Candidate gene studies in rheumatoid arthritis

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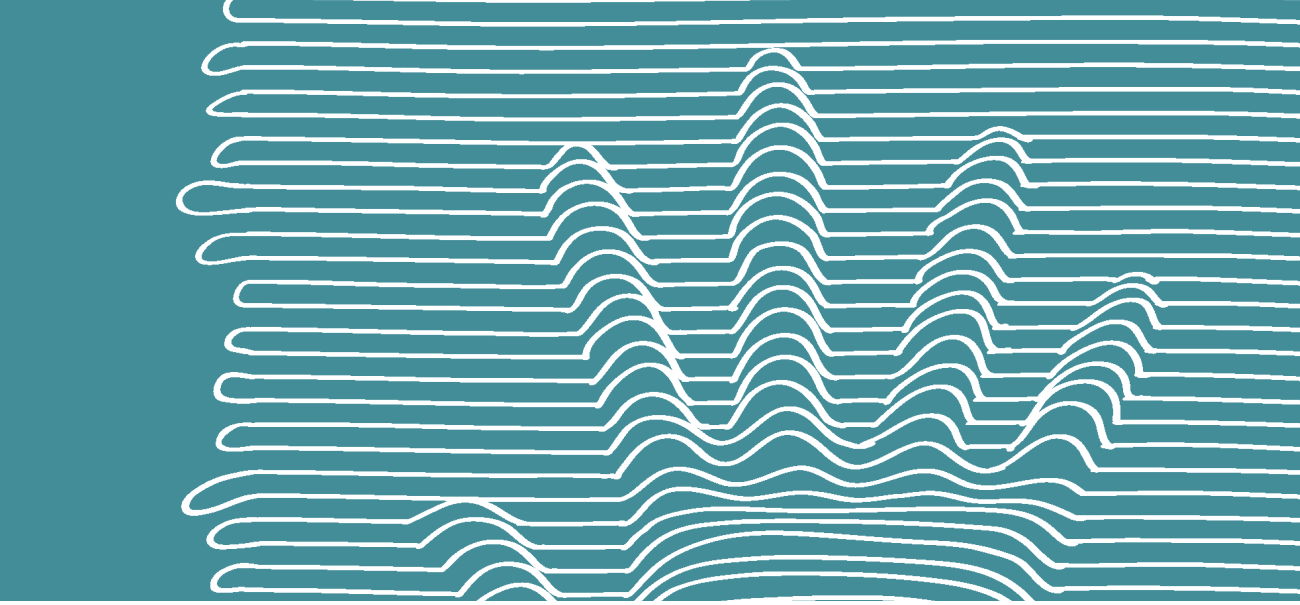

CHAPTER 4

Non-HLA genes modulate the risk of rheumatoid arthritis associated with HLA-DRB1 in a susceptible North American Native population

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ABSTRACT

Objective

Most of the genetic risk for rheumatoid arthritis (RA) is conferred by 'shared epitope' (SE), encoding alleles of HLA-DRB1. Specific North American Native (NAN) populations have RA prevalence rates of 2-5%, representing some of the highest rates estimated worldwide. As many NAN populations also demonstrate a high background frequency of SE, we sought to determine whether other genetic factors contribute to disease risk in this predisposed population.

Methods

RA patients (n = 333) and controls (n = 490) from the Cree/Ojibway NAN population in Central Canada were HLA-DRB1 typed and tested for 21 single-nucleotide polymorphisms (SNPs) that have previously been associated with RA, including PTPN22, TRAF1-C5, CTLA4, PADI4, STAT4, FCRL3, CCL21, MMEL1-TNFRSF14, CDK6, PRKCQ, KIF5A-PIP-4K2C, IL2RB, TNFAIP3, IL10-1082G/A and REL.

Results

Our findings indicate that SE is prevalent and represents a major genetic risk factor for RA in this population (82% cases versus 68% controls, odds ratio = 2.2, 95% confidence interval 1.6-3.1, $P < 0.001$). We also demonstrate that in the presence of SE, the minor allele of MMEL1-TNFRSF14 significantly reduces RA risk in a dominant manner, whereas TRAF1-C5 increases the risk.

Conclusion

These findings point to the importance of non-HLA genes in determining RA risk in a population with a high frequency of disease predisposing HLA-DRB1 alleles.

