Molecular Biology Reports (2018) 45:1557–1564
https://doi.org/10.1007/s11033-018-4257-x

SHORT COMMUNICATION

CXCL13 polymorphism is associated with essential hypertension in Tatars from Russia

Yanina R. Timasheva[1,2] · **Timur R. Nasibullin**[1] · **Ilsiyar A. Tuktarova**[1] · **Vera V. Erdman**[1] · **Olga E. Mustafina**[1,3]

Received: 20 March 2018 / Accepted: 12 July 2018 / Published online: 17 July 2018
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Abstract
Essential arterial hypertension is a disease with distinct yet unexplored inflammatory component. Our aim was to assess the role of chemokine genes and their interaction in its development. Genotyping of polymorphic markers in six chemokine genes (*CXCL13, CCL8, CCL16, CCL17, CCL18*, and *CCL23*) was performed in the group of 522 men of Tatar ethnic origin from the Republic of Bashkortostan, Russia (213 patients with essential hypertension and 309 healthy individuals without history of cardiovascular disease). We found a strong association of *CXCL13* rs355689*C allele with essential hypertension under additive (OR 0.56, $P_{FDR}=0.008$) and dominant (OR 0.41, P_{FDR} 4.38×10^{-4}) genetic model. The analysis of gene–gene interactions revealed 12 allele/genotype combinations that remained significantly associated with essential hypertension after correction for multiple testing was applied, and each of these combinations included *CXCL13* rs355689 polymorphism. Our results indicate that *CXCL13* rs355689 polymorphism is strongly associated with essential hypertension in the ethnic group of Tatars, alone and in combination with polymorphic markers in other chemokine genes.

Keywords Essential hypertension · Chemokines · Genetic testing · Risk prediction

Introduction

Essential, primary, or idiopathic hypertension (EH) is a chronic elevation of blood pressure in the absence of any causes of secondary hypertension or monogenic forms. EH is a highly heterogeneous disorder with multifactorial origin, caused by a combination of genetic, environmental, and lifestyle factors. Evidence that vascular inflammation is present in patients with hypertension may suggest a role for inflammatory mediator genes in its etiopathogenesis.

Chemokines (chemotactic cytokines) are a family of small proteins that mediate cellular interactions during inflammation via binding to specific receptors [1]. Chemokines share significant structural homology and display remarkable functional similarity. They are classified into four subgroups according to the arrangement of the N-terminal two cysteine residues: CXC, CC, (X)C, and CX3C [2]. The genomic organization of chemokine genes forming large clusters reflects evolutionary pressures, when temporary redundancy created by gene duplication resulted in the development of proteins with new specialized functions [3]. Consequently, chemokines display redundant effects on the target cells (no chemokine's actions are confined exclusively to a single leukocyte population and conversely, any particular leukocyte population typically possesses receptors for various chemokines), and their interaction with receptors also shows significant promiscuity (most receptors can bind to multiple ligands and most ligands are capable of binding to more than one receptor) [4]. It has been demonstrated

✉ Yanina R. Timasheva
ianina_t@mail.ru

Timur R. Nasibullin
NasibullinTR@yandex.ru

Ilsiyar A. Tuktarova
iltuk@mail.ru

Vera V. Erdman
danivera@mail.ru

Olga E. Mustafina
anmareg@mail.ru

1 Laboratory of Physiological Genetics, Institute of Biochemistry and Genetics of Ufa Federal Research Centre of Russian Academy of Sciences, October Avenue 71, Ufa, Russian Federation 450054

2 Department of Medical Genetics and Fundamental Medicine, Bashkir State Medical University, Ufa, Russian Federation

3 Department of Genetics and Fundamental Medicine, Bashkir State University, Ufa, Russian Federation

that chemokine receptors CCR2 and CXCR2 are instrumental in blood pressure elevation, macrophage accumulation, and vascular remodeling in animal models of hypertension [5, 6]. Increased chemokine levels were detected in serum (MCP-1 (CCL2) and CXCL10) and vitreous bodies (IL8 (CXCL8) and MCP-1) of patients with EH [7, 8]. Previously, we discovered an altered transcriptional activity of a number of chemokine genes, including *CCL16, CCL17, CCL18, CCL23, CCL8*, and *CXCL13*, in peripheral blood leukocytes of EH patients [9]; although no association has been reported between these gene loci and EH in genome-wide association studies performed to date.

In this study, we aimed to analyze an association of the polymorphic loci in six chemokine genes (*CCL16, CCL17, CCL18, CCL23, CCL8*, and *CXCL13)* with EH, and to study the interactions between these polymorphisms.

Materials and methods

The study was approved by the Ethics Committee of the Institute of Biochemistry and Genetics USC RAS, written informed consent was obtained from all participants participant in accordance with the Declaration of Helsinki. The study group was comprised of men in order to minimize the heterogeneity of the study sample and considering that sex differences are important risk factors for the development of cardiovascular disease. Both patients and control subjects belonged to the Tatar ethnic group and permanently resided in the Republic of Bashkortostan (Russian Federation). The ethnicity of all participants was confirmed by administering questionnaires that included data on the ethnicity and the place of birth of their ancestors in three generations. All patients (213 men with EH, mean age 42.24 ± 8.27) underwent a full clinical evaluation at the Department of Arterial Hypertension at Republic Centre of Cardiology (Ufa, Russian Federation), EH was diagnosed in accordance with the 2013 ESH/ESC guidelines for management of arterial hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg). For each participant, three consecutive blood pressure measurements in both upper arms were taken upon admittance at 1–2 min intervals, after 5 min rest in sitting position, with a standard auscultatory sphygmomanometer. Phase I and V (disappearance) of Korotkoff sounds were identified as systolic and diastolic blood pressure, respectively. In addition, blood pressure measurement was performed twice daily (morning and evening) over the course of treatment. All patients underwent complete clinical assessment, physical examination, laboratory and instrumental investigations (fasting plasma glucose, serum lipids, electrolytes, creatinine, haemoglobin and haematocrit, electrocardiogram, echocardiogram, carotid artery ultrasound, etc). Patients with diabetes, chronic lung, gastrointestinal, renal disease or metabolic disorders were not included in the study. The control group included 309 healthy individuals (mean age 43.58 ± 7.13) without history of cardiovascular disease, recruited at the Republic Centre of Blood Transfusion (Ufa, Republic of Bashkortostan, Russian Federation).

