Clinical Immunology 161 (2015) 157–162

Contents lists available at ScienceDirect

Clinical Immunology

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

Genetic associations of leptin-related polymorphisms with systemic lupus erythematosus

Jian Zhao [a], Hui Wu [a], Carl D. Langefeld [b], Kenneth M. Kaufman [c,d], Jennifer A. Kelly [e], Sang-Cheol Bae [f], Marta E. Alarcón-Riquelme for the BIOLUPUS and GENLES networks [e,g,1], Graciela S. Alarcón [h], Juan-Manuel Anaya [i], Lindsey A. Criswell [j], Barry I. Freedman [k], Diane L. Kamen [l], Gary S. Gilkeson [l], Chaim O. Jacob [m], Judith A. James [e,n,o], Joan T. Merrill [p], Patrick M. Gaffney [e], Kathy Moser Sivils [e,n], Timothy B. Niewold [q], Michelle A. Petri [r], Seung Taek Song [f], Hye-jin Jeong [f], Rosalind Ramsey-Goldman [s], John D. Reveille [t], R. Hal Scofield [e,o,u], Anne M. Stevens [v,w], Susan A. Boackle [x,y], Luis M. Vilá [z], Deh-Ming Chang [aa], Yeong Wook Song [ab], Timothy J. Vyse [ac], John B. Harley [c,d], Elizabeth E. Brown [h,ad], Jeffrey C. Edberg [h], Robert P. Kimberly [h], Bevra H. Hahn [a], Jennifer M. Grossman [a], Betty P. Tsao [a,*], Antonio La Cava [a,*]

[a] Department of Medicine, University of California Los Angeles, Los Angeles, CA, United States
[b] Department of Biostatistical Sciences and Center for Public Health Genomics, Wake Forest School of Medicine, Winston-Salem, NC, United States
[c] Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States
[d] US Department of Veterans Affairs Medical Center, Cincinnati, OH, United States
[e] Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, United States
[f] Department of Rheumatology, Hanyang University Hospital for Rheumatic Diseases, Seoul, South Korea
[g] Pfizer-Universidad de Granada-Junta de Andalucía Center for Genomics and Oncological Research, Granada, Spain
[h] Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, United States
[i] Center for Autoimmune Diseases Research, Universidad del Rosario, Bogotá, Colombia
[j] Rosalind Russell/Ephraim P. Engleman Rheumatology Research Center, University of California San Francisco, San Francisco, CA, United States
[k] Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC, United States
[l] Medical University of South Carolina, Charleston, SC, United States
[m] Department of Medicine, University of Southern California, Los Angeles, CA, United States
[n] Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States
[o] Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States
[p] Clinical Pharmacology, Oklahoma Medical Research Foundation, Oklahoma City, OK, United States
[q] Department of Immunology, Mayo Clinic, Rochester, MN, United States
[r] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States
[s] Northwestern University Feinberg School of Medicine, Chicago, IL, United States
[t] Rheumatology and Clinical Immunogenetics, University of Texas Health Science Center at Houston, Houston, TX, United States
[u] US Department of Veterans Affairs Medical Center, Oklahoma City, OK, United States
[v] Department of Pediatrics, University of Washington, Seattle, WA, United States
[w] Center for Immunity and Immunotherapies, Seattle Children's Research Institute Seattle, WA, United States
[x] University of Colorado School of Medicine, Aurora, CO, United States
[y] US Department of Veterans Affairs Medical Center, Denver, CO, United States
[z] Department of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico
[aa] National Defense Medical Center, Taipei City, Taiwan
[ab] Seoul National University, Seoul, South Korea
[ac] King's College London, London, UK
[ad] Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, United States

* Corresponding authors at: Division of Rheumatology, David Geffen School of Medicine, University of California Los Angeles, 1000 Veteran Ave. 32-59, Los Angeles, CA, United States.
E-mail addresses: btsao@mednet.ucla.edu (B.P. Tsao), alacava@mednet.ucla.edu (A. La Cava).