INTRODUCTION

Rheumatoid arthritis (RA) affects populations worldwide, with prevalence rates of 0.5-1% typically estimated in European and United States populations, and lower rates in African, Chinese and Japanese populations[1]. However, several North American Native (NAN) populations have substantially higher prevalence rates, estimated as high as 5.3% in the Pima of Arizona and 2.4% in the Tlingit of Alaska[2-4]. The shared epitope (SE) hypothesis postulates that RA predisposing alleles of the HLA-DRB1 locus encode for a positively charged QK(R)RAA sequence in position 70-74 in the third hypervariable region of the molecule[5]. In most populations, the SE sequence is found in HLA-DRB1*04 alleles. In NAN populations, SE-bearing alleles are primarily HLA-DRB1*1402 and *0404[6-10]. High background population frequencies of the SE may, in part, explain the higher prevalence rates of RA in NAN populations, compared with other populations. For example, frequencies of the SE vary from 28 to 38% in United States and United Kingdom Caucasians, but frequencies as high as 60 to 95% have been reported in several NAN populations, where they have been studied[8,11].

In European populations, the contribution of HLA to genetic risk of RA has been estimated at 37%[12]. The availability of the human genome sequence, high through-put single-nucleotide polymorphisms (SNP) technologies and large cohorts of RA patients has allowed the identification of associations with 20-30 new non-HLA gene regions, using both candidate gene and genome wide association scans (GWAS). These have included PTPN22, CTLA4, TRAF1-C5, STAT4, TNFAIP3, CD40, MMEL1-TNFRSF14 and REL[13,14]. Interestingly, these studies have suggested ethnic differences in the patterns of genetic susceptibility, necessitating confirmation of associations in different populations[15-17]. Examples are a PTPN22-R620W polymorphism which is strongly associated with RA and other autoimmune diseases in Caucasian populations, but is absent in Orientals, and an association with PADI4 SNP found in Japanese populations which is not detected in most Caucasian populations, despite the presence of these gene polymorphisms[15-20]. Our own studies of the Cree/Ojibway population in Central Canada have suggested a 2-3% prevalence rate of diagnosed RA, and a 60-70% frequency of SE alleles in the background population[7,8,21]. We sought to determine additional non-HLA genes that may contribute to the high frequency of RA detected in this population. We, therefore, genotyped NAN RA patients and controls for a spectrum of SNPs that were shown to be disease associated, using candidate gene and GWAS approaches in other populations. Our results indicate that several of these SNPs may contribute to disease risk in this population, with an SNP in the MMEL1-TNFRSF14 region having a particularly strong protective effect in SE positive individuals.

PATIENTS AND METHODS

Study subjects

We recruited RA patients and controls of Cree, Ojibway or Ojicree ethnic background. To be included in the study, all patients and controls had at least three of four grandparents of this ethnic background by self-report. A total of 338 RA patients were recruited from rheumatology clinics in urban areas of Central Canada, Winnipeg and Saskatoon, as well as rural areas, Norway House and St Theresa Point, Manitoba. A total of 516 controls from the same geographic areas were recruited by one of three methods: an accompanying spouse of an RA proband, a participant in a Health Fair, or through random sampling of the study communities. The latter approach accounted for most of the controls recruited from the rural locations. All controls had no history of RA or other autoimmune diseases.

The study protocol was approved by the Research Ethics Board of the University of Manitoba and by the Tribal Band Councils of the study communities. All study subjects gave informed consent after having the study details explained in their language of choice.

HLA-DRB1 testing

HLA-DRB1 testing was performed by polymerase chain reaction, using sequence-specific oligonucleotide primers and sequence-based typing as previously described[8]. The following DRB1 alleles were included as SE-bearing alleles: DRB1*0101, 0102, 0401, 0404, 0405, 0408, 0410, 1001 and 1402. Other SE-bearing alleles were not found in this population.

SNP testing in non-HLA genes

The complete list of SNP evaluated in this study is shown in Tables 2 and 3. These were selected on the basis of previously published genome wide association data, indicating SNP association with RA[13,14]. Genotyping was undertaken in the laboratory of Dr Katherine Siminovitch at the University of Toronto, and the multiplexed SNP assays were performed on the Sequenom Mass Array iPLEX platform (Sequenom, Inc., San Diego, CA, USA). Allele-specific extension products were plated onto a SpectroCHIP (Sequenom, Inc.) subjected to mass spectrometric analysis, and the genotypes were identified using SpectroCALLER software (Sequenom, Inc.).

