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Genetic Variation and Coronary Atherosclerosis in Patients with Systemic Lupus Erythematosus

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Summary

Coronary artery disease is the major cause of mortality in patients with systemic lupus erythematosus (SLE). Increased cardiovascular risk in SLE is not explained by traditional risk factors. We examined the hypothesis that genetic variation contributes to the presence of coronary atherosclerosis in patients with SLE. The genotypes of single-nucleotide polymorphisms (SNP) in 152 candidate genes linked with autoimmune or cardiovascular risk were determined in 125 patients with SLE. Coronary artery calcium (CAC), a measure of coronary atherosclerosis, was detected in 32 patients (26%) by electron-beam computed tomography. Polymorphism in twenty of the candidate genes (*ADAM33*, *ADIPOQ*, *CCL5*, *CCR7*, *CDKN2B*, *CSF1*, *IL4*, *IL12A*, *IL23R*, *INS*, *IRF5*, *MIF*, *MS4A1*, *PTGS1*, *PTPN22*, *RETN*, *SELE*, *TNFSF4*, *TNFRSF11B*, and *VCAM1*) were nominally associated with the presence of CAC (p-values = 0.001–0.047 after adjustment for age, sex and race). Some of these are known susceptibility genes for SLE and others have been implicated in cardiovascular disease in other populations. No association withstood false discovery rate adjustment. Replication studies in additional cohorts of patients with SLE may be informative.

Keywords

systemic lupus erythematosus; genetic variation; SNP; atherosclerosis

Introduction

Premature coronary artery disease (CAD) is a major cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE).¹ The increased prevalence of atherosclerosis in SLE is not fully explained by traditional cardiovascular risk factors,² and efforts to improve cardiovascular risk prediction are important. While many biomarkers have been associated with increased risk for atherosclerosis in SLE, information on the contribution of genetic markers to CAD risk is limited. Most attempts to identify variants as

risk factors for cardiovascular disease in patients with SLE have been limited to the study of single genes.

Thus, we undertook a more extensive candidate gene approach to more comprehensively test the hypothesis that genetic variation accounts for a portion of the unexplained cardiovascular risk in SLE. We selected polymorphisms located in genes implicated in autoimmunity, inflammation and cardiovascular disease (CVD) and tested the hypothesis that these variants were associated with coronary atherosclerosis in patients with SLE.

Methods

Patients with SLE (n=125), enrolled in an ongoing study to evaluate the prevalence and risk factors associated with coronary atherosclerosis in SLE, were examined. Details of the study have been previously described.³ In summary, patients met the 1997 American College of Rheumatology classification criteria for SLE⁴ and were 18 years or older. Coronary artery calcium (CAC), a non-invasive measurement of coronary atherosclerosis, was detected by electron beam computed tomography scan with an Imatron C-150 scanner (GE/Imatron, San Francisco, CA).

After literature review, 653 single nucleotide polymorphisms (SNPs) in 152 genes (see Appendix for complete list), were selected based on relevance to autoimmune, inflammatory and cardiovascular disorders. SNPs evaluated included published candidates and tagging SNPs. The latter were selected based upon HapMap CEU subject data encompassing 20 kb to each flank of a given gene, using LD select ($r^2 > 0.8$, minor allele frequency > 0.05). DNA was extracted from whole blood samples using Gentra Puregene DNA Purification reagents and standard protocols on an Autopure LS instrument (Qiagen). Quantification was done with a Nanodrop 2000 instrument (Thermo Scientific). Samples were diluted to 50 ng/Gl and genotyping was done by GoldenGate assay on a Beadstation 500GX instrument (Illumina).

Baseline characteristics in SLE patients are presented as mean ($\pm$ SD), median (interquartile range) or frequency (%), as appropriate. The association between presence of CAC and individual SNPs was assessed with logistic regression and odds ratios presented after adjustment for age, sex and race. A two-sided p-value of < 0.05 was considered significant. To adjust for multiple comparisons, a false discovery rate (FDR) threshold of 20% was pre-specified. Analyses were conducted using STATA 12.0 (Stata Corp., College Station). SNAP (SNP annotation and proxy search; www.broadinstitute.org) was used to estimate pair-wise linkage disequilibrium (LD) between individual SNPs showing association within the same gene.

