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Contribution of putative genetic factors and candidate gene variants to inter-individual variation of circulating fractalkine (CX3CL1) levels in a large UK twins' sample

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ABSTRACT

Objective: Soluble fractalkine (sFRACT) is involved in the pathogenesis of several clinical diseases. Our major objective was to determine to what extent its variation is governed by genetic factors and whether this genetic variation could be attributable to SNPs in five candidate genes: CX3CL1, CX3CR1, ADAM10, ADAM17 and AREG.

Methods: Plasma levels of sFRACT and 38 SNPs, with minor allele frequency >0.1 were examined in a large twin sample drawn from the general UK population. The discovery sample included 3306 middle-aged females: 1172 MZ twins and 2134 DZ twins. A replication sample of 1675 twins was used to validate the major association results obtained in genetic association analysis in the discovery sample. We implemented variance component analysis to estimate contribution of putative genetic, (including above SNPs) and environmental factors to sFRACT variation.

Results: sFRACT was found not to vary with either age or BMI. Putative genetic factors (heritability) explained 43.6 ± 3% of the total variation of plasma sFRACT levels. However, we found no evidence of association between sFRACT and any of the examined SNPs, despite having >85% power to detect an association of just 1% of the variance explained. The results in the discovery and replication samples were in good agreement suggesting these findings are real.

Conclusion: Our results suggest involvement of genetic factors to inter-individual variation of sFRACT levels in a general human population. However, further studies are required to determine genetic polymorphisms affecting sFRACT variation.

1. Introduction

Chronic inflammatory diseases are multifactorial diseases involving a variety of molecular factors, including cytokines, and in particular chemokines, such as fractalkine (FRACT). FRACT is a recently described chemokine that plays a major regulatory role in the recruitment and trafficking of immune cells to target locations [1] and has therefore become an attractive subject to study in immune mediated inflammatory conditions [2]. FRACT exists in two forms: a type 1 transmembrane protein [3] and a soluble form (sFRACT). Both membrane bound FRACT and sFRACT exert appropriate adhesion and migration of leukocyte cells via a FRACT receptor, CX3CR1 [4,5]. It is currently well established that elevated levels of sFRACT are associated with a variety of common, chronic inflammatory conditions [6–10]. However, the main factors governing sFRACT variation in a general human population are not well documented. Thus, for example, while for the variety of other cytokines, including chemokines, contribution of genetic factors to their variation has been estimated in several studies [11–15] such data on sFRACT are extremely limited at present. We are aware of only one study, conducted by our group on a family based isolated sample, which reported that genetic factors may explain about 41.6% of the sFRACT variation [9]. Furthermore,

Abbreviations: ADAM10, ADAM metallopeptidase 10; ADAM17, ADAM metallopeptidase 17; AREG, amphiregulin; DZ, dizygotic twin; LRT, likelihood ratio test; MZ, monozygotic twin; sFRACT, soluble fractalkine; VCA, variance component analysis.

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while elevated levels of adipose tissue FRACT were shown to be associated with human obesity [8], we are not aware of any study reporting such an association between sFRACT and obesity, or other components of body composition. Consequently, the primary aim of this study was to examine to what extent main intrinsic factors such as age, body composition and putative genetic factors influence plasma levels of sFRACT variation in a general human population. The additional aim was to test whether the estimated genetic heritability could be attributable to DNA polymorphisms at several candidate genes. To achieve this aim we selected five candidate genomic regions selected because of known relevance to FRACT metabolism: FRACT (CX3CL1), fractalkine receptor (CX3CR1), ADAM metallopeptidase 10 (ADAM10), ADAM metallopeptidase 17 (ADAM17) and amphiregulin (AREG).

