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

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Objective. **To assess whether genetically determined Amerindian ancestry predicts increased presence of risk alleles of known susceptibility genes for systemic lupus erythematosus (SLE).**

Methods. **Single-nucleotide polymorphisms (SNPs) within 16 confirmed genetic susceptibility loci for SLE were genotyped in a set of 804 Mestizo lupus patients and 667 Mestizo 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 between ancestry and the presence of risk alleles was analyzed using linear regression.**

Results. **A meta-analysis of the genetic association of the 16 SNPs across populations showed that *TNFSF4*, *STAT4*, *ITGAM*, and *IRF5* were associated with lupus in a Hispanic Mestizo cohort enriched for European and Amerindian ancestry. In addition, 2 SNPs**

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within the major histocompatibility complex region, previously shown to be associated in a genome-wide association study in Europeans, were also associated in Mestizos. Using linear regression, we predicted an average increase of 2.34 risk alleles when comparing an SLE patient with 100% Amerindian ancestry versus an SLE patient with 0% Amerindian ancestry ($P < 0.0001$). SLE patients with 43% more Amerindian ancestry were predicted to carry 1 additional risk allele.

Conclusion. **Our results demonstrate that Amerindian ancestry is associated with an increased number of risk alleles for SLE.**

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 African ancestry in the US and Europe have been shown to have an earlier age at onset of SLE, a higher frequency of severe SLE-associated renal disease, and a higher frequency of relapses of SLE than individuals of European ancestry (1–8). While socioeconomic factors play a role in the increased morbidity and mortality among Hispanic individuals, the question of whether the presence of genetically defined ancestry correlates with an increased frequency of risk alleles for lupus has never been analyzed. We have previously shown that an increased proportion of Amerindian genome increases the risk for SLE (9), and this observation was confirmed in another study (10). Further, a strong genetic association between *IRF5* and SLE in Mexican individuals, combined with an increased frequency of homozygosity for the risk haplotype, has been reported (11).

In the present work we analyzed 804 Mestizo individuals with lupus for genetic association with polymorphisms within 16 confirmed SLE susceptibility loci (12–31) and investigated whether the frequency of risk alleles correlates with a higher proportion of genetically determined Amerindian ancestry as defined using a set of admixture informative markers. We found that, in Mestizo SLE patients, Amerindian ancestry increases the odds of having more lupus risk alleles as compared with European ancestry.

PATIENTS AND METHODS

Cases and controls. A total of 804 patients with SLE and 667 healthy controls were studied. Three hundred seventy-three of the SLE cases and 272 of the controls were from the Lupus Family Registry and Repository at Oklahoma Medical Research Foundation (OMRF) (http://lupus.omrf.org). The great majority of these individuals are of Mexican ancestry and were born in and/or living in the US. Two hundred forty-two SLE cases and 240 controls were from a multicenter collaboration in Argentina (the Argentine Lupus Collaboration [Appendix A]); these subjects have been previously reported and were used in analyses of genetic associations for *STAT4* (12), *IRF5* (13), *BANK1* (19), and *TNFSF4* (20). The remaining subjects are individuals reported here for the first time, from the Latin American Collaboration on Lupus, which is enrolling and studying SLE patients from Latin America on an ongoing basis. These subjects comprise 101 SLE cases and 64 controls from throughout Mexico (specifically, from the cities of Guadalajara, Morelia, Culiacan, and Mexico City) and 88 cases and 91 controls from Lima, Peru. All cases fulfilled the American College of Rheumatology classification criteria for SLE (32).

Genotyping. Genotyping was performed using the Illumina Custom Bead system on an iSCAN instrument. Genotypes for the following single-nucleotide polymorphisms (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 class III major histocompatibility complex [MHC] region), rs1270942 (*CFB*, within the class III MHC region), rs2070197 (*IRF5*), rs13277113 (*C8ORF13-BLK* region), rs1800450 (*MBL2*), rs4963128 (*KIAA1542*), and rs1143679 (*ITGAM*) (12–31).

In addition, 347 admixture informative markers were used to genotype all individuals (33–35) (see Supplementary Table 1, available in the online version of this article at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). We selected a panel of admixture informative markers that had large frequency differences between European populations and Amerindian populations. In addition, the inter-marker distance between 2 adjacent admixture informative markers was at least 1 Mb, to ensure that the admixture informative markers were not in linkage disequilibrium in the parental populations.