Results

Patients with SLE were 41 ± 12 years old, 91% were women and 64% were Caucasian. The median (IQR) SLE disease activity score was 4 (0–6) and the median SLICC damage index was 1 (0–2). The presence of CAC was detected in 33 of the 125 SLE patients (26%). Consistent with our previous report⁵, older age ($p < 0.001$) and male sex ($p = 0.03$) were more common in patients with than in those without CAC, but there were no statistically

significant differences in other traditional cardiovascular risk factors such as smoking, hypertension, or diabetes. Table 1 shows the 33 SNPs in 20 genes that were associated with the presence of CAC. After adjustment for age, race and sex, there were significant associations between CAC and SNPs in the genes encoding desintegrin and metalloproteinase domain-containing protein-33 (*ADAM33*), adiponectin (*ADIPOQ*), chemokine (C-C motif) ligand-5 (*CCL5*), chemokine (C-C motif) receptor-7 (*CCR7*), cyclin-dependent kinase-4 inhibitor-B (*CDKN2B*), colony stimulating factor-1 (*CSF1*), interleukin-4 (*IL4*), interleukin 12-A (*IL12A*), interleukin-23 receptor (*IL23R*), insulin (*INS*), interferon regulator factor-5 (*IRF5*), macrophage migration inhibitory factor (*MIF*), membrane-spanning 4 domains, subfamily-A member-1 (*MS4A1*), prostaglandin-endoperoxide synthase-1 (*PTGS1*), protein tyrosine phosphatase, non-receptor type-22 (*PTPN22*), resistin (*RETN*), e-selectin (*SELE*), tumor necrosis factor ligand superfamily member-4 (*TNFSF4*), osteoprotegerin (*TNFRSF11B*), and vascular cell adhesion molecule-1 (*VCAM1*). None of these associations remained after FDR correction at 20%. The complete list of SNPs is included in the Appendix; Figure 1 shows the p-values of each of the associations explored in this study.

We performed a sensitivity analysis of the association of the selected SNPs and CAC, adjusting for Framingham risk scores and race, besides the primary analysis (which included age, race, and sex). Our findings were, in general, similar to the ones presented in the primary analysis and 20 SNPs remained associated with the presence of CAC.

Several of the genes including *ADIPOQ*, *CSF1*, *IL12A*, *INS*, *IRF5*, *MIF*, *PTPN22*, *RETN*, and *TNFSF4* contained more than one SNP associated with CAC. They were not in very strong linkage disequilibrium ($r^2 < 0.80$).

Discussion

To the best of our knowledge, this is the first study examining the association of variants in more than 150 selected genes and coronary atherosclerosis in patients with SLE. Our results suggest that variants in genes encoding several cytokines, growth and regulatory factors, and adhesion molecules may be associated with the presence of coronary atherosclerosis in patients with SLE. Some of these are known susceptibility genes for SLE and others have been implicated in cardiovascular disease in the general population.

Cytokines are small proteins that regulate cell signaling. Several genes encoding cytokines, including IL-4, IL-12 α , TNFSF-4 and CCL-5, were nominally associated with atherosclerosis in our study. IL-4 is an anti-inflammatory cytokine, particularly important during aging,⁶ IL-12 α is a pro-inflammatory cytokine produced by dendritic cells, macrophages and B cells in response to microbial pathogens,⁷ TNFSF-4 is a member of the tumor necrosis factor superfamily that promotes survival of CD4⁺T cells⁸ and CCL-5 is a potent chemoattractant of immune cells.⁹ The effect of cytokine signaling is mediated through receptors. Two SNPs located in genes encoding CCR-7 and IL-23R were linked with atherosclerosis in patients with SLE in our study. CCR-7 is a major conductor of immune cell trafficking during the primary immune response and is key in maintaining central and peripheral tolerance.¹⁰

IL-23R is a common biomarker for several autoimmune diseases. In regard to CVD, the association between variants in *IL-23R* and myocardial infarction in the general population have been tested, but no evidence for association with rs11209026 was found.¹¹

We found that variants in two genes encoding adipokines were associated with atherosclerosis in patients with lupus. *ADIPOQ* encodes adiponectin, a molecule with antidiabetic, anti-inflammatory and antiatherogenic effects.¹² *RETN* encodes resistin, an adipokine associated with acute coronary syndromes in the general population.¹³ Two cross-sectional studies of the role of resistin in patients with SLE have yielded different results. A study of 159 women with severe SLE reported higher resistin concentrations in SLE than in 70 controls and an association with coronary artery calcification.¹⁴ Another study reported that resistin concentrations were not higher in SLE patients than controls and the association between resistin and CAC was of borderline significance after adjustment for age and sex ($p=0.06$).¹⁵

Colony stimulating factor-1 is a growth factor, regulating survival, proliferation and differentiation of hematopoietic cells of the monocyte-macrophage series.¹⁶ Its concentration is increased in lupus in mice, and increased concentrations of CSF-1 have been reported in active lupus nephritis in humans.^{17, 18} Because macrophages are critical cells in the development of atherosclerotic plaque, and because CSF-1 also favors a proatherogenic environment and plaque development in the general population,¹⁹ this growth factor may have a role in both atherosclerosis and autoimmunity. Four SNPs of apparent independent effect (low pair-wise LD) in *CSF1* were associated with CAC in our study.