Metabolic relevance to sFRACT include: 1. CX3CL1 is a functional gene responsible for the transcription of FRACT. Polymorphism within the genomic region may alter FRACT levels via regulation of transcriptional elongation, splicing, RNA stability and translation [16]. 2. Two SNPs, rs3732378 and rs3732379 in CX3CR1 were of special interest to this study. These polymorphisms are non-synonymous substitutions, which are in strong LD with each other [17] and have been found to be associated with the defective cell to cell adhesive function mediated by membrane-bound FRACT and impaired signaling and chemotactic activity of the soluble form [18]. Contrary to this finding, Daoudi and colleagues study [19] reported enhanced adhesion of membrane bound FRACT to its receptor but no effect on sFRACT. These results provided a basis for our first working hypothesis that sFRACT level variation may be influenced by these polymorphisms. 3. ADAM10 and ADAM17 genes were selected since these enzymes are responsible for the cleavage and formation of sFRACT. In addition to ADAM17 role in FRACT cleavage, it also plays an important role in the cleavage and production of soluble tumor necrosis factor-α from its precursor, a transmembrane molecule [20,21]. A previous study identified SNPs within the ADAM17 gene, C-154A and Ser747Leu, which influenced variation in soluble TNF-α plasma levels [22]. We therefore hypothesized that polymorphisms in ADAM17 and/or ADAM10 may also influence the protein ability to produce the soluble form of FRACT and therefore influence plasma sFRACT levels. 4. The last candidate gene is amphiregulin (AREG). In our previous study we reported a highly significant and substantial correlation between the plasma levels of sFRACT and AREG [9]. Although the functional relationship between AREG and sFRACT is not clear, this study also suggested that this correlation was caused by common putative genetic factors, simultaneously affecting circulating variation of both molecules, and therefore suggested existence of the common genetic polymorphisms associated with variation of them both.

2. Material and methods

2.1. Sample

The population examined in the present study was from the Twins UK adult registry (http://www.twinsuk.ac.uk), a community based study drawn from the UK population. Ethics committee approval was obtained and the participants gave informed consent. This sample has been described elsewhere [23]. We took two samples from the entire available twins' sample: 1. The discovery sample included 3306 middle-aged females: 1172 MZ twins and 2134 DZ twins. 1076 subjects were below the average typical age range of menopause (45–55 years), 838 were within this range and 1392 above 55 years old. The following data were analyzed: anthropometrical measurements and blood samples. 2. The replication sample consisted of 528 pairs of DZ and 153 pairs of MZ twins with differing dates of blood draws between twin pairs within the family, with an average of 6.5 years apart. Contrary to this, in the discovery sample the blood samples from both twins in the family were collected at the same date. In addition, 313 unrelated female twins, whose second twin was not available to this study, were included in the replication sample.

2.2. Biochemical assays

Venous blood samples were taken after an overnight fast. Within 1 h of collection, samples were centrifuged to obtain plasma, frozen in aliquots, and stored at −80 °C until analyzed. sFRACT levels were measured by sandwich Enzyme-linked immunosorbent assay (ELISA) using DuoSet ELISA development kit (R&D Systems Minneapolis, MN, USA) as described by us elsewhere [9]. All the observed measurements were above the minimal detection sensitivity (63.0 pg/ml). The inter- and intra- assay coefficients of variation in our analyses were: 6.3% and 3.5%, respectively.

2.3. SNP selection

The genotype data were based in genome-wide association scans performed in the Twins UK cohort previously and were analyzed using the illumina (San Diego, USA) 317 K and 610 K SNP arrays, with a call rate of genotype $\geqslant$98%. In all candidate genes, except CX3CR1, SNPs within intronic regions and/or SNPs near the 3′ and 5′ area of each gene were available, and tested for association with individual variations in sFRACT levels. The genomic positions of these genes were determined using the HapMap (release #28), and are presented in Table 1. All available SNPs within each genomic region were downloaded from HapMap to the Haploview program, and selected for minor allele frequency (MAF) > 0.1. The corresponding genotype distributions were tested for Hardy–Weinberg equilibrium, and in case of significant deviations ($P < 0.01$) were excluded from the association analysis. In total, 38 SNPs were selected for this study.