DNA was isolated from 8 ml of whole venous blood using standard phenol-chloroform extraction. Genotyping was performed using polymerase chain reaction (PCR) with subsequent restriction analysis or PCR allele-specific (a fragment containing target sequence was used as an internal positive control). PrimerSelect 5.05 software (DNAStar Inc., Madison, WI, USA) was applied to design primer sets (Table 1). Gene sequences for primer designing were obtained from NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/SNV). Each 10 µl PCR reaction mixture contained 30 mM Tris–HCl, pH 8.6/25 °C, 16.6 mM $(NH_4)_2SO_4$, 2.5M$MgCl_2$ 0.2 mM of each dNTPs (Thermo Fischer Scientific, Lithuania), 0.2 mM of both primers, 0.5 U of Taq-polymerase enzyme and 20 ng of DNA template. The amplification was performed in a T100™ thermal cycler (BioRad, USA) programmed for initial denaturation step (95 °C for 1 min) followed by 28 cycles of amplification (denaturation at 95 °C for 20 s, primer annealing at specific temperature (Table 1) for 30 s, elongation at 72 °C for 30 s) and a final extension (72 °C for 4 min). As part of quality control, results were verified by re-genotyping of randomly selected samples; all results were identical to initially obtained genotyping data. PCR products were separated by the electrophoresis on 2% agarose gel and identified using Mega-Bioprint 1100 gel documentation system (Vilber Lourmat, France).

Study data were stored, managed, and analyzed using IBM SPSS Statistics 21.0 program. Sample size required to detect an association with odds ratio (OR) of 1.5 was determined using QUANTO software version 1.2.4 taking into account the minimally acceptable 80% power level, minor allele frequencies, and the disease prevalence in the studied population. Average prevalence of EH in men in the Republic of Bashkortostan was 48.6% [10]. Testing for Hardy–Weinberg expectations was performed for each SNV using Arlequin 3.0 software. Association between the studied genetic loci and EH was tested using logistic regression analysis under additive and dominant genetic models implemented in PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) with age and BMI as covariates [11]. Dominant model assumes that having one copy of an allele does the same for the disease risk as carrying two copies, while additive model assumes that two copies of an allele have twice the effect on phenotype compared to one copy. The analysis of association between allele and/or genotype combinations and EH was performed by APSampler 3.6.0, the program itself is available at http://apsampler.sourceforg

Table 1 Primer sequences and PCR conditions for amplification of the studied SNPs

SNV	Chromosome position	Locus name	Primers, restriction enzyme	Primer annealing temperature, °C	Alleles fragment length, bp
rs355689	4:78507797	*CXCL13*	C 3′-caggacaggatctctgacagc-5′ T 3′-caggacaggatctctgacagt-5′ F 3′-gggacctaacaaaacaggcag-5′ R 3′-tccactgaagccaggaaaatc-5′	68	AS 86 IC 220
rs223828	16:57447414	*CCL17*	3′-tcggaggcagataaagcatgg-5′ 3′-ctctgtagctggagagcatcc-5′ *Taq*I	65	T 276 C 205+71
rs3138035	17:32645949	*CCL8*	A 3′-cccccacagcttcaagacca-5′ C 3′-cccccacagcttcaagaccc-5′ F 3′-cacctaaggaccaagggctg-5′ R 3′-tgaaggctcatggcttcagat-5′	66	AS 122 IC 216
rs854680	17:34309051	*CCL16*	G 3′-gtattagcatacactgtgacag-5′ T 3′-gtattagcatacactgtgacat-5′ F 3′-cagtccaaagtccgaggtcc-5′ R 3′-caggattacagagcccagac-5′	62	AS 127 IC 241
rs854655	17:34345223	*CCL23*	3′-gaggaagttacagggcagagg-5′ 3′-gtccccatgtgtacaggctatt-5′ *BamH*I	66	C 188 + 104 A 292
rs2015086	17:34391617	*CCL18*	T 3′-ccttctggggtatgagctgtt-5′ C 3′-ccttctggggtatgagctgtc-5′ F 3′-catggtgcagacgaggacaag-5′ R 3′-tgggctgagaactcacatgac-5′	66	AS 118 IC 228

SNV single nucleotide variant, Chromosome position (bp) according to GRCh37.p13, *AS* allele-specific amplification product, *IC* internal control

e.net/, more detailed description can be found elsewhere [12]. Briefly, APSampler (Allelic Pattern Sampler) is a program that utilizes a Markov chain Monte Carlo method based on Bayesian approaches, which allows to identify combinations of allelic variants of multiple loci that are associated with the studied trait. The false discovery rate (FDR) method was applied to adjust for multiple testing [13].