VCAM-1 is an immunoglobulin-like adhesion molecule expressed on activated endothelial cells. It is both critical in the development of atherosclerosis in the general population,²⁰ and higher serum VCAM-1 concentrations are associated with greater coronary calcium scores in SLE.²¹

PTPN22 encodes a tyrosine phosphatase that is expressed only in immune cells and is one of the strongest risk factors associated with autoimmune disease.²² Genetic variants of *PTPN22* increase the risk of atherosclerosis in the general population.²³ Our study identified two SNPs in this gene associated with CAC in patients with SLE.

Recently, a total of 34 independent risk variants at 32 CAD susceptibility loci have been identified by GWAS studies in the general population;²⁴ the variants that we report in this study did not overlap with any on that list. In patients with lupus, variants in *MBL2*, the gene encoding mannose-binding lectin, have been associated with increased disease susceptibility, risk of infections, and atherosclerosis.²⁵ In a study of 91 SLE patients, those who were homozygous for the functional *MLB2* gene (O/O genotype, $n=7$) had a seven-fold increased risk of arterial thrombosis²⁶ and variants in the same gene have been associated with subclinical atherosclerosis, as measured by common carotid intima media thickness;²⁷ but not with the presence of cerebrovascular, cardiovascular, or peripheral arterial organ damage in another study.²⁸ Our study did not find an association with variants in *MBL2* and CAC.

The results should be interpreted considering the limitations of the study. First, the sample size is small for genetic association studies, and none of the associations reached a modest 20% FDR threshold adjustment for multiple comparisons. Therefore, these findings can only be viewed as exploratory, and replication of our findings in independent cohorts will be necessary. Second, although there is an excellent correlation between coronary artery calcification detected by computed tomography and coronary artery atherosclerosis found by pathology;²⁹ it reflects a relatively late stage of plaque development. Thus, early coronary events may occur in the absence of calcification, especially in young patients. Finally, while the contribution of single SNPs as markers for atherosclerosis in this patient population is of interest, the development of risk scores with multiple validated SNPs may have even better potential as a clinical strategy to stratify cardiovascular risk.

In conclusion, our results suggest that polymorphisms in several genes including *ADAM33*, *ADIPOQ*, *CCL5*, *CCR7*, *CDKN2B*, *CSF1*, *IL4*, *IL12A*, *IL23R*, *INS*, *IRF5*, *MIF*, *MS4A1*, *PTGS1*, *PTPN22*, *RETN*, *SELE*, *TNFSF4*, *TNFRSF11B*, and *VCAM1* were associated with the presence of CAC in patients with SLE. The involvement of these genes is supported in part by published reports in lupus and the general population. Replication of these findings in additional cohorts will be both necessary and informative.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Reference List

1. Manzi S, Meilahn EN, Rairie JE, et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol.* 1997; 145(5):408–415. [PubMed: 9048514]
2. Esdaile JM, Abrahamowicz M, Grodzicky T, et al. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum.* 2001; 44(10):2331–2337. [PubMed: 11665973]
3. Asanuma Y, Oeser A, Shintani AK, et al. Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *N Eng J Med.* 2003; 349(25):2407–2415.
4. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997; 40(9):1725. [PubMed: 9324032]
5. Asanuma Y, Oeser A, Shintani AK, et al. Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *N Engl J Med.* 2003; 349(25):2407–2415. [PubMed: 14681506]
6. Gadani SP, Cronk JC, Norris GT, Kipnis J. IL-4 in the Brain: A Cytokine To Remember. *J Immunol.* 2012; 189(9):4213–4219. [PubMed: 23087426]
7. Vignali DA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. *Nat Immunol.* 2012; 13(8):722–728. [PubMed: 22814351]

