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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

Liran Franco [a], Frances M.K. Williams [b], Svetlana Trofimov [a], Gabriela Surdulescu [b], Timothy D. Spector [b], Gregory Livshits [a,b,*]

[a] *Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Israel*
[b] *Department of Twin Research and Genetic Epidemiology, King's College London, UK*

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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.

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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.

* Corresponding author. Address: Human Population Biology Research Unit, Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel. Fax: +972 3 640 8287.

E-mail addresses: Lyranf@gmail.com (L. Franco), frances.williams@kcl.ac.uk (F.M.K. Williams), artem@post.tau.ac.il (S. Trofimov), gabriela.l.surdulescu@kcl.ac.uk (G. Surdulescu), tim.spector@kcl.ac.uk (T.D. Spector), gregl@post.tau.ac.il (G. Livshits).

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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^2[b]
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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