Population structure determination. Population structure was analyzed with STRUCTURE, version 2.3.1 (36), which implements a model-based clustering method for inferring population substructure using admixture informative markers. We set most of the 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 K 3–5, as described (35). The best-fitting K was 4, as a mixture of 4 populations: African, European, Asian, and Amerindian.

We selected genotypes from European, Amerindian, Asian, and African individuals in the HapMap version 3 data set (37) as potential ancestral populations. Subjects were excluded if they showed >10% African or Asian ancestry, in order to enrich for 2 ancestral populations, European and Amerindian. Among the samples, 45 individuals were excluded from further analyses.

Principal components analysis. To account for confounding population substructure or admixture in the studied population, we used principal components analysis (38–41) as implemented in HelixTree, using genotype data from the 347 admixture informative markers. The first 3 principal compo-

Table 1. Average ancestry proportions of the population sets studied

Population	No. of individuals	Amerindian	South European	North European	African
Yoruba (YRI; HapMap version 3)	167	0.001	0.000	0.001	0.998
European (CEU; HapMap version 3)	165	0.003	0.003	0.994	0.000
Spain	1,062	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

* OMRF = Oklahoma Medical Research Foundation.

nents explained 71.7% of the variance among the first 10 principal components and had eigenvalues of 42.1, 21.3, and 8.3. The eigenvalues for principal components 4–10 showed a plateau, suggesting that the first 3 principal components accounted for most of the populations' substructure in this analysis. All individuals who were not clustering with the main Amerindian cluster (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 performed using PLINK, version 1.0.7 (42). First, quality control filters were applied to remove SNPs with differential rates of missing data between cases and controls ($P < 0.05$), significant deviation from Hardy-Weinberg equilibrium in controls ($P < 0.001$), or a minor allele frequency of <1%. Allele frequencies of the remaining SNPs (16 of 16) were tested by chi-square test for significant association within each study population. The meta-analysis of all of the populations was conducted using standard methods based on the Cochran-Mantel-Haenszel test (43). The Breslow-Day test (44) was performed for all SNPs, to assess heterogeneity of the odds ratios in different populations. The pooled odds ratio was calculated according to a fixed-effects model (Mantel-Haenszel meta-analysis) for SNPs with homogeneity between populations, as well as a random-effects model (DerSimonian-Laird) when heterogeneity was present, using StatsDirect software, version 2.4.6. Alternatively, we also derived principal components on a population-specific basis using HelixTree, version 7.2.3, and applied an adjustment for the first 5 principal components.

Regression analysis. We used linear regression to model the relationship between the proportion Amerindian ancestry and the number of SLE risk alleles. Our initial model included proportion Amerindian ancestry, sex, and the interaction between sex 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 2 remaining predictor variables. Since we were interested in the association between the number of risk alleles and the proportion Amerindian ancestry, we removed sex from the model as neither predictor variable was significant while both were fit. Our final model included the proportion Amerindian ancestry as a predictor of 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% among OMRF Hispanics, 24.7% among Argentines (consistent with what we had described previously) (45), 52.9% among Mexicans, and 72.6% among Peruvians. OMRF Hispanics differed from the Latin American subjects from Mexico, Peru, and Argentina in that the former group had a higher proportion of North European ancestry, suggesting that some of the 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, the Latin American groups had a substantial proportion of South European ancestry (Table 1), as expected by the known history of these populations.

For individual ancestry proportions, there were no differences between cases and controls in the 4 clusters. In addition, we did not observe any differences after comparing the clusters with and without prior populations.