Data analysis

The genotyping data for all individuals tested were subjected to quality control and individuals with genotype call rates less than 95% were removed. For each marker, genotype data quality was verified by testing the Hardy-Weinberg equilibrium in the control samples. The SNPs included in the study met the minimum quality control standards, with call rate greater than 95%, MAFs more than 1% and deviation from the Hardy-Weinberg equilibrium at $P > 0.0001$.

The SNP associations with RA were tested using the Plink software version 1.06 (http://pngu.mgh.harvard. edu/~purcell/plink/summary.shtml) and SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA). As all the SNP associations with RA have been previously made in at least one population, no correction for multiple comparisons was made.

In total, there were 854 NAN individuals (338 RA cases and 516 controls). In all, 31 individuals were removed for low genotyping, and after quality control, 333 cases and 490 controls were retained for analysis. The statistical power was evaluated by the software 'The CaTS Power Calculator' (Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA) for this study, with a total of 823 subjects consisting 333 cases and 490 controls. We assumed the estimated disease prevalence of 0.025, disease allele frequency 0.19, genotypic relative risk 1.5 and Type-1 error alpha 0.05.

Association analyses were performed using allelic, genotypic, minor allele dominant (major allele recessive) and minor allele recessive models. In analyzing the SE positive subjects 631 individuals (277 RA cases and 354 controls) were included. In all, 28 individuals were removed for low genotyping, and after quality control, 273 cases and 330 controls were retained. As with the entire population, association analyses were undertaken with all genetic models for this data set. Interaction between SE and MMEL1-TNFRSF14 was further tested using a multiplicative interaction model (risk factor 1 negative + risk factor 2 negative versus risk factor 1 positive + risk factor 2 positive), as previously described[34]. A logistic regression model was also tested, in which an interaction term between SE and the AA genotype of MMEL1-TNFRSF14 was added.35 The power to detect the association is 89% using an additive model and is 75% using a dominant model.

RESULTS

The demographic and clinical features of the patient and control populations are shown in Table 1. As is typical of other RA populations, this was a predominantly female population. Although there was also a female predominance in the control population, the frequency was significantly lower than in the RA patients (82 versus 63%, P<0.01). As we have previously shown in this population, the available RA autoantibody data indicated that, approximately 90% of this cohort was rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) positive[22]. The overall prevalence of one or two copies of SE (SE/x) was 82 and 68% for RA patients and controls, respectively (P<0.01). The number of patients and controls with two copies of SE (SE/SE) was 32 and 23%, respectively. The two most common SE alleles were HLA-DRB1*0404 (43 and 32% in RA cases and controls, respectively) and *1402 (31 and 29% in RA cases and controls, respectively).

Table 1. *Clinical and immunogenetic characteristics of the study populations*

	RA (n = 333) %	Controls (n = 490) %	P-value
Females	82	63	<0.01
RF positive	88 (223/255)	-	-
Anti-CCP positive	91 (74/81)	-	-
SE/x genotype	82	68	< 0.01
SE/SE genotype	32	23	< 0.01

Abbreviations: Anti-CCP, anti-cyclical citrullinated protein; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope.

We sought to determine non-HLA genes that may contribute to the development of RA in the NAN population. We tested SNPs that have been previously shown to be associated with RA; using candidate gene and GWAS approaches, these studies having been performed primarily in Caucasian populations. Table 2 compares the minor allele frequencies (MAFs) detected in the NAN control population to that reported in the GWAS.23 The genotype distribution and MAF for the SNPs detected in the NAN RA cases and controls is shown in Table 3. The data in Table 3 indicate that genotypic distribution for MMEL1-TNFRSF14, FCRL3 and CCL21 differed significantly between controls and RA patients. The MAF for MMEL1-TNFRSF14 tended to be lower in RA patients (odds ratio (OR) 0.82, 95% confidence interval (CI) 0.7-1.0, P = 0.08) while being higher for TRAF1-C5 (OR 1.23, 95% CI 1.0-1.5, P = 0.06).

