coefficient estimates for the bivariate BMI X sIL-6r model also parsed the genetic effects specific to sIL-6r from those shared with BMI. This model similarly points to a shared covariance between genetic and unshared environmental effects. However, most of the additive genetic effects explaining the sIL-6r phenotype were independent of BMI, thus very distinct from the pattern of genetic constraints found for IL-6.

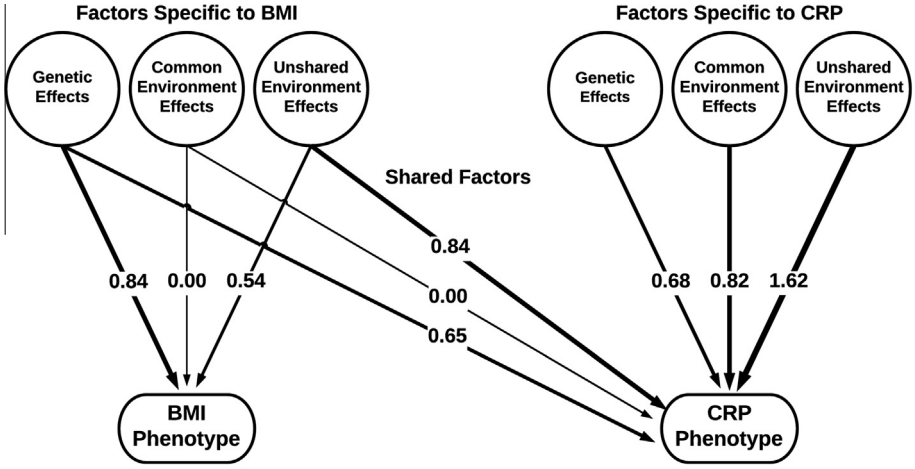

Fig. 3. The most parsimonious path coefficient estimates for the bivariate BMI X CRP model also indicated shared covariance between genetic and unshared environmental effects. Unlike the bivariate model for IL-6, however, the phenotypic variance attributed to the additive genetic effects was split between those shared with BMI and some independent of BMI.

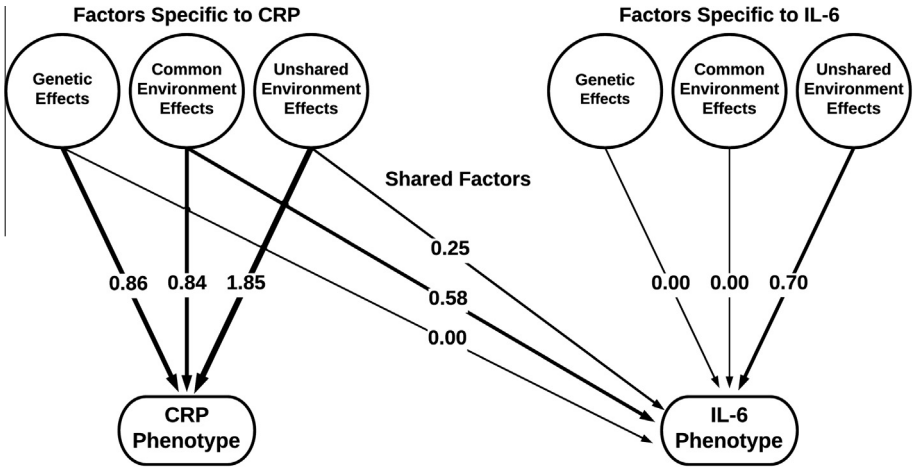

Fig. 4. The path coefficient estimates for the bivariate CRP X IL-6 model that most parsimoniously fit the data indicated a lack of genetic covariance between CRP and IL-6, attributing the phenotypic covariance to common and unshared environmental effects.

SEI (Table 2). This case/control analysis confirmed the importance of heritable pathways related to obesity, and indicated that the 4 matching criteria resulted in IL-6 values similar to the twins, whereas there was no evidence that these 4 attributes increased the likelihood of a correlation in sIL-6r between cases and controls. In addition, as shown in Table 2, the ICC for IL-6 between the MZ twins was not greater than for the DZ twins, demonstrating that the degree of relatedness did not influence IL-6 more than the concordance for obesity. In contrast, the sIL-6r levels were more similar for MZ twins, while DZ twins were divergent, supporting the greater heritability of sIL-6r. When a MZ participant was matched to an unrelated control by age, gender, BMI and SEI, the resulting ICC for IL-6 was nearly the same as for the MZ co-twin (Fig. 5a and b). On the other hand, the sIL-6r of a twin was not significantly correlated with the value seen in the unrelated, matched control (Fig. 5c and d). MZ and DZ twins were highly concordant for smoking history ($r = 0.57$, $p < 0.001$ and $r = 0.41$, $p = 0.02$, respectively). However, a history of smoking or abstinence did not significantly affect either the IL-6 or sIL-6r levels in this cohort.