To appreciate the coverage of each of the genomic regions, we performed a Tagger test (using Haploview) for each region separately and with all the available SNPs. This was done by forcing the inclusion of selected SNPs from our database and by pairwise tagging, with simultaneous fixation of the r^2 threshold to 0.8. The tests captured 71%, 82%, 88% and 100% of all the SNPs within AREG, ADAM10, CX3CL1 and ADAM17, respectively. Remarkable was the fact that SNPs not captured by r^2 threshold of 0.8 were in complete

Table 1
Genomic location and SNP summary used in the study via HapMap and Haploview program.

Gene Name	Genomic location (base pair)	SNP location[a]	Average r^{2b}
CX3CL1	16:55,963,915–55,976,455	16:55,961,001–55,981,359	$r^2 = 0.97$
CX3CR1	3:39,279,990–39,296,531	3:39,282,166–39,282,260	$r^2 = 0.54$
ADAM10	15:56,675,802–56,829,469	15:56,653,448–56,838,189	$r^2 = 0.92$
ADAM17	2:9,546,864–9,613,368	2:9,530,740–9,616,764	$r^2 = 0.99$
AREG	4:75,529,717–75,709,506	4:75,501,711–75,726,735	$r^2 = 0.95$

SNP – single nucleotide polymorphism.
[a] Genomic range of selected SNPs.
[b] r^2 represents average measure of linkage disequilibrium inside the genomic location captured by selected SNPs.

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Table 2
Study SNP position and main association analysis results observed in the discovery sample.

Genomic region	SNP	Position (bp)	MAF	$\chi 2$[a]	*P*-value	Location[b]
CX3CL1 (Chr 16)	rs223812	55961001	0.26	0.345	0.55	Upstream
	rs170364	55967435	0.24	0.375	0.54	Intronic
	rs170361	55972124	0.19	0.373	0.54	Intronic
	rs4151117	55974635	0.21	0.470	0.49	3′ UTR
	rs8102	55976226	0.25	0.707	0.40	3′ UTR
	rs614230	55976787	0.35	0.264	0.69	3′ Near gene
	rs8063316	55981359	0.20	0.252	0.61	Downstream
CX3CR1 (Chr 3)	rs3732378	39282166	0.17	0.005	0.94	Missense
	rs3732379	39282260	0.27	0.042	0.83	Missense
ADAM10 (Chr 15)	rs4774308	56653448	0.37	0.496	0.48	Downstream
	rs1869135	56669261	0.22	0.849	0.35	Downstream
	rs2305421	56690375	0.14	1.914	0.16	Intronic
	rs1427282	56701618	0.35	0.013	0.91	Intronic
	rs8043406	56701924	0.32	0.076	0.78	Intronic
	rs7174386	56716605	0.14	1.743	0.18	Intronic
	rs1427281	56746565	0.25	0.464	0.49	Intronic
	rs7176436	56752223	0.10	0.218	0.64	Intronic
	rs8027998	56784541	0.15	0.171	0.67	Intronic
	rs4238331	56799425	0.29	0.028	0.87	Intronic
	rs383902	56821466	0.33	0.097	0.75	Intronic
	rs694335	56838189	0.16	0.675	0.41	upstream
ADAM 17 (Chr 2)	rs2001660	9530892	0.42	0.206	0.65	3′ near gene
	rs6705408	9547682	0.41	0.265	0.60	3′ UTR
	rs12473402	9550169	0.41	0.228	0.63	Intronic
	rs2276338	9563240	0.41	0.302	0.58	Intronic
	rs1056204	9564542	0.30	0.330	0.56	Intronic
	rs11891922	9567417	0.41	0.342	0.55	Intronic
	rs10179642	9601147	0.15	0.894	0.34	Intronic
	rs6432017	9611326	0.34	0.121	0.72	Intronic
	rs6432018	9639347	0.44	1.231	0.26	Upstream
AREG (Chr 4)	rs1494878	75501711	0.43	0.001	0.98	Upstream
	rs6447000	75596222	0.34	0.021	0.87	NA
	rs4694691	75602196	0.48	0.614	0.43	Intronic
	rs7656798	75606837	0.44	0.334	0.56	Intronic
	rs9790716	75613628	0.39	0.007	0.93	Intronic
	rs4694196	75623566	0.28	0.014	0.90	Intronic
	rs7696397	75640651	0.35	0.574	0.46	Intronic
	rs2609200	75698084	0.26	0.001	0.98	NA