[1] The BIOLUPUS network is composed of Johan Frostegård (Huddinge, Sweden), Lennart Truedsson (Lund, Sweden), Enrique de Ramón (Málaga, Spain), José M. Sabio (Granada, Spain), María F. González-Escribano (Sevilla, Spain), Javier Martin (Granada, Spain), Norberto Ortego-Centeno (Granada, Spain), José Luis Callejas (Granada, Spain), Julio Sánchez-Román (Sevilla, Spain), Sandra D'Alfonso (Novara, Italy), Sergio Migliarese (Napoli, Italy), Gian-Domenico Sebastiani (Rome, Italy), Mauro Galeazzi (Siena, Italy), Torsten Witte (Hannover, Germany), Bernard R. Lauwerys (Louvain, Belgium), Emoke Endreffy (Szeged, Hungary), László Kovács (Szeged, Hungary), Carlos Vasconcelos (Porto, Portugal) and Berta Martins da Silva (Porto, Portugal). The members of GENLES Network are Hugo R. Scherbarth, Pilar C. Marino, Estela L. Motta, Susana Gamron, Cristina Drenkard, Emilia Menso, Alberto Allievi, Guillermo A. Tate, Jose L. Presas, Simon A. Palatnik, Marcelo Abdala, Mariela Bearzotti, Alejandro Alvarellos, Francisco Caeiro, Ana Bertoli, Sergio Paira, Susana Roverano, Cesar E. Graf, Estela Bertero, Cesar Caprarulo, Griselda Buchanan, Carolina Guillerón, Sebastian Grimaudo, Jorge Manni, Luis J. Catoggio, Enrique R. Soriano, Carlos D. Santos, Cristina Prigione, Fernando A. Ramos, Sandra M. Navarro, Guillermo A. Berbotto, Marisa Jorfen, Elisa J. Romero, Mercedes A. Garcia, Juan C Marcos, Ana I. Marcos, Carlos E. Perandones, Alicia Eimon, Sanatorio Parque and Cristina G. Battagliotti in Argentina; Eduardo Acevedo and Mariano Cucho in Perú; Ignacio García de la Torre, Mario Cardiel Ríos, José Francisco Moctezuma and Marco Maradiaga Ceceña in Mexico.

http://dx.doi.org/10.1016/j.clim.2015.09.007

ARTICLE INFO

Article history:
Received 12 September 2015
accepted with revision 12 September 2015
Available online 16 September 2015

Keywords:
Systemic lupus erythematosus
Leptin pathway
Gene polymorphisms

ABSTRACT

Leptin is abnormally elevated in the plasma of patients with systemic lupus erythematosus (SLE), where it is thought to promote and/or sustain pro-inflammatory responses. Whether this association could reflect an increased genetic susceptibility to develop SLE is not known, and studies of genetic associations with leptin-related polymorphisms in SLE patients have been so far inconclusive. Here we genotyped DNA samples from 15,706 SLE patients and healthy matched controls from four different ancestral groups, to correlate polymorphisms of genes of the leptin pathway to risk for SLE. It was found that although several SNPs showed weak associations, those associations did not remain significant after correction for multiple testing. These data do not support associations between defined leptin-related polymorphisms and increased susceptibility to develop SLE.

1. Introduction

The etiopathogenesis of systemic lupus erythematosus (SLE) generally considers an involvement of environmental factors (including epigenetic changes) that could trigger abnormal autoimmune responses, facilitated by sex and hormones, in individuals that carry a predisposing genetic background [1]. Therefore, in SLE multiple genetic variants could create favorable conditions for a heightened sensitivity of autoreactive cells to an increased stimulation/activation.

Our group and others have previously shown that leptin is abnormally elevated in SLE patients [2,3]. We also showed that leptin in mice could promote SLE autoimmunity [4,5]. Whether these findings underlie genetic association(s) between selected leptin polymorphisms and SLE is not known. A recent study suggested an association of a leptin receptor gene polymorphism (LEPRQ223R) with increased susceptibility of SLE in 100 Kashmiri individuals [6]. Since analyses on larger numbers of SLE patients and in multiple ethnic groups would better delineate the possibility of association(s) between leptin-related genes and increased risk for SLE, we performed genetic association studies for single nucleotide polymorphisms (SNPs) within multiple leptin-related genes. Criteria for selection were based on the following considerations. Leptin gene (*LEP*) polymorphisms [7] were studied because of their possible roles in abnormal function/catabolism of leptin. For leptin receptor (*LEPR*), which exists in six alternatively spliced forms with cytoplasmic domains of different length [8], we assessed polymorphisms of all isoforms [9] because any of them might influence catabolism and/or sustain leptin activity. The polymorphism of *PPARG* [10–12] was also studied because leptin can downregulate PPAR-γ expression for a subsequent increase in the release of pro-inflammatory IL-1β, IL-6 and TNF-α [13,14]. Finally, the polymorphism of the growth hormone secretagogue receptor *GHSR* was studied because its opposing action on leptin results in the inhibition of the same pro-inflammatory cytokines [15,16].

