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Genetically Determined Amerindian Ancestry Correlates with Increased Frequency of Risk Alleles for Systemic Lupus Erythematosus

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Abstract

Objectives—To analyze if genetically determined Amerindian ancestry predicts the increased presence of risk alleles of known susceptibility genes for systemic lupus erythematosus.

Methods—Single nucleotide polymorphisms within 16 confirmed genetic susceptibility loci for SLE were genotyped in a set of 804 Mestizo lupus patients and 667 Mestizo normal healthy controls. In addition, 347 admixture informative markers were genotyped. Individual ancestry proportions were determined using STRUCTURE. Association analysis was performed using PLINK, and correlation of the presence of risk alleles with ancestry was done using linear regression.

Results—A meta-analysis of the genetic association of the 16 SNPs across populations showed that *TNFSF4*, *STAT4*, *PDCD1*, *ITGAM*, and *IRF5* were associated with lupus in a Hispanic-Mestizo cohort enriched for European and Amerindian ancestry. In addition, two SNPs within the MHC region, previously associated in a genome-wide association study in Europeans, were also associated in Mestizos. Using linear regression we predict an average increase of 2.34 risk alleles when comparing a lupus patient with 100% Amerindian ancestry to an SLE patient with 0% American Indian Ancestry ($p < 0.0001$). SLE patients with 43% more Amerindian ancestry are predicted to carry one additional risk allele.

Conclusion—Amerindian ancestry increased the number of risk alleles for lupus.

Introduction

Differences in the prevalence and severity of systemic lupus erythematosus (SLE) between various ethnicities are well documented. In particular, individuals of self-reported Hispanic (or Mestizo), Asian or of African ancestry in the United States and Europe have been shown to have an earlier age of onset, a higher frequency of severe renal disease and a higher frequency of relapses than individuals of European ancestry (1–8). While socioeconomic factors do play a role in the increased morbidity and mortality of Hispanic individuals, it has never been analyzed if the presence of genetically defined ancestry does correlate with an increased frequency of risk alleles for lupus. We have previously shown that the increased proportion of Amerindian genome increases the risk for SLE (9). This observation has been confirmed (10). Further, we also described the strong genetic association between *IRF5* and SLE in Mexican individuals combined with an increased frequency of homozygosity for the risk haplotype (11).

In the present work we have analyzed 804 Mestizo individuals with lupus for genetic association with polymorphisms within 16 confirmed SLE susceptibility loci (12–31) and queried if the frequency of risk alleles correlates with a higher proportion of genetically determined Amerindian ancestry defined using a set of admixture informative markers.

Herein, we describe that Amerindian ancestry increases the odds of having more lupus risk alleles as compared to European ancestry in Mestizo lupus patients.

Materials and Methods

Cases and controls

A total of 804 patients with SLE and 667 healthy controls were from two main sources: one source was the OMRF collection from the Lupus Family Registry and Repository (LFRR; <http://lupus.omrf.org>) comprising 373 cases with SLE and 272 controls. The great majority of these individuals are of Mexican ancestry born or living in the United States. The second source were cases recruited within a multicenter collaboration from Argentina: 242 cases and 240 controls that have been previously reported and were used in the analysis of genetic associations described previously for *STAT4* (12), *IRF5* (13), *BANK1* (19), and *TNFSF4* (20). The remaining of samples are individuals reported here for the first time from an ongoing collection of SLE patients from Latin America known as GENLES. These comprise 101 SLE cases and 64 controls collected throughout Mexico (specifically from the cities of Guadalajara, Morelia, Culiacán and Mexico City) and 88 cases and 91 controls from the city of Lima, Peru, in South America.

All cases fulfilled the American College of Rheumatology classification criteria for lupus in its latest version (32).

Genotyping

Genotyping was performed using the Illumina Custom Bead system on the iSCAN instrument. Genotypes for the following SNPs within 16 confirmed susceptibility genes for SLE were used: rs2476601 (*PTPN22*), rs1801274 (*FCGR2A*), rs2205960 (*TNFSF4*), rs7574865 (*STAT4*), rs231775 (*CTLA4*), rs11568821 (*PDCD1*), rs6445975 (*PXK*), rs10516487 (*BANK1*), rs907715 (*IL21*), rs3131379 (*MSH5* within the MHC class III region), rs1270942 (*CFB*, within the MHC class III), rs2070197 (*IRF5*), rs13277113 (*C8ORF13-BLK* region), rs1800450 (*MBL2*), rs4963128 (*KIAA1542*) and rs1143679 (*ITGAM*) (12–31).