Results

The observed allele frequencies for the *CXCL13, CCL8, CCL16, CCL17, CCL18*, and *CCL23* loci are shown in Table 2. Genotype frequency distribution for all studied SNVs was in accordance with Hardy–Weinberg equilibrium ($P > 0.05$). *CXCL13* rs355689*C allele has shown an association with EH under additive and dominant genetic models (OR 0.56, $P_{FDR} = 0.008$, and OR 0.41, $P_{FDR} = 4.38 \times 10^{-4}$, respectively). No significant association was detected for the individual markers of *CCL8, CCL16, CCL17, CCL18*, and *CCL23* genes.

Analysis of association between EH and allele/genotype combinations revealed six bi- and six tri- component combinations distributed differently in the group of EH patients and in control group (Table 3). Interestingly, all the combinations that remained significantly associated with EH after correction for multiple testing was applied included the *CXCL13* rs355689 variant. *CXCL13**T/T genotype was part of the combinations predisposing to the development of EH, while allele *CXCL13**C was present in the genotype/allele combinations that were associated with decreased risk of hypertension (Table 3). *CCL16* rs854680*T allele was found only in combinations associated with increased risk of EH, while *CCL8* rs3138035*C allele was featured exclusively in patterns with protective effect against the disease (Table 3). *CCL17* rs223828 polymorphism exhibited allele-specific action, with C allele being part of combinations predisposing to EH, and T allele being present in one combination associated with decreased risk of the disease (Table 3). *CCL18* rs2015086*T/T genotype and T allele were detected in both types of patterns, displaying ambivalent effect (Table 3).

Discussion

Having genotyped SNVs in six chemokine genes in the groups of patients with EH and normotensive controls, we found a significant association between *CXCL13* rs355689 polymorphism and hypertension. This SNP has been tested for an association with a number of traits, including SBP, DBP, coronary artery disease, BMI, obesity and type 2 diabetes, but the observed P-values did not reach the GWAS significance level ($P < 5^{-08}$) [14–17]. However, its minor

Table 2 Genotyping results and the analysis of association between the studied SNPs and essential hypertension

SNV	Locus name	N cases/N controls	EA/NEA	EAF cases/controls	HWE *P*-value	Additive			Dominant		
						OR (95% CI)	*P*-value	P_{FDR}	OR (95% CI)	*P*-value	P_{FDR}
rs355689	*CXCL13*	186/294	C/T	17.5/28.4	0.287	**0.56 (0.39–0.80)**	**0.001**	**0.008**	**0.41 (0.26–0.64)**	**7.29×10^{-5}**	**4.38×10^{-4}**
rs223828	*CCL17*	200/291	T/C	14/17.2	0.866	0.73 (0.49–1.08)	0.117	0.234	0.69 (0.44–1.08)	0.104	0.312
rs3138035	*CCL8*	208/306	T/C	39.7/35	0.267	1.31 (0.98–1.75)	0.067	0.198	1.31 (0.87–1.97)	0.190	0.379
rs854680	*CCL16*	208/309	G/T	20.7/20.2	0.124	1.01 (0.71–1.41)	0.979	0.979	1.12 (0.75–1.69)	0.573	0.645
rs854655	*CCL23*	182/237	C/A	8/9.3	0.974	0.85 (0.48–1.48)	0.560	0.671	0.87 (0.49–1.56)	0.645	0.645
rs2015086	*CCL18*	209/304	C/T	11.5/9.7	0.223	1.18 (0.74–1.89)	0.495	0.671	1.21 (0.74–1.96)	0.446	0.645

P values of less than 0.05 were considered significant are shown in bold

SNV single nucleotide variant, *EA* effect (risk) allele, *NEA* non-effect allele, *EAF* effect allele frequency, %, *HWE P-value* Hardy–Weinberg P-value, *OR* odds ratio, *CI* confidence interval. The OR is aligned to the SNV risk allele

allele was reported to be inversely associated with the risk of non-Hodgkin B-cell lymphoma in HIV patients, and with the serum levels of CXCL13 [18]. *CXCL13* rs355689 polymorphism was also associated with impaired lung function in patients with cystic fibrosis [19]. CXCL13 regulates the adhesion of B cells and subsets of T cells to lymphoid follicles via binding to its receptor CXCR5 [20]. It has been demonstrated that CXCL13/CXCR5 interaction is involved in cardiac remodeling following pressure overload in *Cxcr5*-knockout mice [21]. Transcriptional activity of *CXCL13* (but not *CXCR5*) was up-regulated in carotid atherosclerosis, and CXCL13 was shown to increase the expression of anti-inflammatory cytokines IL-10 and TGF-beta, and the expression of tissue inhibitor of metalloproteinases-1 in monocytes [22]. CXCL13 also exerted anti-apoptotic effect on lipid-exposed monocytes and smooth muscle cells [23].

CCL17 is expressed by dendritic cells, activates chemokine receptor CCR4, and is present in atherosclerotic plagues. In *Apoe*-knockout mice, CCL17 deficiency results in significant attenuation of atherosclerosis [24]. An association was reported between the promoter polymorphism of the *CCL17* gene, rs223828, and the incidence of aneurysm in patients with Kawasaki disease [25]. The carriers of rs223828*C/C genotype had higher risk of coronary artery aneurysm formation [25]. Haplotype consisting of *CCL17* rs223828*T allele and a *CCL22* rs223889*A allele was associated with decreased susceptibility to multiple sclerosis [26]. However, no correlation was found between *CCL17* rs223828 genotypes and the serum chemokine level [27]. In our study, *CCL17* rs223828*C/C was detected as part of combinations associated with increased risk of EH, while *CCL17* rs223828*T allele in combination with *CXCL13* rs355689*C allele and *CCL8* rs3138035*C allele had a protective effect against the development of hypertension.