Haplotype-tagging SNPs selected from these genes were genotyped by a customized SNP genotyping-array and assessed for association with SLE in 15,706 case-control subjects from four different ancestral groups including European American (EA), African American (AA), East Asian (AS) and Hispanic enriched for the Amerindian-European admixture (HS).

2. Materials and methods

2.1. Subjects' samples collection and SNP genotyping

To test association of *LEPR*, *PPARG*, *GHSR* and *LEP* with SLE, we used a large collection of samples from case-control subjects from multiple ethnic groups. These samples were from the collaborative Large Lupus Association Study 2 (LLAS2) and were contributed by participating institutions in the United States, Asia and Europe. All SLE patients met the American College of Rheumatology (ACR) criteria for the classification of SLE [17]. LLAS2 samples were processed at the Lupus Genetics Studies Unit of the Oklahoma Medical Research Foundation (OMRF). SNP genotyping was carried out on the Illumina iSelect platform. Subjects with individual genotyping call rate <0.90 were removed because of low data quality. Subjects that were duplicated or first degree related were also removed. Both principal component analysis and global ancestry estimation based on 347 ancestry informative markers (AIMs) were used to detect population stratification and admixture, as described in another LLAS2 report [18]. After removing genetic outliers, a final dataset of 15,706 unrelated subjects (8269 cases vs. 7437 controls) was obtained.

According to genetic ancestry, subjects were grouped into four ancestral groups including European American (3966 cases vs. 3543 controls), African American (1527 cases vs. 1812 controls), East Asian (1272 cases vs. 1270 controls) and Hispanic enriched for the Amerindian-European admixture (1504 cases vs. 812 controls).

The study was approved by the Human Subject Institutional Review Boards or the Ethic Committee of each institution. All subjects were enrolled after informed consent had been obtained.

2.2. SNP selection and statistical analysis

To avoid the genotyping of all SNPs for the genes of interest yet capture the majority of diversity within each region, we selected haplotype tag SNPs for genotyping according to the Hapmap Project (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/; HapMap public release #24 of 11/26/2008). In addition, SNPs with potential functional consequences were selected as well for testing. In total, we selected 9 SNPs for *LEP*, 17 SNPs for *LEPR*, 5 SNPs for *GHSR* and 16 SNPs for *PPARG*, at an average density of 8.2 kb per SNP. 32 SNPs that passed data cleaning and quality control measures (7 SNPs for *LEP*, 10 SNPs for *LEPR*, 3 SNPs for *GHSR* and 12 SNPs for *PPARG*, Table 1) were genotyped on the Illumina iSelect platform and subsequently used for genetic association test.

The Hardy–Weinberg equilibrium (HWE) test threshold was set at $P > 0.01$ for controls and $P > 0.0001$ for cases. SNPs failing the HWE test were excluded from association test. SNPs showing genotyping missing rate >5% or showing significantly different genotyping missing rate between cases and controls (missing rate >2% and $P_{missing} < 0.05$) were excluded from association test. In each ancestral group, SNPs were assessed for association with SLE under a logistic regression model adjusting for gender and the first 3 principal components estimated using AIMs. The trans-ancestry meta-analysis was conducted across all four ancestral groups. For each SNP, if the Cochran's Q statistic showed no evidence of genetic heterogeneity ($P > 0.05$), a fixed effect model was applied. Otherwise, a random effect model was used. All analyses described above were performed using PLINK v1.07 [19]. Pairwised LD values shown in Fig. 1 were calculated using Haploview 4.2 [20]

3. Results

3.1. Genetic association between leptin-related polymorphisms and human SLE

To test the possibility of common leptin-related variants predisposing to SLE, genetic association studies using htSNPs for the four selected