In addition, 347 admixture informative markers (AIMs) were also used to genotype all individuals (Supplementary table 1) (33–35). We have selected a panel of AIMs that had large frequency differences between European populations and Amerindian populations. In addition, the inter-marker distance between two adjacent AIMs was at least 1Mb to ensure that the AIMs were not in linkage disequilibrium in the parental populations.

Population structure determination—Population structure was analyzed with STRUCTURE v2.3.1 (36), which implements a model-based clustering method for inferring population substructure using AIMs. We set most of parameters to their default values as advised in the user's manual. Specifically, we chose the admixture model and the option of correlated allele frequencies between populations as suggested by Falush et al. (36). The range of possible populations we tested was from $K=3$ to 5 as described (37). The best fitting K was 4, as a mixture of four populations: African, European, Asiatic and Amerindian. We selected genotypes from European (CEU), Amerindian (MEX), Asiatic (CHB) and African (YRI) individuals from HapMap version 3 dataset as potential ancestral populations (38). Outliers were excluded when they showed more than 10% African or Asian ancestry, in order to enrich for two ancestral populations, European and Amerindian. Among the samples 45 individuals were excluded from further analyses.

Principal Component Analysis—To account for confounding population substructure or admixture in the studied population we used principal component analysis (PCA) (39–42) as implemented in HelixTree using genotype data from the 347 AIMs. The first three PCs explained 71.7% of the variance among the first 10 PCs and had the following eigenvalues

42.1, 21.3 and 8.3. The eigenvalues for PC4-PC10 showed a plateau, suggesting that the first three PCs account for most of the populations' substructure in this analysis. All individuals who were not clustering with the main Amerindian cluster (deviation of more than 4 SD from cluster centroids) were excluded from subsequent analysis. Using this method we identified 23 outlier individuals (15 healthy controls and 8 SLE patients).

Statistical Genetic Analysis

The genetic association analysis was done using PLINK v1.0.7 (43). First quality control filters were applied to remove SNPs with differential missing rate between cases and controls ($P < 0.05$), significant deviation from Hardy-Weinberg equilibrium in controls ($P < 0.001$) or a minor allele frequency $< 1\%$. Allele frequencies of the remaining SNPs (16 of 16) were tested for significant association by a χ^2 test within each study population. The Meta-analysis of the all populations was conducted using standard methods based on Cochran-Mantel-Haenszel test (44). The Breslow-Day test (45) was performed for all SNPs to assess heterogeneity of the odds ratios in different populations. The pooled OR was calculated according to a fixed-effects model (Mantel-Haenszel meta-analysis) for SNPs with homogeneity between populations as well as random effects model (DerSimonian-Laird) when heterogeneity was present, using the StatsDirect v2.4.6 software.

Alternatively, we also derived principal components on a population-specific basis using HelixTree software v7.2.3, and applied an adjustment for the five first principal components.

Regression analysis—We used linear regression to model the relationship between the proportion of Amerindian ancestry and the number of SLE risk alleles. Our initial model included the proportion of Amerindian ancestry, gender, and the interaction between gender and Amerindian ancestry as predictor variables for the number of SLE risk alleles. There was no evidence of interaction, so we refit the model with the two remaining predictor variables. Since we were interested in the association between the number of risk alleles and the proportion of Amerindian ancestry, we removed gender from the model as neither predictor variables were significant while both were fit. Our final model included the proportion of Amerindian ancestry as a predictor for the number of SLE risk alleles. All linear modeling assumptions were assessed and met.

Results

Population structure analyses showed the following mean proportions of Amerindian ancestry for each of the sets included (Table 1): Amerindian ancestry was 30.7% for OMRF Hispanics, 24.7% for Argentine, in agreement with what we had described previously (46), 52.3% for Mexicans and 72.6% for Peruvians. OMRF Hispanics differed from the Latin American set by having higher proportions of North European ancestry, suggesting that some of these samples may include second or third generation Mexican-Americans where inclusion of the European-American genetic pool, mainly of North European ancestry has occurred. On the other hand, Latin American sets had clearly a South European proportion, as expected by the known history of these populations (Table 1).