We first determined the genetic association with each of the 16 SLE SNPs, for the overall group of Hispanic cases and the overall group of Hispanic controls. Association was observed for *TNFSF4*, *STAT4*, *IRF5*, *MSH5*, *CFB*, and *ITGAM*, and a trend toward association was observed for *PDCD1* (Table 2). The SNPs for *C8orf13-BLK*, *BANK1*, and *PXK* showed a significant degree of heterogeneity across the different country sets ($P < 0.0001$, $P = 0.023$, and $P = 0.001$, respectively), and this could have contributed to the fact that the final meta-analysis did not show a genetic association for these variants. This is particularly true for the *C8orf13-BLK* SNP, but it might not explain the

Table 2. Meta-analysis of the genetic association of 16 risk gene polymorphisms in Hispanic subjects*

Gene/SNP	GG, no. (%)	AG, no. (%)	AA, no. (%)	Allele G, no. (%)	Allele A, no. (%)	OR (95% CI)	*P*
PTPN22/rs2476601							
SLE patients (n = 794)	712 (89.7)	81 (10.2)	1 (0.1)	1,595 (94.8)	83 (5.2)	1.233 (0.866–1.754)	0.2832
Controls (n = 648)	596 (91.8)	49 (7.6)	3 (0.6)	1,241 (95.6)	55 (4.4)		
FCGR2A/rs1801274							
SLE patients (n = 767)	184 (25.8)	385 (50.2)	198 (24)	781 (51)	753 (49)	0.885 (0.762–1.027)	0.1182
Controls (n = 640)	178 (22.7)	317 (49.5)	145 (27.8)	607 (47.4)	673 (52.6)		
TNFSF4/rs2205960							
SLE patients (n = 794)	310 (39)	381 (48)	103 (13)	1,001 (63)	587 (37)	1.488 (1.269–1.745)	1.65×10^{-6}
Controls (n = 649)	329 (50.7)	265 (40.8)	55 (8.5)	923 (71)	375 (28.9)		
STAT4/rs7574865							
SLE patients (n = 727)	268 (36.9)	350 (48.1)	109 (15)	886 (61)	568 (39)	1.41 (1.2–1.659)	5.81×10^{-5}
Controls (n = 595)	255 (42.9)	276 (46.4)	64 (10.8)	786 (66.1)	404 (33.9)		
CTLA4/rs231775							
SLE patients (n = 783)	294 (37.5)	364 (46.5)	125 (16)	952 (60.8)	614 (39.2)	0.976 (0.838–1.137)	0.7882
Controls (n = 640)	246 (38.4)	300 (46.9)	94 (14.7)	792 (62)	488 (38)		
PDCD1/rs11568821							
SLE patients (n = 778)	671 (86.2)	102 (13.1)	5 (0.6)	1,444 (92.8)	112 (7.2)	0.758 (0.576–0.997)	0.0571
Controls (n = 636)	529 (83.2)	99 (15.6)	8 (1.3)	1,157 (91)	115 (9)		
PXK/rs6445975							
SLE patients (n = 785)	332 (42.3)	350 (44.6)	103 (13.1)	1,114 (64.6)	556 (35.4)	1.077 (0.8–1.45)	0.622
Controls (n = 647)	290 (44.8)	280 (43.3)	77 (11.9)	860 (66.5)	434 (33.5)		
BANK1/rs10516487							
SLE patients (n = 753)	536 (71.2)	190 (25.2)	27 (3.6)	1,262 (83.8)	244 (16.2)	0.711 (0.425–1.189)	0.194
Controls (n = 612)	402 (65.7)	179 (29.2)	31 (5.1)	983 (80.3)	241 (19.7)		
IL21/rs907715							
SLE patients (n = 781)	353 (45.2)	345 (44.2)	83 (10.6)	1,051 (67.3)	511 (32.7)	1.107 (0.942–1.299)	0.2298
Controls (n = 635)	307 (48.3)	267 (42)	16 (19.6)	881 (69.4)	389 (30.6)		
MSH5/rs3131379							
SLE patients (n = 796)	692 (86.9)	102 (12.8)	2 (0.3)	1,486 (93.3)	106 (6.7)	1.773 (1.255–2.505)	0.0013
Controls (n = 651)	602 (92.5)	48 (7.4)	1 (0.2)	1,252 (96.2)	50 (3.8)		
CFB/rs1270942							
SLE patients (n = 796)	698 (87.7)	96 (12.1)	2 (0.3)	1,492 (93.7)	100 (6.3)	1.881 (1.311–2.698)	0.0007
Controls (n = 652)	608 (93.3)	43 (6.6)	1 (0.2)	1,259 (96.5)	45 (3.5)		
IRF5/rs2070197							
SLE patients (n = 768)	507 (66)	233 (30.3)	28 (3.6)	1,247 (81.2)	289 (18.8)	2.058 (1.632–2.595)	1.65×10^{-9}
Controls (n = 536)	421 (78.5)	104 (19.4)	11 (2.1)	946 (88.2)	126 (11.8)		
C8orf-BLK/rs13277113							
SLE patients (n = 753)	232 (31)	362 (48.3)	155 (20.7)	826 (55.1)	672 (44.9)	1.228 (0.771–1.955)	0.3869
Controls (n = 611)	252 (41.2)	262 (42.9)	97 (15.9)	766 (62.7)	456 (37.3)		
MBL2/rs1800450							
SLE patients (n = 793)	510 (64.3)	253 (31.9)	30 (3.8)	1,273 (80.3)	313 (19.7)	1.058 (0.878–1.276)	0.5831
Controls (n = 648)	424 (65.4)	195 (30.1)	29 (4.5)	1,043 (80.5)	253 (19.5)		
KIAA1542/rs4963128							
SLE patients (n = 762)	375 (49.2)	311 (40.8)	76 (10)	1,061 (69.6)	463 (30.4)	0.983 (0.835–1.157)	0.8761
Controls (n = 632)	358 (51.7)	280 (40.4)	55 (7.9)	996 (72)	390 (28)		
ITGAM/rs1143679							
SLE patients (n = 795)	538 (67.7)	234 (29.4)	23 (2.9)	1,310 (82.4)	280 (17.6)	2.232 (1.767–2.818)	6.22×10^{-11}
Controls (n = 650)	541 (83.2)	102 (15.7)	7 (1.1)	1,184 (91.1)	116 (8.9)		