CCL8, CCL16, CCL23 and *CCL18* genes are located in humans on chromosome 17. CCL8 is expressed in fibroblasts and endothelial cells, and specializes on recruiting monocytes, granulocytes, and effector T-cells, acting via CCR1, CCR2, CCR3 and CCR5 receptors [23]. Polymorphism rs3138035 located in the 5′-flanking region of *CCL8* gene was found to confer significantly decreased risk of death by non-small cell lung cancer; the protective effect was more pronounced in smokers [28]. Notably, the patients with non-small cell lung cancer carrying *CCL8* rs3138035*C/C genotype had an increased risk of death compared to the heterozygotes and the carriers of T/T genotype, while in our study, *CCL8* rs3138035*C was found in all the combinations associated with the decreased risk of EH.

CCL16 acts as a chemoattractant for lymphocytes, dendritic cells, and monocytes, and increases their adhesive properties. CCL16 is up-regulated by interleukin 10 (IL-10) in activated monocytes, and exerts its biological effects through binding to CCR1, CCR2, CCR5, and CCR8

Table 3 Genotype and allele combinations of the studied loci associated with EH

Combinations					Control, %	Cases, %	P_{FDR}	OR	CI_{OR}
CXCL13 rs355689	*CCL18* rs2015086	*CCL8* rs3138035	*CCL17* rs223828	*CCL16* rs854680					
Predisposing									
T/T	T		C		46.55	69.57	2.08×10^{-4}	2.63	1.77–3.89
T/T	T			T	47.40	69.73	1.2×10^{-4}	2.56	1.73–3.77
T/T			C	T	45.32	67.93	1×10^{-4}	2.56	1.73–3.77
T/T	T				49.48	71.35	7.83×10^{-5}	2.54	1.72–3.77
T/T			C		47.48	69.57	8.12×10^{-5}	2.53	1.71–3.74
T/T				T	47.96	69.73	7.84×10^{-5}	2.50	1.70–3.69
Protective									
C	T/T	C			38.19	16.30	1.39×10^{-4}	0.32	0.2–0.5
C	T	C			46.53	24.46	1.39×10^{-4}	0.37	0.25–0.56
C		C	T		19.13	4.37	8.92×10^{-5}	0.19	0.09–0.42
C	T/T				36.33	15.68	1.24×10^{-4}	0.36	0.23–0.55
C	T				50.17	28.11	9.42×10^{-5}	0.39	0.26–0.58
C		C			46.05	25.00	9.24×10^{-5}	0.39	0.26–0.59

P_{FDR} P value adjusted for multiple comparisons, *OR* odds ratio, CI_{OR} 95% confidence interval

(only in mouse) [2]. *CCL16* rs854680 polymorphism is reportedly associated with systemic lupus erythemathosis [29].

CCL18 is constitutively expressed at low levels in monocytes and macrophages, but its transcriptional activity is significantly up-regulated in atherosclerotic plagues [30]. The carriers of C allele of the rs2015086 SNV in the *CCL18* promoter region had higher *CCL18* gene expression and higher serum CCL18 levels, as well as diminished survival in idiopathic pulmonary fibrosis [30]. *CCL18* rs2015086 was also associated with increased plasma CCL3 levels [31]; this polymorphism is also in linkage disequilibrium ($r^2=0.911$, D' = 0.966) with another SNV located near *CCL18* gene, rs854462, which significantly influences PARC level [32].

CCL23 is an inflammatory chemokine present at high concentrations in serum and is known to promote angiogenesis and induces chemotaxis of immune cells and chemotactic migration of endothelial cells through activation of CCR1 [33]. Expression of *CCL23* in peripheral blood leukocytes is induced by IL4 and IL13, acting via STAT6 binding site located between −698 and −689 relative to the transcriptional start site gene [34]. Interestingly, we found that *CCL23* rs854655 was in linkage disequilibrium ($r^2=0.975$, D' = 0.994) with *CCL23* rs1003645 polymorphism (Met123Val), which was shown to be associated with plasma circulating proteins at a GWAS significance level [32, 35]. However, in our study, we detected no association with *CCL23* rs854655 SNV with EH when analyzed separately, nor when performing the analysis of gene–gene interaction.

Conclusion

Our study has demonstrated significant association between *CXCL13* rs355689 and EH in men of Tatar ethnicity. This polymorphism was also detected as a core element in the patterns associated with the risk of EH, with T/T genotype being part of unfavorable combinations, and C allele being present in protective combinations. The identified patterns associated with EH also featured polymorphic variants of *CCL8, CCL16, CCL17*, and *CCL18* genes. Although relatively modest study sample diminishes the statistical power of our study, the results suggest a role for chemokines in the development of hypertension. If our findings are confirmed by a replication study on an independent population, then an additional research of functional relationship between these genes could further elucidate the molecular mechanisms underlying hypertension.

Acknowledgements The study was supported by Federal Agency for Scientific Organizations program for support of the bioresource collections.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

Ethical approval The study was approved by the Ethics Committee of the Institute of Biochemistry and Genetics USC RAS.

Informed consent Informed consent was obtained from all participants participant in accordance with the Declaration of Helsinki.