For individual ancestry proportions, there were no differences between cases and controls in the four clusters. In addition, we did not observe any differences after comparing the clusters with and without population priors.

Using all cases and controls we first determined the genetic association between all Hispanic cases and controls and SLE with the 16 SNPs. Association was observed for *TNFSF4*, *STAT4*, *IRF5*, *MSH5*, *CFB*, and *ITGAM* and a trend of association was observed for *PDCD1* (Table 2). The SNPs for *C8orf13-BLK*, *BANK1* and *PXK* showed a significant degree of

heterogeneity ($P < 0.0001$; $P = 0.023$; and $P = 0.001$, respectively) across the different country sets and this could have contributed to the fact that the final meta-analysis did not provide a genetic association for these genetic variants. This is particularly true for the *C8orf13-BLK* SNP, however might not explain the results for *BANK1* and *PXK*, where it could relate to insufficient power for detection of the genetic association.

We have previously shown that Amerindian ancestry increases the risk for lupus (9), and this was later confirmed (10). Therefore we analyzed if the individual proportion of Amerindian ancestry had any effect on the number of risk alleles.

Linear regression (Figure 1) showed that on average, we predict a 2.34 increase of risk alleles of a subject with 100% Amerindian ancestry as compared to a subject with 0% of such ancestry. In other words, an individual with 43% more Amerindian ancestry will on average have one additional risk allele.

Discussion

It has been consistently shown that individuals of Mestizo (Hispanic) descent have a more severe clinical lupus disease accompanied with earlier age of onset and severe renal disease. Mestizos are a very heterogeneous group of individuals with different cultural backgrounds but in general a common mother tongue, Spanish. The complexity of the Mestizo population does not allow for appropriate genetics studies unless such complexity is taken into consideration (1). With the aim to investigate if genes identified in lupus in Europeans also play a role in the disease in Mestizos, we have selected a group from Latin American countries with an enrichment of Amerindian and European ancestries based on population history and a set of Hispanics from the United States primarily originating from Mexico.

In general, the populations of Mexico, Peru and Argentina have lower African ancestry and primarily European and Amerindian ancestry. Our collection also includes samples from Southern Europe (Spain and Portugal) as reference, so we were able to discern between North and South Europeans. In this regard, OMRF Hispanics showed a high proportion of North European ancestry, in line with recent inclusion of European-American gene pool.

Testing of the 16 SNPs representing risk variants of lupus susceptibility genes described in Europeans, we confirmed the genetic association previously found in *IRF5*, *STAT4*, *TNFSF4*, *ITGAM* and to a lesser degree the two SNPs within the MHC region and *PDCD1*. Interestingly, the two SNPs used here for the MHC were the same included in the GWAS that detected the highest genetic association in Europeans. Here, the genetic associations of the non-MHC variants was stronger than for the MHC, suggesting two possibilities: either the MHC effect originates from the European admixture in the Amerindian background and it is “diluted” and/or, other Amerindian genes play a very important role in disease susceptibility in Hispanics and in some way substitute for the strong effect that the MHC has in Europeans. However these do not tag MHC haplotypes and cannot be seen as representing the main effect on the MHC region in this population. For this, dense coverage of the region would be required. Such studies are underway. We are at present performing a GWAS in Hispanic-Mestizo individuals to answer this question. Of the remaining genetic association it is important to point out that this replication is not completely independent: the Argentine samples have been used previously in our work on *BANK1*, *IRF5*, *TNFSF4* and *STAT4* (12,13,19,20). In fact, our previous work (9) showed an increased frequency of Amerindian genome in patients with SLE in this same set of Argentine patients. Here we observe a very similar average in the proportion of Amerindian genome between cases and controls, but we also have included new samples. The previous work used a completely different set of

AIMS, although the number was smaller. At this point we are unable to explain the reason for this controversy.

As the individual sets of Mexican and Peruvian samples are new but each is relatively small, the associations were not discernible at the individual cohort level. The Peru sample showed a weak association for *FCGR2A* ($P = 0.02$), *IRF5* ($P = 0.004$) and *ITGAM* ($P = 0.01$), while the Mexico set showed association with *BANK1* ($P = 0.0002$) and *ITGAM* ($P = 0.001$). Most of the contribution to the genetic associations observed in the meta-analysis was provided by the Argentine and the OMRF Hispanic sets.