Table 2. *Comparison of the MAF for SNPs detected with >5% frequency in the NAN control population with the frequency previously published in GWAS of Caucasian populations.*

Gene	SNP	Population	Minor	Major	MAF	OR	P-value
MMEL1-TNFRSF14*	rs3890745	NAN	G	A	0.31	0.82	0.079
		Caucasian	C	T	0.32	0.89	3.6E—6
REL	rs13031237	NAN	T	G	0.23	0.91	0.393
		Caucasian[b]	T	G	0.37	1.13	7.9E—7
STAT4	rs7574865	NAN	T	G	0.29	1.01	0.981
		Caucasian[b]	T	G	0.22	1.16	2.9E—7
CTLA4	rs3087243	NAN	A	G	0.37	1.06	0.601
		Caucasian[b]	A	G	0.44	0.87	1.2E—8
TNFAIP4	rs10499194	NAN	T	C	0.12	1.22	0.199
		Caucasian[b]	T	C	0.27	1.91	7.4E—4
CCL21	rs2812378	NAN	C	T	0.38	0.92	0.994
		Caucasian[b]	G	A	0.34	1.10	1.0E—4
TRAF1-C5	rs3761847	NAN	G	A	0.29	1.23	0.062
		Caucasian[b]	G	A	0.43	1.13	2.1E—7
KIF5A-PIP4K2C	rs1678542	NAN	G	C	0.30	0.94	0.547
		Caucasian[b]	G	C	0.38	0.91	2.0E—4

Abbreviations: GWAS, genome wide association scans; MAF, minor allele frequencies; NAN, North American Natives; OR, odds ratio; SNP, single-nucleotide polymorphisms. [a]Bases used for genotyping the minor allele of rs3890745 are the complementary bases to those reported in GWAS, but the data indicate a comparable MAF in both populations.[b]Caucasian MAF data based on data published in Stahl et al.[23]

Table 3. *Genotype distribution and MAF for the panel of non-HLA SNP tested in the entire population of NAN RA patients (n = 333) and ontrols (n = 490)*

Gene	SNP	Minor	Major	Genotype distribution			MAF				
				Controls	Cases	P-value	Controls	Cases	OR	95% CI	P-value
MMEL1-TNFRSF14	rs3890745	G	A	43/232/215	33/123/177	0.012	0.32	0.28	0.82	0.7-1.0	0.079
PADI4	rs2240340	G	A	114/255/121	84/159/90	0.481	0.49	0.49	0.99	0.8-1.2	0.941
PTPN22	rs6679677	A	C	1/34/455	1/29/303	NA	0.04	0.05	1.28	0.8-2.2	0.323
PTPN22	rs2476601	A	G	1/32/457	1/27/305	NA	0.03	0.04	1.27	0.7-2.2	0.358
FCRL3	rs11264799	T	C	23/182/285	28/101/203	0.025	0.23	0.24	1.02	0.8-1.3	0.859
FCRL3	rs7528684	G	A	64/222/204	56/123/154	0.045	0.36	0.35	0.98	0.8-1.2	0.858
IL10-1082G/A	rs1800896	G	A	15/149/326	14/104/215	0.639	0.18	0.20	1.11	0.9-1.4	0.429
REL	rs702873	A	G	96/252/142	70/158/104	0.558	0.45	0.45	0.98	0.8-1.2	0.865
REL	rs13031237	T	G	21/186/283	16/111/206	0.395	0.23	0.21	0.91	0.7-1.2	0.393
STAT4	rs3024921	A	T	0/19/470	0/11/322	NA	0.02	0.02	0.85	0.4-1.9	0.665
STAT4	rs7574865	G	T	55/176/259	31/132/170	0.460	0.29	0.29	1.01	0.8-1.3	0.981
CTLA4	rs3087243	A	G	66/226/198	45/161/126	0.763	0.37	0.38	1.06	0.7-1.3	0.601
CTLA4	rs6748358	A	C	78/231/180	51/157/125	0.961	0.40	0.39	0.97	0.8-1.2	0.781
TNFAIP4	rs10499194	T	C	11/84/394	8/70/255	0.371	0.11	0.13	1.22	0.9-1.7	0.199
TNFAIP4	rs6920220	A	G	1/40/449	1/30/302	NA	0.04	0.05	1.13	0.7-1.9	0.618
CDK6	rs42041	G	C	3/56/431	8/41/284	NA	0.06	0.09	1.39	0.9-2.1	0.086
CCL21	rs2812378	C	T	82/207/201	41/170/122	0.031	0.38	0.38	0.92	0.7-1.1	0.994
TRAF1-C5	rs3761847	G	A	40/193/257	36/142/155	0.183	0.28	0.32	1.23	1.0—1.5	0.062
TRAF1-C5	rs10818488	A	G	40/193/257	36/142/155	0.183	0.28	0.32	1.23	1.0—1.5	0.062
PRKCQ	rs4750316	C	G	1/29/460	3/28/302	NA	0.03	0.05	1.65	1.0-2.8	0.047
KIF5A-PIP4K2C	rs1678542	G	C	51/189/249	26/137/170	0.414	0.30	0.28	0.94	0.8-1.2	0.547
IL2RB	rs3218253	T	C	2/28/460	0/33/300	NA	0.03	0.05	1.54	0.9-2.6	0.084