References

1. Cardona SM, Garcia JA, Cardona AE (2013) The fine balance of chemokines during disease: trafficking, inflammation, and homeostasis. In: Cardona A, Ubogu E (eds) Chemokines. Springer, New York, pp 1–16
2. Zlotnik A, Yoshie O (2012) The chemokine superfamily revisited. Immunity 36(5):705–716
3. Zlotnik A, Yoshie O, Nomiyama H (2006) The chemokine and chemokine receptor superfamilies and their molecular evolution. Genome Biol 7(12):243
4. Colobran R, Pujol-Borrell R, Armengol MP, Juan M (2007) The chemokine network. I. How the genomic organization of chemokines contains clues for deciphering their functional complexity. Clin Experim Immunol 148(2):208–217
5. Bush E, Maeda N, Kuziel WA, Dawson TC, Wilcox JN, DeLeon H, Taylor WR (2000) CC chemokine receptor 2 is required for macrophage infiltration and vascular hypertrophy in angiotensin II-induced hypertension. Hypertension 36(3):360–363. https://doi.org/10.1161/01.Hyp.36.3.360
6. Wang L, Zhao XC, Cui W, Ma YQ, Ren HL, Zhou X, Fassett J, Yang YZ, Chen Y, Xia YL, Du J, Li HH (2016) Genetic and pharmacologic inhibition of the chemokine receptor CXCR2 prevents experimental hypertension and vascular dysfunction. Circulation 134(18):1353–1368. https://doi.org/10.1161/circulationaha.115.020754
7. Antonelli A, Fallahi P, Rotondi M, Ferrari SM, Romagnani P, Ghiadoni L, Serio M, Taddei S, Ferrannini E (2008) High serum levels of CXC chemokine ligand 10 in untreated essential hypertension. J Hum Hypertens 22:579. https://doi.org/10.1038/jhh.2008.15
8. Tucci M, Quatraro C, Frassanito MA, Silvestris F (2006) Deregulated expression of monocyte chemoattractant protein-1 (MCP-1) in arterial hypertension: role in endothelial inflammation and atheromasia. J Hypertens 24(7):1307–1318. https://doi.org/10.1097/01.hjh.0000234111.31239.c3
9. Timasheva Y, Nasibullin T, Tuktarova I, Zakirova A, Mustafina O (2011) Altered expression profile and the association of cytokine genes with essential hypertension. J Hypertens 29:e332–e333
10. Lukmanova TV, Karamova IM, Sharafutdinova NK (2007) Prevalence of arterial hypertension, associated clinical conditions, and target organ damage in the Republic of Bashkortostan. Ration Pharmacother Cardiol 3(6):6–11
11. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira Manuel AR, Bender D, Maller J, Sklar P, de Bakker Paul IW, Daly Mark J, Sham Pak C (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3):559–575. https://doi.org/10.1086/519795
12. Favorov AV, Andreewski TV, Sudomoina MA, Favorova OO, Parmigiani G, Ochs MF (2005) A Markov chain Monte Carlo technique for identification of combinations of allelic variants underlying complex diseases in humans. Genetics 171(4):2113–2121. https://doi.org/10.1534/genetics.105.048090
13. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B (Methodological) 57:289–300
14. Peden JF, Hopewell JC, Saleheen D, Chambers JC, Hager J, Soranzo N, Collins R, Danesh J, Elliott P, Farrall M, Stirrups K, Zhang WH, Hamsten A, Parish S, Lathrop M, Watkins H, Clarke R, Deloukas P, Kooner JS, Goel A, Ongen H, Strawbridge RJ, Heath S, Malarstig A, Helgadottir A, Ohrvik J, Murtaza M, Potter S, Hunt SE, Delepine M, Jalilzadeh S, Axelsson T, Syvanen AC, Gwilliam R, Bumpstead S, Gray E, Edkins S, Folkersen L, Kyriakou T, Franco-Cereceda A, Gabrielsen A, Seedorf U, Eriksson P, Offer A, Bowman L, Sleight P, Armitage J, Peto R, Abecasis G, Ahmed N, Caulfield M, Donnelly P, Froguel P, Kooner AS, McCarthy MI, Samani NJ, Scott J, Sehmi J, Silveira A, Hellenius ML, van't Hooft FM, Olsson G, Rust S, Assmann G, Barlera S, Tognoni G, Franzosi MG, Linksted P, Green FR, Rasheed A, Zaidi M, Shah N, Samuel M, Mallick NH, Azhar M, Zaman KS, Samad A, Ishaq M, Gardezi AR, Memon FU, Frossard PM, Spector T, Peltonen L, Nieminen MS, Sinisalo J, Salomaa V, Ripatti S, Bennett D, Leander K, Gigante B, de Faire U, Pietri S, Gori F, Marchioli R, Sivapalaratnam S, Kastelein JJP, Trip MD, Theodoraki EV, Dedoussis GV, Engert JC, Yusuf S, Anand SS, The Coronary Artery Disease (C4D) Genetics Consortium (2011) A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet 43(4):339–344. https://doi.org/10.1038/ng.782
15. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sober S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WH, Sjogren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Nguyen KD, Lehtimaki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CS, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, Aspelund T, Garcia M, Chang YP, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kahonen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JA, Kottgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grassler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Soler Artigas M, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stancakova A, Raffel LJ, Yao J, Kathiresan S, O'Donnell CJ, Schwartz SM, Ikram MA, Longstreth WT Jr, Mosley TH, Seshadri S, Shrine NR, Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJ, van Gilst WH, Janipalli CS, Mani KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikainen LP, Soininen P, Tukiainen T, Wurtz P, Ong RT, Dorr M, Kroemer HK, Volker U, Volzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kumar MV, Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Fowkes FG, Charchar FJ, Schwarz PE, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der

Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Tai ES, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Zhu X, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJ, Altshuler D, Loos RJ, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JI, Rettig R, Uda M, Strachan DP, Witteman JC, Hartikainen AL, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Jarvelin MR, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ, Johnson T (2011) Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature 478(7367):103–109. https://doi.org/10.1038/nature10405