PDCDI deserves further discussion. We identified *PDCDI* as a susceptibility gene for lupus after linkage analysis in Icelandic and Swedish multiplex families and we described a polymorphism in intron 4 associated with SLE with a replication in European-Americans, Swedish and Mexican cases with SLE (31). A second independent report replicated this genetic association in Mexican pediatric SLE patients (47) and we recently described a correlation between surface levels of *PDCDI* protein (PD-1) in CD4+CD25+ T cells and the associated variants (known as PD1.3) (48). Here, the association was only observed in the Argentine SLE cases and controls ($P = 0.013$), a set never before analyzed for this polymorphism. Importantly, and possibly affecting our results is the fact that the Argentine set is the most European and is possibly also why the association is detectable in that set. Finally, no association was observed for *CTLA4*, *IL21*, *MBL2* and *KIAA1542*, while *BLK* showed, as mentioned, extensive heterogeneity. The negative results of the meta-analysis for *BLK* should be viewed with caution.

What is the significance of the increased risk for individuals with Amerindian genome to carry risk alleles of lupus susceptibility genes identified in Europeans? First, it is possible that in Hispanics/Mestizos, the “European” risk alleles interact with genes important in the Amerindian background. This is somewhat reminiscent of what happens in the New Zealand mouse strains, where the New Zealand White background interacts with genes found in the New Zealand Black background leading to a strong and florid lupus-like disease in the resultant F1 strain (49,50). To some degree Mestizo individuals from Latin America behave as a sort of genetic F1 where unknown genetic interactions might occur leading to an increased risk to develop severe SLE in the admixed population. On the other hand, our results might also be explained by an enrichment of European risk alleles due to positive selection.

From the data presented here we can suggest that the admixture may in part be responsible for the increased susceptibility for the disease and that the Amerindian background genome contributes to this increased risk. The identification of genes of Amerindian origin contributing to the increased risk for the disease is clearly justified.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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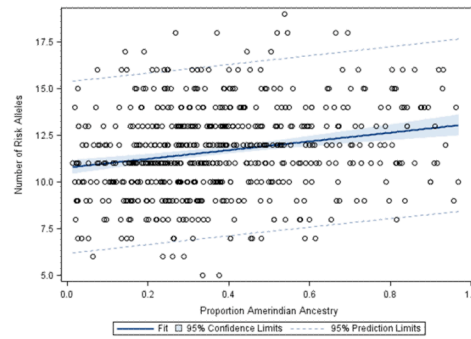


Figure 1. Scatter plot of the input data overlaid with the fitted regression line, 95% confidence limits, and 95% prediction limits. The 95% confidence limits in the plot are point-wise limits that cover the mean number of risk alleles for a particular proportion of Amerindian ancestry with probability 0.95. The 95% prediction limits illustrate the point-wise limits for a future measurement of risk alleles for a given proportion of Amerindian ancestry with probability 0.95.

Table 1

Average Ancestry Proportions of the Population Sets Used

Population	Nr. Individuals	Amerindian	South European	North European	African
Yoruba (HapMap3)	167	0.001	0.000	0.001	0.998
CEU (HapMap3)	165	0.003	0.003	0.994	0.000
Spain	1062	0.013	0.868	0.114	0.004
Portugal	386	0.008	0.863	0.126	0.003
Mexico	165	0.529	0.353	0.096	0.022
Peru	179	0.726	0.190	0.052	0.032
Argentina	482	0.247	0.645	0.100	0.008
OMRF Hispanic	645	0.307	0.454	0.153	0.085

Table 2
 Meta-analysis of the Genetic Association of 16 risk gene polymorphisms in Hispanics