4. Discussion

By taking advantage of the fact that monozygotic twins are genetically more similar than dizygotic twins, our analyses demonstrated that the genetic constraints on IL-6 and its soluble receptor are clearly distinct. In addition, the association between BMI and IL-6 was more evident than for sIL-6r. This differential relationship with adiposity affected the heritability estimates. By fitting bivariate ACE models, we calculated variance and covariance components, parsing out genetic and environmental effects specific to IL-6, sIL-6r, and CRP from those linked to the heritability of BMI. The approach more clearly revealed the degree of covariance in the additive genetic effects shared by IL-6 and BMI. Optimized path coefficients did not expose unique effects specific to the genetic control of IL-6, but rather an overlap of the heritable processes influencing both IL-6 and BMI in adults. These analyses also extend our understanding of the differential influence exerted by BMI on CRP. While the regression analyses did show that BMI affected serum CRP levels to a greater extent than IL-6, the heritability models indicated that the effects of BMI on IL-6 were largely due

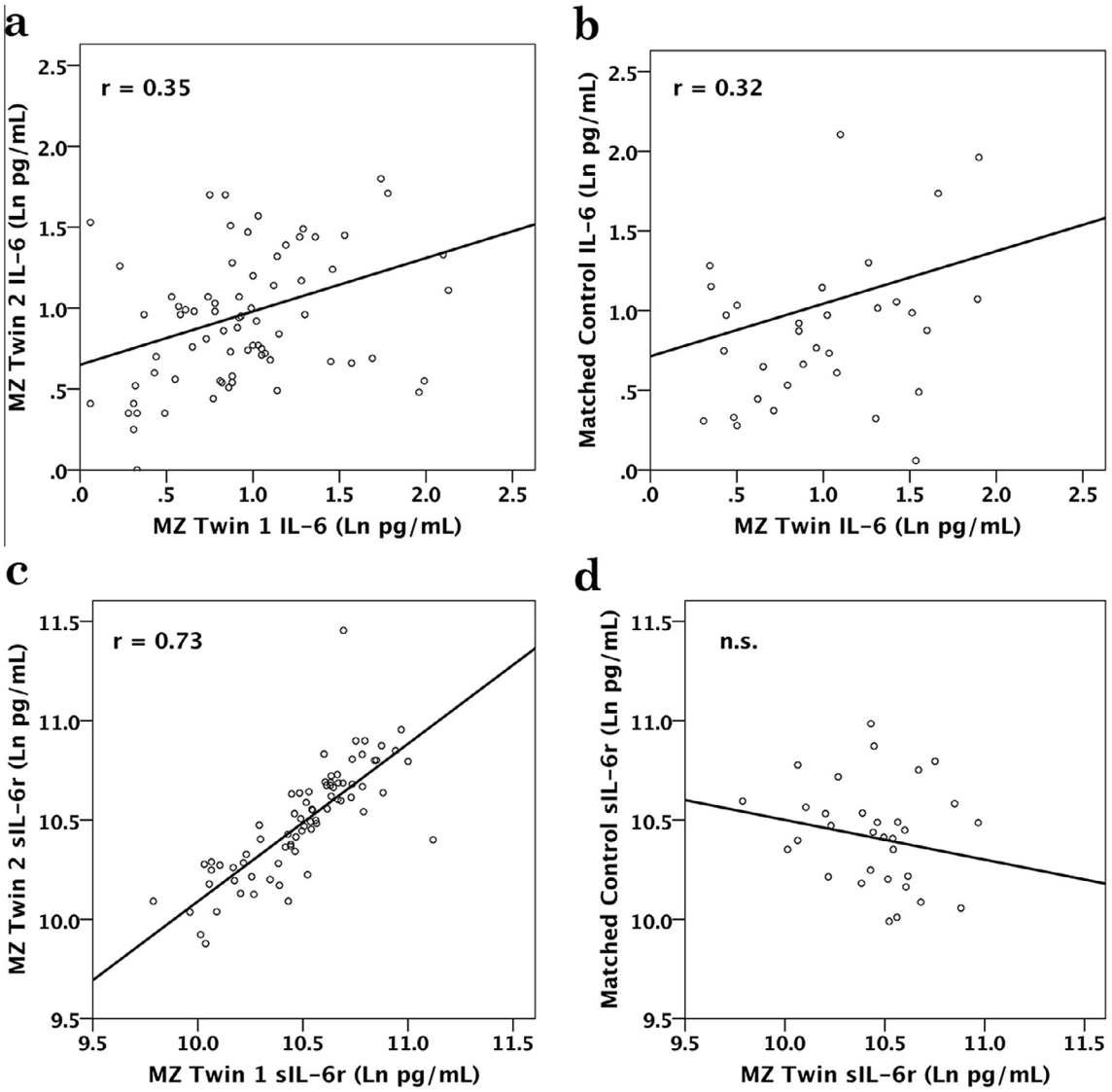

Fig. 5. Intra-class correlations are shown for IL-6 between MZ co-twins and between MZ twins and unrelated, matched controls, as well as for the sIL-6r associations. The correlation for IL-6 for MZ co-twins (a) was nearly identical to the correlation between MZ twins and unrelated controls matched on age, gender, BMI and SEI (b). Levels of sIL-6r in MZ co-twins were highly correlated (c), while sIL-6r values were not significantly correlated between MZ cases and their unrelated, matched controls (d).

to shared genetics. In contrast, the effect of BMI on CRP was split between shared genetics and environmental influences. The genetic effects acting on CRP were evenly distributed between those shared with BMI and those unique to CRP genetics, whereas the additive genetic effects influencing IL-6 phenotype appeared to be more exclusively tied to BMI. In spite of the close relationship between CRP and IL-6, our heritability estimates point to environmental factors as the main source of covariance. Hence, in the unstimulated state without infection or chronic disease, the genetics underlying body adiposity appears to influence IL-6 and CRP levels in the blood through independent pathways.

In order to further validate our conclusions on the differential constraints regulating IL-6 and sIL-6r, we conducted a case/control analysis, with each twin matched to an unrelated individual on the basis of gender, age, BMI, and education. Given the strong influence these 4 variables have on IL-6, the ICCs attained for the matched controls were of the same magnitude as for the actual co-twin siblings. In contrast, the sIL-6r values were markedly discordant, confirming that the genetic constraint on the soluble receptor was less influenced by life style and other environmental factors.