[a] $\chi 2$ – test for association between selected SNPs and sFRACT levels. Markers rs170631 and rs8102 were not available, therefore imputation was performed. The imputation qualities for these markers are 0.961 and 0.975, respectively.
[b] Locations of SNPs were located from UCSC Genome Browser and Haploview program. MAF – Minor allele frequency, bp – base pair, NA – Not available.

linkage disequilibrium (LD) by D' estimate with the selected SNPs, which is likely to reflect that r^2 is more sensitive to allele frequencies than D' [24]. Two SNPs, rs3732378 and rs3732379 in CX3CR1 were tested for association with sFRACT levels. As mentioned above, these polymorphisms are non-synonymous substitutions, which are in strong LD with each other [17], and have yet to be studied in relation with sFRACT circulating levels. Table 2 shows the summary of SNP selection. SNP location consequence to transcript was located via UCSC Genome Browser and Haploview.

2.4. Statistical and quantitative genetic analysis

Preliminary statistical analysis included descriptive statistics of the phenotype and potential covariate, which was done using SPSS statistical analysis software, version 19 (SPSS, Chicago, IL, USA). To examine the contribution of the putative genetic factors to sFRACT variation, we first performed a family correlation analysis. Further determination of phenotypic variation was done by implementing variance component analysis (VCA), using MAN-2011 package (MAN, Tel-Aviv, Israel). MAN-software takes into account a family structure of the sample, including both members of each pair of MZ and DZ twins and unrelated singletons. It simultaneously estimates effect of the covariates (if any), as well as contribution of the major components of variation. According to quantitative genetic theory [25], the total phenotypic variation (V_{PH}) may be decomposed into a number of major components of variation: V_{AD} – reflects contribution of the additive genetic factors, V_{TW} – effect of common environment, shared by twins, and V_{RS} is unexplained residual variation. A maximum likelihood ratio test (LRT) was used as a fitting technique to compare a general model including all possible parameter estimates vs. a more parsimonious model, excluding potentially non-significant parameters. To estimate contribution of the selected SNPs effect on sFRACT variation, the corresponding best fitting and most parsimonious model was modified by their inclusion as covariates. The SNP-specific genotypes were scored by the number of minor alleles, as 0, 1 and 2, and the corresponding regression coefficients were estimated. Prior to testing association we conducted power analysis of the study discovery sample to detect significant association ($P < 0.05$), using Monte–Carlo simulations. We tested a variety of the selected SNP effects (0%, 0.5%, 1%, 10%), i.e. percentages of the quantitative trait variance attributable to the SNP, with MAF = 0.2, 0.4, and residual heritability, $h^2 = 0.40$. The simulations showed that even under the assumption of SNP effect = 1.0% and MAF = 0.4, we have >85% power to detect an association.

3. Results

The basic descriptive statistics of both study samples, including plasma levels of sFRACT are presented in Table 3, by Twin zygosity,

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Table 3
Descriptive statistics of sFRACT (pg/ml) plasma levels in the discovery sample (Panel a) and the replication sample (Panel b).

Variable	Mean	Min	Max	SD	Mean	Min	Max	SD	*P*-value [a]
	MZ (n = 1172)				DZ (n = 2134)				
Panel a									
sFRACT (pg/ml)	2533.63	64.32	41302.25	5075.16	2450.32	51.43	47761.84	5297.58	0.291
Age (years)	48.79	16.12	84.60	15.11	50.69	18.46	82.13	13.28	0.001
Weight (kg)	66.87	37.90	125.00	12.39	68.28	35.10	140.90	13.36	0.003
BMI (kg/m^2)	25.51	15.22	52.39	4.92	25.92	15.22	52.39	4.91	0.025
Panel b									
	MZ (n = 1150)				DZ (n = 525)				
sFRACT (pg/ml)	2336.18	53.38	40961.72	4717.58	2684.64	68.13	41277.04	5520.43	0.103
Age (years)	54.90	17.98	82.42	12.51	51.87	18.42	85.50	12.22	0.001
Weight (kg)	67.42	42.20	128.10	12.43	67.60	40.80	118.20	12.47	0.782
BMI (kg/m^2)	25.91	15.88	45.32	4.70	25.78	15.55	45.88	4.62	0.596

BMI – Body Mass Index, MZ – monozygotic, DZ – dizygotic.
[a] Independent sample *t*-test (*P* value) for mean values between MZ and DZ twins.