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; MAF, minor allele frequency; NAN, North American Natives; OR, odds ratio; RA, rheumatoid arthritis; SNP, single-nucleotide polymorphism. P-value for genotype distribution was tested using w2-test, df = 2.

We explored models other than the genotypic and allelic models that may potentially better explain the association between individual SNPs and RA, in particular minor allele dominant and minor allele recessive models. These models examine associations on the basis of the homozygous and heterozygous states for each SNP, as gene dosage may impact on risk. Figure 1 compares the OR detected for each SNP using an allelic model (Figure 1a) and a minor allele dominant model (Figure 1b). The latter model compares the heterozygous + minor allele homozygous states to the homozygous major allele state (Aa + aa versus AA). This analysis further demonstrates the significant protective effect of the MMEL1-TNFRSF14 in minor allele dominant manner.

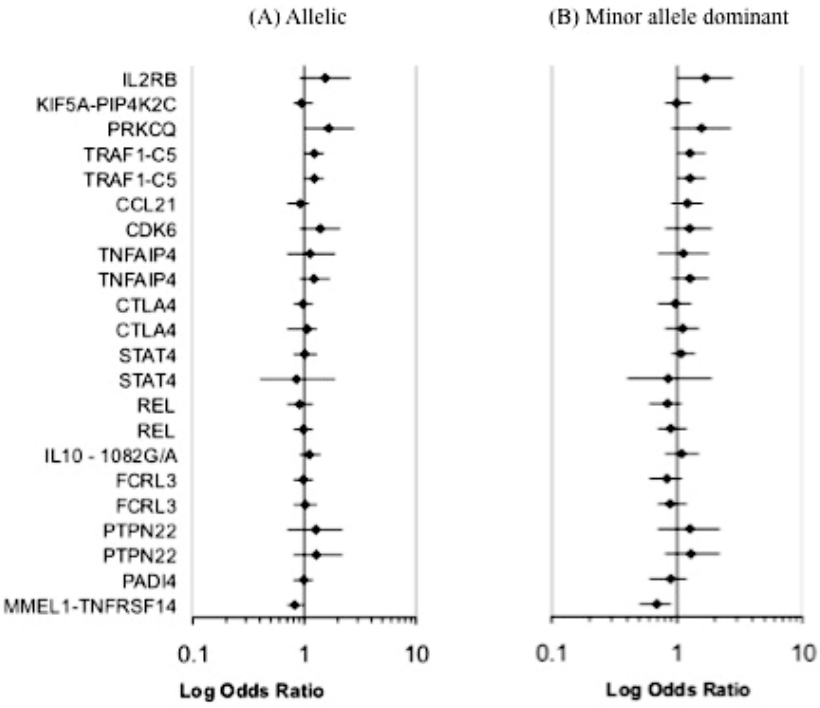

Figure 1. *Log odds ratio for association between a panel of non-HLA SNPs and RA in a NAN population. (a) Represents an allelic model; (b) represents a minor allele dominant model (AA versus Aa + aa). MMEL1-TNFRSF14 is significantly protective in the minor allele dominant model.*

Because of the high frequency of SE alleles in this population, we analyzed the panel of SNPs in the SE positive cases and controls. These data are shown in Table 4. On the basis of this analysis, we tested MMEL1-TNFRSF14 and TRAF1-C5 in minor allele dominant and recessive models. These data are shown in Table 5 and demonstrate the strength of the RA association in the minor allele dominant model, particularly when comparing only SE positive cases and controls. In the case of TRAF1-C5 minor allele, the association was positive, whereas the association with the MMEL1-TNFRSF14 minor allele was in the opposite direction.