* SNP = single-nucleotide polymorphism; OR = odds ratio; 95% CI = 95% confidence interval; SLE = systemic lupus erythematosus.

results for *BANK1* and *PXK*, which 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 investigated whether the proportion of Amerindian ancestry in an individual had any effect on the number of risk alleles. Linear regression (Figure 1) showed that, on average, one could predict an increase of 2.34 SLE risk alleles in a subject with 100% Amerindian ancestry as compared with a subject with 0% of such ancestry ($P < 0.0001$), and an individual with 43% more Amerindian ancestry would have, on average, 1 additional risk allele.

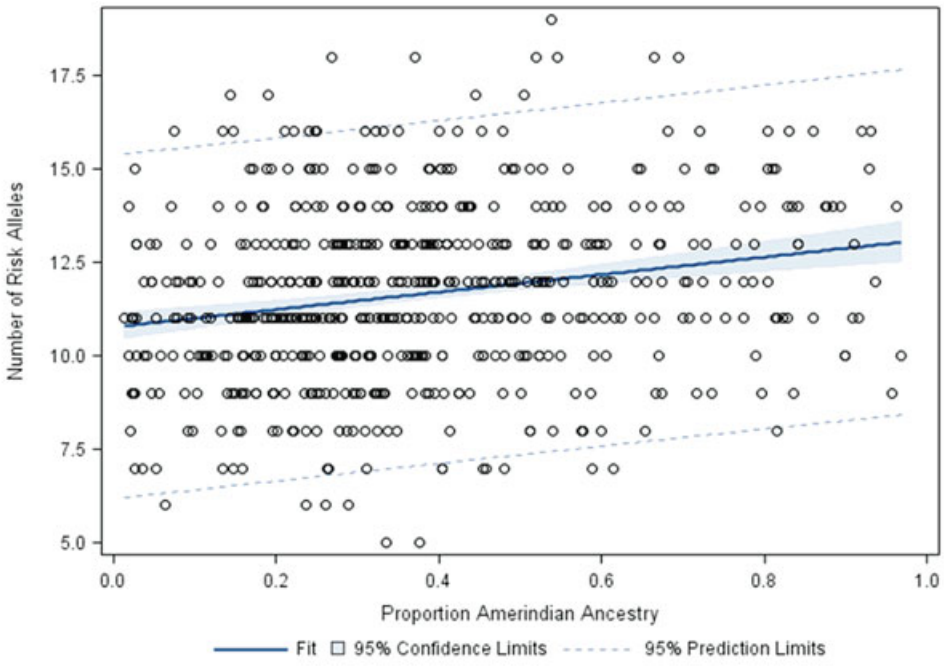

Figure 1. Scatterplot of the input data, overlaid with the fitted regression line, 95% confidence limits, and 95% prediction limits. The 95% confidence limits in the plot are pointwise limits that cover the mean number of risk alleles for a particular proportion of Amerindian ancestry with probability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

DISCUSSION

It has been consistently shown that patients of Mestizo (Hispanic) descent have more severe clinical lupus disease, severe SLE-related renal disease, and earlier age at onset. 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 of investigating whether genes identified as being related to lupus in Europeans also play a role in the disease in Mestizos, we selected a group from Latin American countries with an enrichment of Amerindian and European ancestries based on population history, and a group of Hispanic subjects from the US, primarily originating from Mexico.