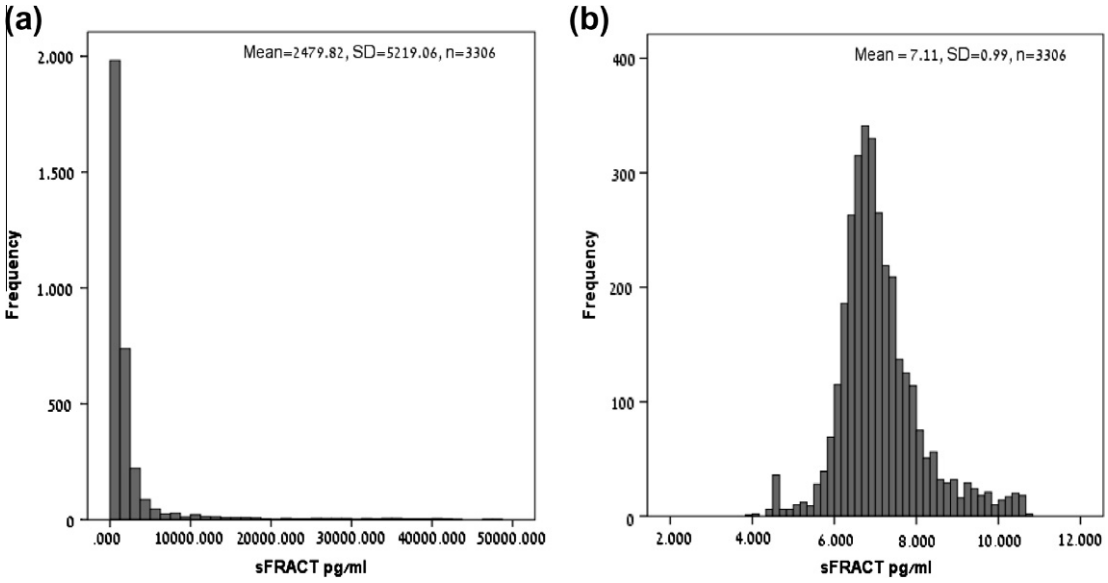

Fig. 1. (a) and (b) Histogram of plasma sFRACT levels before and after log-transformation in the discovery sample.

for the discovery and replication samples. The sFRACT circulating levels varied widely, between 51.43 pg/ml and 47761.84 pg/ml in the total sample, with 2468.23 pg/ml and 1016.33 pg/ml for mean and median estimates with no significant differences by zygosity ($P > 0.1$). The mean levels in the discovery and replication sample were comparable (2479.8 ± 90.75 pg/ml vs. 2445.4 ± 121.77 pg/ml, respectively). Other sample characteristics, age, weight and BMI were all also similar for various subsamples' comparison. sFRACT levels of subjects aged below the typical average age of menopause (45–55) were not statistically significantly different when compared to sFRACT levels of subjects within or above this age group (data not shown).

In general, the distribution of plasma sFRACT showed significant deviation from normality (Fig 1a), with significant estimates of skewness and kurtosis ($P < 0.001$ in all instances). Consequently the data were log-transformed to normalize sFRACT distribution (Fig 1b). Testing the correlations within twin pairs by zygosity, showed that both correlations were highly significant ($P < 0.001$) and correlations between the MZ twins were substantially higher than between DZ twins (Table 4) and the difference was statistically significant ($Z = 4.97$, $P < 0.001$). These findings suggest involvement of familial, likely genetic factors to inter-individual variation of sFRACT levels. To examine this hypothesis further we

Table 4
Correlations of sFRACT within twin pairs by zygosity in the discovery sample.