8. Baum PR, Gayle RB III, Ramsdell F, et al. Molecular characterization of murine and human OX40/OX40 ligand systems: identification of a human OX40 ligand as the HTLV-1-regulated protein gp34. *EMBO J.* 1994; 13(17):3992–4001. [PubMed: 8076595]
9. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature.* 1990; 347(6294):669–671. [PubMed: 1699135]
10. Worbs T, Förster R. A key role for CCR7 in establishing central and peripheral tolerance. *Trends in Immunology.* 2007; 28(6):274–280. [PubMed: 17462953]
11. Mangino M, Braund P, Singh R, et al. Association analysis of IL-12B and IL-23R polymorphisms in myocardial infarction. *J Mol Med.* 2008; 86(1):99–103. [PubMed: 17901940]
12. Maahs DM, Ogden LG, Kinney GL, et al. Low Plasma Adiponectin Levels Predict Progression of Coronary Artery Calcification. *Circulation.* 2005; 111(6):747–753. [PubMed: 15699257]
13. Jamaluddin MS, Weakley SM, Yao Q, Chen C. Resistin: functional roles and therapeutic considerations for cardiovascular disease. *Br J Pharmacol.* 2012; 165(3):622–632. [PubMed: 21545576]
14. Baker JF, Morales M, Qatanani M, et al. Resistin levels in lupus and associations with disease-specific measures, insulin resistance, and coronary calcification. *J Rheumatol.* 2011; 38(11):2369–2375. [PubMed: 21885493]
15. Chung CP, Long AG, Solus JF, et al. Adipocytokines in systemic lupus erythematosus: relationship to inflammation, insulin resistance and coronary atherosclerosis. *Lupus.* 2009; 18(9):799–806. [PubMed: 19578104]
16. Rettenmier CW, Roussel MF, Sherr CJ. The colony-stimulating factor 1 (CSF-1) receptor (c-fms proto-oncogene product) and its ligand. *J Cell Sci Suppl.* 1988; 9:27–44. [PubMed: 2978516]
17. Menke J, Iwata Y, Rabacal WA, Basu R, Stanley ER, Kelley VR. Distinct Roles of CSF-1 Isoforms in Lupus Nephritis. *J Am Soc Nephrol.* 2011; 22(10):1821–1833. [PubMed: 21885670]
18. Yang PT, Xiao WG, Zhao LJ, et al. Increase in the level of macrophage colony-stimulating factor in patients with systemic lupus erythematosus. *Ann Rheum Dis.* 2008; 67(3):429–430. [PubMed: 18292107]
19. Irvine KM, Andrews MR, Fernandez-Rojo MA, et al. Colony-stimulating factor-1 (CSF-1) delivers a proatherogenic signal to human macrophages. *Journal of Leukocyte Biology.* 2009; 85(2):278–288. [PubMed: 19004987]
20. Ley K, Huo Y. VCAM-1 is critical in atherosclerosis. *J Clin Invest.* 2001; 107(10):1209–1210. [PubMed: 11375406]
21. Rho YH, Chung CP, Oeser A, et al. Novel cardiovascular risk factors in premature coronary atherosclerosis associated with systemic lupus erythematosus. *J Rheumatol.* 2008; 35(9):1789–1794. [PubMed: 18634156]
22. Burn GL, Svensson L, Sanchez-Blanco C, Saini M, Cope AP. Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? *FEBS Letters.* 2011; 585(23):3689–3698. [PubMed: 21515266]
23. Pertovaara M, Raitala A, Juonala M, et al. Autoimmunity and atherosclerosis: functional polymorphism of PTPN22 is associated with phenotypes related to the risk of atherosclerosis. The Cardiovascular Risk in Young Finns Study. *Clinical & Experimental Immunology.* 2007; 147(2):265–269. [PubMed: 17223967]
24. Prins BP, Lagou V, Asselbergs FW, Snieder H, Fu J. Genetics of coronary artery disease: Genome-wide association studies and beyond. *Atherosclerosis.* 2012; 225(1):1–10. [PubMed: 22698794]
25. Monticeli O, Mucenic T, Xavier R, Brenol J, Chies JA. The role of mannose-binding lectin in systemic lupus erythematosus. *Clin Rheumatol.* 2008; 27(4):413–419. [PubMed: 18214570]
26. Ohlenschlaeger T, Garred P, Madsen HO, Jacobsen S. Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. *N Engl J Med.* 2004; 351(3):260–267. [PubMed: 15254284]
27. Troelsen LN, Garred P, Christiansen B, Torp-Pedersen C, Jacobsen S. Genetically Determined Serum Levels of Mannose-Binding Lectin Correlate Negatively with Common Carotid Intima-Media Thickness in Systemic Lupus Erythematosus. *The Journal of Rheumatology.* 2010; 37(9):1815–1821. [PubMed: 20595266]