Our heritability estimates for IL-6 in blood do contrast with some of the commonly held assumptions derived from other approaches. For example, it has been reported previously that both CRP and IL-6 levels are similar in twins, a finding that will emerge when the influence of adiposity is not taken into account, nor statistically considered as a contributing factor (Rooks et al., 2012; Wörns et al., 2006). In addition, there is a substantial literature reporting that allele polymorphisms affect IL-6 release, but that effect is most apparent in the context of inflammatory disorders, or when cells are activated *in vitro* by a proinflammatory stimulant (Bennermo et al., 2004; Brull et al., 2001; Burzotta et al., 2001; Shah et al., 2013). IL-6 gene-related polymorphisms include the SNPs rs1800795 and rs1800796 (Chatzikyriakidou et al., 2013; Chen et al., 2012; Vaughn et al., 2013). Although these polymorphisms do affect inflammatory responses in patients (Bruunsgaard et al., 2004; Sen et al., 2011; Walston et al., 2007), they do not appear to have a strong influence on basal IL-6 in the blood of a healthy individual. In addition, these SNPs do not have a strong effect on IL-6 transcription at baseline or even a large influence when the cells are activated by proinflammatory stimulants (Smith et al., 2012). That may help to explain why studies of the association between the -176C/G SNP and cardiovascular disease have been inconsistent (Brull et al., 2001; Burzotta et al., 2001; Lieb et al., 2004; Nauck et al., 2002). Similar concerns have also been raised about the predictive power of IL-6 related SNPs in meta-analyses of the literature on inflammatory disease (Dai et al., 2012; Di Bona et al., 2009; Lee et al., 2012; Nikolopoulos et al., 2008; Yang et al., 2012).

In contrast, several polymorphisms that affect the production and shedding of the soluble IL-6 receptor, including rs2228145, rs2228146, rs2229238, rs4072391, rs4537545 and rs8192284, reliably account for variation in basal levels (Ferreira et al., 2013; Lamas et al., 2013; Marinou et al., 2010; Rafiq et al., 2007; Reich et al., 2007; Rodríguez-Rodríguez et al., 2011; Sasayama et al., 2012; Wang et al., 2005). These SNPs have already been linked to poor prognosis in clinical conditions, including chronic inflammation, metabolic and psychological disorders (Ferreira et al., 2013; Hamid et al., 2004; Huth et al., 2006; Lamas et al., 2013; Marinou et al., 2010; Sasayama et al., 2012; Stephens et al., 2012; Zhang et al., 2013). Genetic admixture mapping also indicates robust correlations of the sIL-6r variation in blood to ancestry (Reich et al., 2007). Although both IL-6 and its soluble receptor play important roles in inflammatory processes, the production of sIL-6r seems to be more tightly regulated, while IL-6 is more sensitive to life style, diet and other factors associated with adiposity.