Sample	All (n = 3306)	MZ (n = 1172)	DZ (n = 2134)
Correlation	0.31	0.41	0.25
P-value	<0.001	<0.001	<0.001

Correlation coefficient within twin pairs, MZ – monozygotic, DZ – dizygotic, n – number of subjects.

Table 5
Summary of variance component analysis of sFRACT levels in the discovery sample.

Parameter	General model	Most parsimonious
V_{AD}	0.31	0.43 ± 0.03
V_{TW}	0.09	(F) 0
V_{RS}	0.58	0.56 ± 0.03
α_0	−0.01	(F) 0
β_1 (age)	−0.03	(F) 0
β_2 (BMI)	0.03	(F) 0
h^2	0.31	0.43

V_{AD} - additive genetic component, V_{TW} - common twin environment, V_{RS} - residual variance component, α_0 and β are regression parameters, intercept and slope, h^2 - heritability (proportion of phenotypic variance attributed to genetic variation), BMI - Body Mass Index.

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conducted model-based VCA with simultaneous adjustment for the potential covariates (age and BMI), as described above. Table 5 provides summary of this analysis. By LRT, the most parsimonious model displayed a significant heritability estimate, explaining 43.6% of the total sFRACT variation. Common twin environment, made only negligible and statistically non-significant contribution (0.09 ± 0.07), and was not retained in the most parsimonious model. Finally, sFRACT did not correlate with age or BMI in either discovery or replication samples. Furthermore, while stratification of BMI in the discovery sample into non-obese (BMI < 30, $n = 2773$) and obese (BMI > 30, $n = 533$) groups showed numerical differences in sFRACT plasma concentrations (2435.27 ± 97.31 pg/ml vs. 2711.62 ± 246.11 pg/ml, respectively) in the expected direction, it did not reach a statistically significant value ($P = 0.148$).

We tested the hypothesis that sFRACT variation is associated with one more of the selected SNPs in the candidate genes. The analysis was first conducted in our discovery sample but revealed no significant associations ($P \geqslant 0.15$, Table 2). In the replication sample, the association results were also all non-significant statistically (not shown), while the variance component estimates were similar to those reported in Table 5.

4. Discussion

Chemokines are a family of small proteins that attract and activate leukocytes to sites of injury and inflammation, and therefore may play an important role as mediators of inflammation [26]. Despite the potentially important role of sFRACT, the contribution of the intrinsic and extrinsic factors on sFRACT variation in the normal population as well as in pathological conditions remains largely unknown.

We examined sFRACT in a large, community-based sample of British women of middle age. We observed the wide range of sFRACT levels variation, from 51.43 pg/ml to 47761.84 pg/ml, quite comparable in both our study samples. The mean levels did not differ by twin zygosity and were very similar to the results in our previous study (2136.54 pg/ml, 63.09–30203.0 pg/ml) of a very different ethnic population [9]. However, these plasma concentrations were substantially higher than those previously reported as control values for various types of morbidity. For example, the control values were reported as 364.67 pg/ml, (±64.81, $n = 15$) in obstructive sleep apnea–hypopnea study [27] and 381.6 pg/ml (±149.3, $n = 26$) in a pulmonary arterial hypertension study [28]. Note, however, much smaller sample sizes were used in these studies, and thus the sample probably did not include the rare high values observed in our large samples (Fig. 1a). Although exact menopausal status was not available in this study, we found no correlation between age and sFRACT. Furthermore we found no significant differences of sFRACT levels between subjects below, within or above the typical average age of menopause. Additionally, we did not observe a significant correlation between sFRACT and BMI in any of our samples, which may be in contrast to Shah and colleagues [8] reporting elevated levels of adipose FRACT in obese individuals and elevated plasma sFRACT in type 2 diabetes subjects. For example, in their unadjusted logistic regression model, there was more than a twofold increase for diabetes for a one SD increase in plasma sFRACT levels. However, this study does not report elevated sFRACT levels in obese individuals in their sample. The lack of correlation between sFRACT and BMI may imply that metabolic changes occurring in type 2 diabetes in addition to obesity, elevates sFRACT plasma concentrations in diabetic individuals. While obesity is now considered a low grade inflammatory state [29], the presence of insulin resistance may further promote inflammation [30] and likely cause the aforementioned association. Since obesity, metabolic syndrome and insulin resistance are all known risk factors for coronary heart disease [29,31], as well as reported association of FRACT with atherosclerosis [32], one may assume that chronic low state of inflammation may lead to elevated levels of sFRACT, consequently contributing to the development of coronary artery diseases. With this said, it would be of importance to examine whether the circulating sFRACT levels differ between obese subjects with and without metabolic syndrome or insulin resistance.