16. Mahajan A, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T, Horikoshi M, Johnson AD, Ng MC, Prokopenko I, Saleheen D, Wang X, Zeggini E, Abecasis GR, Adair LS, Almgren P, Atalay M, Aung T, Baldassarre D, Balkau B, Bao Y, Barnett AH, Barroso I, Basit A, Been LF, Beilby J, Bell GI, Benediktsson R, Bergman RN, Boehm BO, Boerwinkle E, Bonnycastle LL, Burtt N, Cai Q, Campbell H, Carey J, Cauchi S, Caulfield M, Chan JC, Chang LC, Chang TJ, Chang YC, Charpentier G, Chen CH, Chen H, Chen YT, Chia KS, Chidambaram M, Chines PS, Cho NH, Cho YM, Chuang LM, Collins FS, Cornelis MC, Couper DJ, Crenshaw AT, van Dam RM, Danesh J, Das D, de Faire U, Dedoussis G, Deloukas P, Dimas AS, Dina C, Doney AS, Donnelly PJ, Dorkhan M, van Duijn C, Dupuis J, Edkins S, Elliott P, Emilsson V, Erbel R, Eriksson JG, Escobedo J, Esko T, Eury E, Florez JC, Fontanillas P, Forouhi NG, Forsen T, Fox C, Fraser RM, Frayling TM, Froguel P, Frossard P, Gao Y, Gertow K, Gieger C, Gigante B, Grallert H, Grant GB, Grrop LC, Groves CJ, Grundberg E, Guiducci C, Hamsten A, Han BG, Hara K, Hassanali N, Hattersley AT, Hayward C, Hedman AK, Herder C, Hofman A, Holmen OL, Hovingh K, Hreidarsson AB, Hu C, Hu FB, Hui J, Humphries SE, Hunt SE, Hunter DJ, Hveem K, Hydrie ZI, Ikegami H, Illig T, Ingelsson E, Islam M, Isomaa B, Jackson AU, Jafar T, James A, Jia W, Jockel KH, Jonsson A, Jowett JB, Kadowaki T, Kang HM, Kanoni S, Kao WH, Kathiresan S, Kato N, Katulanda P, Keinanen-Kiukaanniemi KM, Kelly AM, Khan H, Khaw KT, Khor CC, Kim HL, Kim S, Kim YJ, Kinnunen L, Klopp N, Kong A, Korpi-Hyovalti E, Kowlessur S, Kraft P, Kravic J, Kristensen MM, Krithika S, Kumar A, Kumate J, Kuusisto J, Kwak SH, Laakso M, Lagou V, Lakka TA, Langenberg C, Langford C, Lawrence R, Leander K, Lee JM, Lee NR, Li M, Li X, Li Y, Liang J, Liju S, Lim WY, Lind L, Lindgren CM, Lindholm E, Liu CT, Liu JJ, Lobbens S, Long J, Loos RJ, Lu W, Luan J, Lyssenko V, Ma RC, Maeda S, Magi R, Mannisto S, Matthews DR, Meigs JB, Melander O, Metspalu A, Meyer J, Mirza G, Mihailov E, Moebus S, Mohan V, Mohlke KL, Morris AD, Muhleisen TW, Muller-Nurasyid M, Musk B, Nakamura J, Nakashima E, Navarro P, Ng PK, Nica AC, Nilsson PM, Njolstad I, Nothen MM, Ohnaka K, Ong TH, Owen KR, Palmer CN, Pankow JS, Park KS, Parkin M, Pechlivanis S, Pedersen NL, Peltonen L, Perry JR, Peters A, Pinidiyapathirage JM, Platou CG, Potter S, Price JF, Qi L, Radha V, Rallidis L, Rasheed A, Rathman W, Rauramaa R, Raychaudhuri S, Rayner NW, Rees SD, Rehnberg E, Ripatti S, Robertson N, Roden M, Rossin EJ, Rudan I, Rybin D, Saaristo TE, Salomaa V, Saltevo J, Samuel M, Sanghera DK, Saramies J, Scott J, Scott LJ, Scott RA, Segre AV, Sehmi J, Sennblad B, Shah N, Shah S, Shera AS, Shu XO, Shuldiner AR, Sigurdsson G, Sijbrands E, Silveira A, Sim X, Sivapalaratnam S, Small KS, So WY, Stancakova A, Stefansson K, Steinbach G, Steinthorsdottir V, Stirrups K, Strawbridge RJ, Stringham HM, Sun Q, Suo C, Syvanen AC, Takayanagi R, Takeuchi F, Tay WT, Teslovich TM, Thorand B, Thorleifsson G, Thorsteinsdottir U, Tikkanen E, Trakalo J, Tremoli E, Trip MD, Tsai FJ, Tuomi T, Tuomilehto J, Uitterlinden AG, Valladares-Salgado A, Vedantam S, Veglia F, Voight BF, Wang C, Wareham NJ, Wennauer R, Wickremasinghe AR, Wilsgaard T, Wilson JF, Wiltshire S, Winckler W, Wong TY, Wood AR, Wu JY, Wu Y, Yamamoto K, Yamauchi T, Yang M, Yengo L, Yokota M, Young R, Zabaneh D, Zhang F, Zhang R, Zheng W, Zimmet PZ, Altshuler D, Bowden DW, Cho YS, Cox NJ, Cruz M, Hanis CL, Kooner J, Lee JY, Seielstad M, Teo YY, Boehnke M, Parra EJ, Chambers JC, Tai ES, McCarthy MI, Morris AP (2014) Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet 46(3):234–244. https://doi.org/10.1038/ng.2897