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

Testing of the 16 SNPs representing risk variants of lupus susceptibility genes described in Europeans confirmed the genetic associations previously found for *IRF5*, *STAT4*, *TNFSF4*, *ITGAM*, and to a lesser degree, the 2 SNPs within the MHC region and *PDCD1*. Interestingly, the 2 SNPs used here for the MHC were the same ones included in the genome-wide association study, and in that study the highest genetic association in Europeans was detected with those genes (16). In the present study, the genetic associations of the non-MHC variants were stronger than for the MHC, suggesting two possibilities: either the MHC effect originates from the European admixture on 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 of the MHC in Europeans. However, these 2 SNPs in the MHC region 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 under way; we are at present performing a genome-wide association study in Hispanic Mestizo individuals to address this question.

With regard to the remaining genetic association it is important to point out that this replication is not completely independent: the samples from the Argentine subjects have been used previously in our work on *BANK1*, *IRF5*, *TNFSF4*, and *STAT4* (12,13,19,20). Our previous work (9) showed an increased frequency of Amerindian genome in patients with SLE in the same set of Argentine subjects, whereas in the present study we observed a very similar average proportion of Amerindian genome between cases and controls; however, we also have included new samples in the present study. The previous work used a completely different, and smaller, set of admixture informative markers. At this point, we are unable to explain the reason for the discrepancy.

Because the sets of Mexican and Peruvian samples used for the first time in this study were each relatively small, the associations were not discernible at the individual cohort level. In the Peruvian sample there was weak association with *FCGR2A* ($P = 0.02$), *IRF5* ($P = 0.004$), and *ITGAM* ($P = 0.01$), while association with *BANK1* ($P = 0.0002$) and *ITGAM* ($P = 0.001$) was shown in the Mexican set. Most of the contribution to the genetic associations observed in the meta-analysis was provided by the Argentine and the OMRF Hispanic cohorts.

PDCD1 warrants further discussion. We identified *PDCD1* 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 replication in European American, Swedish, and Mexican cases (31). A second independent study replicated this genetic association in Mexican pediatric SLE patients (46), and a correlation between surface levels of *PDCD1* protein (programmed death 1 [PD-1]) in CD4+CD25+ T cells and the associated variants (known as PD-1.3) was recently described (47). In the present study, the association was observed only in the Argentine SLE cases and controls ($P = 0.013$), a set not previously analyzed for this polymorphism. Important, and possibly affecting our results, is the fact that the Argentine set had the highest proportion of European ancestry; this may also be the reason the association was detectable in that set. Finally, no association with *CTLA4*, *IL21*, *MBL2*, or *KIAA1542* was observed, while *BLK* showed, as mentioned above, extensive heterogeneity. The negative results for *BLK* in the meta-analysis should be viewed with caution.

What is the significance of the increased risk, among individuals with Amerindian genome, of carrying risk alleles of lupus susceptibility genes identified in Europeans? First, it is possible that in Hispanics/Mestizos, the "European" risk alleles interact with genes that are important on the Amerindian background. This is somewhat reminiscent of what happens in 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 F_1 strain (48,49). In that scenario Mestizo individuals from Latin America would, to some degree, behave as a sort of genetic F_1, where unknown genetic interactions might occur, leading to an increased risk of developing 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 to SLE, and that the Amerindian background genome contributes to this increased risk. Studies to identify genes of Amerindian origin that contribute to the increased risk of the disease are clearly warranted.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Alarcón-Riquelme had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Analysis and interpretation of data. Sanchez, Webb, Rasmussen, Kaufman, Moctezuma, Cucho-Venegas, Martin, Richardson, Sawalha, Alarcón-Riquelme.

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