Gene	SNP	AA	%	Aa	%	aa	%	Allele A	%	Allele a	%	P value	OR	95% CI
<i>PTPN22</i>	rs2476601 (C>T)	SLE (n= 794)	712	89.7	81	10.2	1	0.1	1595	94.8	83	5.2		
		Controls (n= 648)	596	91.8	49	7.6	3	0.6	1241	95.6	55	4.4	0.2832	1.233
<i>FCGR2A</i>	rs1801274 (C>T)	SLE (n= 767)	184	25.8	385	50.2	198	24	781	51	753	49		
		Controls (n= 640)	178	22.7	317	49.5	145	27.8	607	47.4	673	52.6	0.1182	0.885
<i>TNFSF4</i>	rs2205960 (G>T)	SLE (n= 794)	310	39	381	48	103	13	1001	63	587	37		
		Controls (n= 649)	329	50.7	265	40.8	55	8.5	923	71	375	28.9	1.65E-06	1.488
<i>STAT4</i>	rs7574865 (G>T)	SLE (n= 727)	268	36.9	350	48.1	109	15	886	61	568	39		
		Controls (n= 595)	255	42.9	276	46.4	64	10.8	786	66.1	404	33.9	5.81E-05	1.41
<i>CTLA4</i>	rs231775 (G>A)	SLE (n= 783)	294	37.5	364	46.5	125	16	952	60.8	614	39.2		
		Controls (n= 640)	246	38.4	300	46.9	94	14.7	792	62	488	38	0.7882	0.976
<i>PDCDI</i>	rs11568821 (G>A)	SLE (n= 778)	671	86.2	102	13.1	5	0.6	1444	92.8	112	7.2		
		Controls (n= 636)	529	83.2	99	15.6	8	1.3	1157	91	115	9	0.0571	0.758
<i>PXK</i>	rs6445975 (T>G)	SLE (n= 785)	332	42.3	350	44.6	103	13.1	1114	64.6	556	35.4		
		Controls (n= 647)	290	44.8	280	43.3	77	11.9	860	66.5	434	33.5	0.622	1.077
<i>BANK1</i>	rs10516487 (G>T)	SLE (n= 753)	536	71.2	190	25.2	27	3.6	1262	83.8	244	16.2		
		Controls (n= 612)	402	65.7	179	29.2	31	5.1	983	80.3	241	19.7	0.194	0.711
<i>IL21</i>	rs907715 (G>A)	SLE (n= 781)	353	45.2	345	44.2	83	10.6	1051	67.3	511	32.7		
		Controls (n= 635)	307	48.3	267	42	16	9.6	881	69.4	389	30.6	0.2298	1.107
<i>MSH5</i>	rs3131379 (C>T)	SLE (n= 796)	692	86.9	102	12.8	2	0.3	1486	93.3	106	6.7		
		Controls (n= 651)	602	92.5	48	7.4	1	0.2	1252	96.2	50	3.8	0.0013	1.773
<i>CFB</i>	rs1270942 (T>C)	SLE (n= 796)	698	87.7	96	12.1	2	0.3	1492	93.7	100	6.3		
		Controls (n= 652)	608	93.3	43	6.6	1	0.2	1259	96.5	45	3.5	0.0007	1.881
<i>IRF5</i>	rs2070197 (T>C)	SLE (n= 768)	507	66	233	30.3	28	3.6	1247	81.2	289	18.8		

Gene	SNP	AA	%	Aa	%	aa	%	Allele A	%	Allele a	%	P value	OR	95% CI	
<i>C8orf-BLK</i>	rs13277113 (G>A)	Controls (n= 536)	421	78,5	104	19,4	11	2,1	946	88,2	126	11,8	1,65E-09	2,058	1,632-2,595
		SLE (n=753)	232	31	362	48,3	155	20,7	826	55,1	672	44,9			
		Controls (n=611)	252	41,2	262	42,9	97	15,9	766	62,7	456	37,3	0,3869	1,228	0,771-1,955
<i>MBL2</i>	rs1800450 (G>A)	SLE (n= 793)	510	64,3	253	31,9	30	3,8	1273	80,3	313	19,7			
		Controls (n= 648)	424	65,4	195	30,1	29	4,5	1043	80,5	253	19,5	0,5831	1,058	0,878-1,276
<i>KIAA1542</i>	rs4963128 (G>A)	SLE (n= 762)	375	49,2	311	40,8	76	10	1061	69,6	463	30,4			
		Controls (n= 632)	358	51,7	280	40,4	55	7,9	996	72	390	28	0,8761	0,983	0,835-1,157
<i>ITGAM</i>	rs1143679 (G>A)	SLE (n= 795)	538	67,7	234	29,4	23	2,9	1310	82,4	280	17,6			
		Controls (n= 650)	541	83,2	102	15,7	7	1,1	1184	91,1	116	8,9	6,22E-11	2,232	1,767-2,818