17. Berndt SI, Gustafsson S, Magi R, Ganna A, Wheeler E, Feitosa MF, Justice AE, Monda KL, Croteau-Chonka DC, Day FR, Esko T, Fall T, Ferreira T, Gentilini D, Jackson AU, Luan J, Randall JC, Vedantam S, Willer CJ, Winkler TW, Wood AR, Workalemahu T, Hu YJ, Lee SH, Liang L, Lin DY, Min JL, Neale BM, Thorleifsson G, Yang J, Albrecht E, Amin N, Bragg-Gresham JL, Cadby G, den Heijer M, Eklund N, Fischer K, Goel A, Hottenga JJ, Huffman JE, Jarick I, Johansson A, Johnson T, Kanoni S, Kleber ME, Konig IR, Kristiansson K, Kutalik Z, Lamina C, Lecoeur C, Li G, Mangino M, McArdle WL, Medina-Gomez C, Muller-Nurasyid M, Ngwa JS, Nolte IM, Paternoster L, Pechlivanis S, Perola M, Peters MJ, Preuss M, Rose LM, Shi J, Shungin D, Smith AV, Strawbridge RJ, Surakka I, Teumer A, Trip MD, Tyrer J, Van Vliet-Ostaptchouk JV, Vandenput L, Waite LL, Zhao JH, Absher D, Asselbergs FW, Atalay M, Attwood AP, Balmforth AJ, Basart H, Beilby J, Bonnycastle LL, Brambilla P, Bruinenberg M, Campbell H, Chasman DI, Chines PS, Collins FS, Connell JM, Cookson WO, de Faire U, de Vegt F, Dei M, Dimitriou M, Edkins S, Estrada K, Evans DM, Farrall M, Ferrario MM, Ferrieres J, Franke L, Frau F, Gejman PV, Grallert H, Gronberg H, Gudnason V, Hall AS, Hall P, Hartikainen AL, Hayward C, Heard-Costa NL, Heath AC, Hebebrand J, Homuth G, Hu FB, Hunt SE, Hypponen E, Iribarren C, Jacobs KB, Jansson JO, Jula A, Kahonen M, Kathiresan S, Kee F, Khaw KT, Kivimaki M, Koenig W, Kraja AT, Kumari M, Kuulasmaa K, Kuusisto J, Laitinen JH, Lakka TA, Langenberg C, Launer LJ, Lind L, Lindstrom J, Liu J, Liuzzi A, Lokki ML, Lorentzon M, Madden PA, Magnusson PK, Manunta P, Marek D, Marz W, Mateo Leach I, McKnight B, Medland SE, Mihailov E, Milani L, Montgomery GW, Mooser V, Muhleisen TW, Munroe PB, Musk AW, Narisu N, Navis G, Nicholson G, Nohr EA, Ong KK, Oostra BA, Palmer CN, Palotie A, Peden JF, Pedersen N, Peters A, Polasek O, Pouta A, Pramstaller PP, Prokopenko I, Putter C, Radhakrishnan A, Raitakari O, Rendon A, Rivadeneira F, Rudan I, Saaristo TE, Sambrook JG, Sanders AR, Sanna S, Saramies J, Schipf S, Schreiber S, Schunkert H, Shin SY, Signorini S, Sinisalo J, Skrobek B, Soranzo N, Stancakova A, Stark K, Stephens JC, Stirrups K, Stolk RP, Stumvoll M, Swift AJ, Theodoraki EV, Thorand B, Tregouet DA, Tremoli E, Van der Klauw MM, van Meurs JB, Vermeulen SH, Viikari J, Virtamo J, Vitart V, Waeber G, Wang Z, Widen E, Wild SH, Willemsen G, Winkelmann BR, Witteman JC, Wolffenbuttel BH, Wong A, Wright AF, Zillikens MC, Amouyel P, Boehm BO, Boerwinkle E, Boomsma DI, Caulfield MJ, Chanock SJ, Cupples LA, Cusi D, Dedoussis GV, Erdmann J, Eriksson JG, Franks PW, Froguel P, Gieger C, Gyllensten U, Hamsten A, Harris TB, Hengstenberg C, Hicks AA, Hingorani A, Hinney A, Hofman A, Hovingh KG, Hveem K, Illig T, Jarvelin MR, Jockel KH, Keinanen-Kiukaanniemi SM, Kiemeney LA, Kuh D, Laakso M, Lehtimaki T, Levinson DF, Martin NG, Metspalu A, Morris AD, Nieminen MS, Njolstad I, Ohlsson C, Oldehinkel AJ, Ouwehand WH, Palmer LJ, Penninx B, Power C, Province MA, Psaty BM, Qi L, Rauramaa R, Ridker PM, Ripatti S, Salomaa V, Samani NJ, Snieder H, Sorensen TI, Spector TD, Stefansson K, Tonjes A, Tuomilehto

J, Uitterlinden AG, Uusitupa M, van der Harst P, Vollenweider P, Wallaschofski H, Wareham NJ, Watkins H, Wichmann HE, Wilson JF, Abecasis GR, Assimes TL, Barroso I, Boehnke M, Borecki IB, Deloukas P, Fox CS, Frayling T, Groop LC, Haritunian T, Heid IM, Hunter D, Kaplan RC, Karpe F, Moffatt MF, Mohlke KL, O'Connell JR, Pawitan Y, Schadt EE, Schlessinger D, Steinthorsdottir V, Strachan DP, Thorsteinsdottir U, van Duijn CM, Visscher PM, Di Blasio AM, Hirschhorn JN, Lindgren CM, Morris AP, Meyre D, Scherag A, McCarthy MI, Speliotes EK, North KE, Loos RJ, Ingelsson E (2013) Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. Nat Genet 45(5):501–512. https://doi.org/10.1038/ng.2606