IL-6 is secreted by many cells, including fibroblasts, hepatocytes, endothelial cells, and, especially, adipocytes, in addition to leukocytes (Hamzic et al., 2013; Lepiller et al., 2013; Saiki et al., 2013; Salman et al., 2013). The diversity of these tissue sources is key to understanding the heritability of IL-6, especially given the strong genetic and familial influences on adiposity (Schousboe et al., 2003; Segal et al., 2008). Although some previous twin studies considered the influence of adiposity on IL-6 heritability by adjusting IL-6 values by BMI or waist-to-hip ratios, they did not account for the shared genetic covariance (de Maat et al., 2004; Sas et al., 2012; Su et al., 2008). Therefore, those genetic estimates were likely inflated by the shared influence with BMI.

Twin analyses have a number of assumptions, including that there is a sharing of 100% of the genetic load by MZ twins versus 50% for DZ twins. It is also assumed that MZ and DZ siblings both share a similar influence of the common environments, and gene-environment interactions are not modeled in the standard twin model (i.e., ACE estimates are estimated as constant for the sample because potential moderator variables are not modeled). Some investigations have questioned the latter assumption based on the view that epigenetic modifications and individual perceptions may lead to a differential experience of even minor environmental events (Stenberg, 2012). Assuming a perfectly homogeneous and common environmental influence is probably not reasonable, so it will be important to verify our conclusions with a larger-scale gene-association study (Purcell, 2002; Tan et al., 2010). In addition, a substantial portion of the variance in IL-6 and CRP still remains to be accounted. Some variance may be attributed to reliability issues when relying on a single blood sample (Navarro et al., 2012). Determining the stability of IL-6 levels over time through multiple samples, would be of value for replicating and extending our heritability estimates. It should also be acknowledged that our participants were entirely American adults of European descent. IL-6 tends to be higher in certain races, including in those of African backgrounds, whereas it tends to be lower in some Asian populations (e.g., Japanese) (Coe et al., 2011). That was one reason why we opted not to include the small number of African-American twins in the current analyses. Similarly, body adiposity, IL-6 and CRP, as well as the relationships between adiposity and these inflammatory markers, vary not only by race, but also by gender (Carroll et al., 2009; Clifton, 2003; Coe et al., 2011). Hence, it is likely that estimates of shared genetic effects will vary by race and gender. Further validation for the driving effects of obesity on IL-6 and CRP would be achieved by controlled interventional studies targeting improvements in diet, exercise and weight reduction.

IL-6 affects not only immunity, but also cell growth, metabolism, and a number of critical brain functions. Further, cytokines are implicated in pain sensitization and sickness, including fever (Burton et al., 2011; May et al., 2009; Patel et al., 2012; Sasayama et al., 2012). Variation in IL-6 levels has been associated with psychological factors, including arousal, stress, and cognitive functioning. Thus, it makes sense that the secretion of IL-6 is dynamic and labile, while its modulatory receptor appears to be more conservatively constrained. This differential relationship is not seen for all cytokines, which often rely on the up- or down-regulation of the receptor to moderate the activity of the ligand (Krupp and Lane, 1981; Marchio et al., 1989; Seo et al., 2000). But it does make IL-6 an ideal biomarker for research on aging and health disparities across populations, because it is especially responsive to adiposity, a critical factor involved in the path to Type 2 diabetes and cardiovascular disease.

Conflict of interest

Amaral, W.Z. has no conflict of interest to declare.
Krueger, R.F. does not have any conflicts of interest.
Ryff, C.D. does not have any conflicts of interest.
Coe, C.L. does not have any conflicts of interest.

Acknowledgments

This research was supported by a grant from the National Institute on Aging (P01 AG020166). The original MIDUS study was supported by the MacArthur Foundation Research Network on Successful Midlife Development. Specimen collection was facilitated by the General Clinical Research Centers program (M01-RR023942 [Georgetown], M01-RR00865 [UCLA]), and at UW from the Clinical and Translational Science Award (CTSA) program of the National Center for Research Resources (1UL1RR025011). The contributions of D. Brar and J. Wochos are gratefully acknowledged. WZA is a predoctoral fellow on a NIMH supported training program (5T32 MH018931).

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