28. Jonsen A, Gullstrand B, Güner N, et al. Genetically determined mannan-binding lectin deficiency is of minor importance in determining susceptibility to severe infections and vascular organ damage in systemic lupus erythematosus. *Lupus*. 2007; 16(4):245–253. [PubMed: 17439930]
29. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy RS, Schwartz RS. Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area. A histopathologic correlative study. *Circulation*. 1995; 92(8):2157–2162. [PubMed: 7554196]

Figure.
Genetic variation and coronary atherosclerosis in patients with systemic lupus erythematosus

Table 1

Genetic Association with Coronary Atherosclerosis in Patients with SLE

Gene	Gene product	SNP	Minor allele	Minor allele frequency [#]	Odds ratio* (95% CI)	p-value
<i>ADAM33</i>	desintegrin and metalloproteinase domain-containing protein-33	rs2787094	C	0.26	2.43 (1.09–5.45)	0.03
<i>ADIPOQ</i>	adiponectin	rs7649121	T	0.16	0.05 (0.01–0.30)	0.001
		rs182052	A	0.34	2.36 (1.11–4.99)	0.03
		rs2107538	T	0.25	0.50 (0.25–0.98)	0.04
<i>CCL5</i>	chemokine (C-C motif) ligand-5	rs2023906	G	0.22	0.30 (0.10–0.92)	0.03
<i>CCR7</i>	chemokine (C-C motif) receptor-7	rs1333049	C	0.42	1.91 (1.01–3.61)	0.047
<i>CDKN2B</i>	cyclin-dependent kinase-4 inhibitor-B	rs333947	A	0.13	4.71 (1.93–11.53)	0.001
<i>CSF1</i>	macrophage colony-stimulating factor-1	rs333970	A	0.49	0.39 (0.20–0.77)	0.006
		rs333968	T	0.18	0.41 (0.19–0.88)	0.02
		rs3093037	T	0.16	0.21 (0.06–0.81)	0.02
		rs2243131	C	0.24	0.42 (0.20–0.87)	0.02
<i>IL12A</i>	interleukin 12- α	rs583911	G	0.40	2.17 (1.04–4.52)	0.04
<i>IL23R</i>	interleukin-23 receptor	rs11209026	A	0.05	9.38 (1.21–72.55)	0.03
<i>IL4</i>	interleukin-4	rs2243248	G	0.09	2.90 (1.02–8.21)	0.045
<i>INS</i>	insulin	rs3842748	G	0.29	0.35 (0.15–0.80)	0.01
		rs2070762	G	0.46	2.43 (1.18–4.98)	0.02
<i>IRF5</i>	interferon regulatory factor 5	rs1874328	G	0.41	0.38 (0.17–0.83)	0.02
		rs10954213	G	0.39	2.12 (1.07–4.20)	0.03
<i>MIF</i>	macrophage migration inhibitory factor	rs875643	A	0.42	0.36 (0.17–0.74)	0.005
		rs738806	G	0.27	3.24 (1.41–7.45)	0.006
<i>MS4A1</i>	membrane-spanning 4 domains, subfamily-A member-1	rs2000636	A	0.37	0.48 (0.25–0.92)	0.03
<i>PTGS1</i>	prostaglandin-endoperoxide synthase-1	rs1236913	T	0.05	0.31 (0.10–0.98)	0.045
<i>PTPN22</i>	protein tyrosine phosphatase, non-receptor type-22	rs2476599	A	0.33	0.40 (0.19–0.83)	0.01
		rs1217414	A	0.34	0.46 (0.22–0.93)	0.03
<i>RETN</i>	resistin	rs1862513	G	0.33	2.94 (1.47–5.93)	0.002
		rs34124816	C	0.05	6.69 (1.51–29.72)	0.01

Gene	Gene product	SNP	Minor allele	Minor allele frequency [#]	Odds ratio [*] (95% CI)	p-value
		rs3745369	C	0.47	2.49 (1.20–5.15)	0.01
<i>SELE</i>	e-selectin	rs5355	A	0.04	0.17 (0.03–0.89)	0.04
<i>TNFRSF11B</i>	osteoprotegerin	rs2073617	G	0.42	0.48 (0.23–0.99)	0.047
<i>TNFSF4</i>	tumor necrosis factor ligand superfamily member 4	rs3850641	G	0.15	3.15 (1.28–7.78)	0.01
		rs1234314	G	0.39	2.56 (1.22–5.35)	0.01
		rs1234315	C	0.45	0.42 (0.20–0.88)	0.02
<i>VCAM1</i>	vascular cell adhesion molecule-1	rs3917009	T	0.07	3.75 (1.53–9.22)	0.004

^{*} Odds ratios for the comparison between minor and major alleles

[#] Observed