18. Hussain S, Zhu W, Chang S-C, Breen EC, Vendrame E, Magpantay L, Widney D, Conn D, Sehl ME, Jacobson L (2012) Serum levels of the chemokine CXCL13, genetic variation in CXCL13 and its receptor CXCR5, and HIV-associated non-Hodgkin B cell lymphoma risk. Cancer Epidemiol Biomarkers Prev. https://doi.org/10.1158/1055-9965.EPI-12-1122
19. Bezzerri V, d'Adamo P, Rimessi A, Lanzara C, Crovella S, Nicolis E, Tamanini A, Athanasakis E, Tebon M, Bisoffi G (2011) Phospholipase C-β3 is a key modulator of IL-8 expression in cystic fibrosis bronchial epithelial cells. J Immunol 186(8):4946–4958
20. Kowarik MC, Cepok S, Sellner J, Grummel V, Weber MS, Korn T, Berthele A, Hemmer B (2012) CXCL13 is the major determinant for B cell recruitment to the CSF during neuroinflammation. J Neuroinflamm 9(1):93
21. Waehre A, Halvorsen B, Yndestad A, Husberg C, Sjaastad I, Nygård S, Dahl CP, Ahmed MS, Finsen AV, Reims H (2011) Lack of chemokine signaling through CXCR5 causes increased mortality, ventricular dilatation and deranged matrix during cardiac pressure overload. PLoS ONE 6(4):e18668
22. Smedbakken LM, Halvorsen B, Daissormont I, Ranheim T, Michelsen AE, Skjelland M, Sagen EL, Folkersen L, Krohg-Sørensen K, Russell D (2012) Increased levels of the homeostatic chemokine CXCL13 in human atherosclerosis–potential role in plaque stabilization. Atherosclerosis 224(1):266–273
23. Struyf S, Proost P, Vandercappellen J, Dempe S, Noyens B, Nelissen S, Gouwy M, Locati M, Opdenakker G, Dinsart C (2009) Synergistic up-regulation of MCP-2/CCL8 activity is counteracted by chemokine cleavage, limiting its inflammatory and anti-tumoral effects. Eur J Immunol 39(3):843–857
24. Weber C, Meiler S, Döring Y, Koch M, Drechsler M, Megens RT, Rowinska Z, Bidzhekov K, Fecher C, Ribechini E (2011) CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice. J Clin Investig 121(7):2898–2910
25. Lee C-P, Huang Y-H, Hsu Y-W, Yang KD, Chien H-C, Yu H-R, Yang Y-L, Wang C-L, Chang W-C, Kuo H-C (2013) TARC/CCL17 gene polymorphisms and expression associated with susceptibility and coronary artery aneurysm formation in Kawasaki disease. Pediatric Res 74(5):545
26. Galimberti D, Scalabrini D, Fenoglio C, De Riz M, Comi C, Venturelli E, Cortini F, Piola M, Leone M, Dianzani U (2008) Gender-specific influence of the chromosome 16 chemokine gene cluster on the susceptibility to multiple sclerosis. J Neurol Sci 267(1):86–90
27. Ghesquières H, Maurer MJ, Casasnovas O, Ansell SM, Larrabee BR, Lech-Maranda E, Novak AJ, Borrel A-L, Slager SL, Brice P (2013) Cytokine gene polymorphisms and progression-free survival in classical Hodgkin lymphoma by EBV status: results from two independent cohorts. Cytokine 64(2):523–531
28. Ma H, Shu Y, Pan S, Chen J, Dai J, Jin G, Hu Z, Shen H (2011) Polymorphisms of key chemokine genes and survival of non-small cell lung cancer in Chinese. Lung Cancer 74(2):164–169
29. Vyshkina T, Sylvester A, Sadiq S, Bonilla E, Perl A, Kalman B (2008) CCL genes in multiple sclerosis and systemic lupus erythematosus. J Neuroimmunol 200(1):145–152
30. Hägg DA, Olson FJ, Kjelldahl J, Jernås M, Thelle DS, Carlsson LM, Fagerberg B, Svensson P-A (2009) Expression of chemokine (C–C motif) ligand 18 in human macrophages and atherosclerotic plaques. Atherosclerosis 204(2):e15–e20
31. Deming Y, Xia J, Cai Y, Lord J, Del-Aguila JL, Fernandez MV, Carrell D, Black K, Budde J, Ma S (2016) Genetic studies of plasma analytes identify novel potential biomarkers for several complex traits. Sci Rep 6:18092
32. Kim S, Swaminathan S, Inlow M, Risacher SL, Nho K, Shen L, Foroud TM, Petersen RC, Aisen PS, Soares H (2013) Influence of genetic variation on plasma protein levels in older adults using a multi-analyte panel. PLoS ONE 8(7):e70269
33. Hwang J, Son K-N, Kim CW, Ko J, Na DS, Kwon BS, Gho YS, Kim J (2005) Human CC chemokine CCL23, a ligand for CCR1, induces endothelial cell migration and promotes angiogenesis. Cytokine 30(5):254–263
34. Novak H, Müller A, Harrer N, Günther C, Carballido JM, Woisetschläger M (2007) CCL23 expression is induced by IL-4 in a STAT6-dependent fashion. J Immunol 178(7):4335–4341
35. Suhre K, Arnold M, Bhagwat AM, Cotton RJ, Engelke R, Raffler J, Sarwath H, Thareja G, Wahl A, DeLisle RK (2017) Connecting genetic risk to disease end points through the human blood plasma proteome. Nat Commun 8:14357