The present study clearly shows that putative genetic factors are major contributors to inter-individual variation of sFRACT. Some 43.6 ± 3% of the total sFRACT variation in our discovery sample was attributable to genetic factors and this finding was confirmed in our replication sample. These estimates are in good agreement with previously published data on a variety of cytokines, including several chemokines. The heritability estimates in these studies vary in wide range, from 10% to 80% depending on the cytokine and the sample [11–15]. For example, Pantsulaia et al. [13] reported that adjusted for potential covariates, 72% of the MCP-1 variance, was attributable to familial effects. Of these about 49% was due to potential genetic factors and the rest due to common household environment. However, there are also studies with negative results. Thus, Berrahmoune et al. [33], using a variance component analysis, found no significant genetic influence on variation of both CRP measurements and their 5-year changes. Our heritability estimate of 0.43 (±0.03) of sFRACT variation is well inside the aforementioned published range of estimates and in a very good agreement with heritability estimate of sFRACT observed by our team in a rural Russian population, 0.40 (±0.07) [9]. However, we are not aware of any association study attempting to identify specific genetic polymorphisms potentially responsible for heritability of sFRACT.

Since no previous studies on this subject were available, we selected five promising candidate genes. We assumed that the structural genes for FRACT and its receptor, the structural genes of the two major enzymes, ADAM10 and ADAM17, responsible for the shedding and production the soluble form of FRACT and the gene for amphiregulin, of which circulating levels are significantly correlated with sFRACT in Leonov et al. study [9], are reasonable candidates for this type of study. However, SNPs selected in these and three other genomic regions (Table 2) showed no sign of association in either of the Twins UK samples, despite the fact that our sample had >85% power to detect association explaining 1% of the sFRACT variation. Since the study samples include apparently healthy adults drawn from the general UK population, we cannot exclude that our negative findings may be due to the absence of inflammatory conditions, known to enhance expression of FRACT, ADAM17 and ADAM10 activity [34–36]. It is also possible that the coverage of the selected genes was not complete, or the effects of the polymorphisms of interest was so minor, that it could not be detectable even in the sample including >3000 measured individuals. Note, however, that non-significant or week association results were previously and repeatedly reported with respect to other cytokines [12,37–40]. Furthermore, regulatory enhancer elements distant from CX3CL1 gene or on other genes may also influence transcription levels. A likely candidate gene is heat shock transcription factors (HSFs). Transcription levels of inflammatory cytokines such as TNF-α, IL-1B and IL-6 are directly regulated via HSFs in mice [41,42], and therefore should be concerned in future studies as a regulatory factor of sFRACT. Finally, it is possible that a consortium based study involving tens of thousands of individuals and implementing GWAS approach is needed for the identification of the functional genes governing sFRACT variation.

In conclusion, our results confirm the involvement of familial, likely genetic, factors to inter-individual variation of sFRACT levels in the general population. This finding motivates future studies to assess relevancy of genetic polymorphisms affecting sFRACT varia-

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tion. Determination of potential polymorphisms will improve our understanding of the underlining mechanisms determining sFRACT levels. In addition, we found no significant correlation between sFRACT and body composition. As a result further studies on sFRACT and obesity related biomarkers such as adipokines are required to determine sFRACT potential role in obese individuals.

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