Table 4. *Genotype distribution and MAF for the panel of non-HLA SNP, tested in the SE-positive population of NAN RA patients (n = 273) and controls (n = 330)*

Gene	SNP	Minor	Major	Genotype distribution			MAF				
				Controls	Cases	P	Controls	Cases	OR	95% CI	P-value
MMEL1-TNFRSF14	rs3890745	G	A	25/163/142	29/92/152	0.001	0.32	0.27	0.79	0.6-1.0	0.070
PADI4	rs2240340	G	A	71/178/81	70/125 /78	0.137	0.48	0.49	1.01	0.8-1.3	0.986
PTPN22	rs6679677	A	C	1/24/305	1/23/249	NA	0.04	0.05	1.17	0.6-2.1	0.583
PTPN22	rs2476601	A	G	1/24/305	1/22/250	NA	0.04	0.04	1.17	0.6-2.1	0.692
FCRL3	rs11264799	T	C	15/122/193	24/82/166	0.040	0.23	0.24	1.05	0.8-1.4	0.724
FCRL3	rs7528684	G	A	45/145/140	46/94/133	0.057	0.36	0.34	0.93	0.7-1.2	0.577
IL10—1082G/A	rs1800896	G	A	10/102/218	12/85/176	0.662	0.18	0.20	1.11	0.8-1.5	0.516
REL	rs702873	A	G	69/172/89	58/125/89	0.243	0.47	0.44	0.91	0.7-1.1	0.355
REL	rs13031237	T	G	17/118/195	12/89/172	0.611	0.23	0.21	0.85	0.6-1.1	0.330
STAT4	rs3024921	A	T	0/9/320	0/6/267	NA	0.01	0.01	0.81	0.3-2.5	0.675
STAT4	rs7574865	G	T	37/119/174	26/96/151	0.731	0.29	0.27	0.91	0.7-1.2	0.412
CTLA4	rs3087243	A	G	44/151/135	36/135/101	0.602	0.36	0.38	1.08	0.9-1.4	0.511
CTLA4	rs6748358	A	C	57/149/123	41/128/104	0.744	0.40	0.38	0.92	0.7-1.2	0.594
TNFAIP4	rs10499194	T	C	6/51/272	8/60/205	0.072	0.10	0.14	1.53	1.1-2.2	0.019
TNFAIP4	rs6920220	A	G	1/24/305	1/22/250	NA	0.04	0.04	1.12	0.6-2.1	0.692
CDK6	rs42041	G	C	0/33/297	3/34/236	NA	0.05	0.07	1.51	0.9-2.5	0.092
CCL21	rs2812378	C	T	57/141/132	35/143/95	0.051	0.39	0.39	1.02	0.8-1.3	0.894
TRAF1-C5	rs3761847	G	A	21/125/184	31/120/122	0.010	0.25	0.33	1.48	1.1-1.9	0.002
TRAF1-C5	rs10818488	A	G	21/125/184	31/120/122	0.010	0.25	0.33	1.48	1.1-1.9	0.002
PRKCQ	rs4750316	C	G	1/17/312	3/26/244	NA	0.03	0.06	2.11	1.1-3.9	0.010
KIF5A-PIP4K2C	rs1678542	G	C	36/127/166	21/113/139	0.375	0.30	0.28	0.91	0.7-1.2	0.482
IL2RB	rs3218253	T	C	1/17/312	0/25/248	NA	0.03	0.05	1.62	0.9-3.1	0.117

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; MAF, minor allele frequency; NAN, North American Natives; OR, odds ratio; RA, rheumatoid arthritis; SE, sharewd epitope; SNP, single-nucleotide polymorphism. P-value for genotype distribution was tested using w2-test, df = 2.

Table 5. *OR for RA association with MMEL1-TNFRSF14 and TRAF-C5, using a minor allele dominant (top panel) and minor allele recessive model (bottom panel), in the entire population of cases and controls (left panels) and SE-positive cases and controls (right panels)*

	All controls and cases			SE positive controls and cases		
	OR	95% CI	P-value	OR	95% CI	P-value
Minor allele dominant model (Aa+aa vs AA)						
MMEL1-TNFRSF14	0.69	0.5-0.9	0.009	0.61	0.4-0.8	0.002
TRAF1-C5	1.27	1.0-1.7	0.096	1.56	1.1-2.2	0.007
Minor allele recessive model (aa vs Aa+AA)						
MMEL1-TNFRSF14	1.14	0.7-1.8	0.620	1.45	0.8-2.5	0.200
TRAF1-C5	1.36	0.9-2.2	0.220	1.89	1.1-3.3	0.040

Abbreviations: CI, confidence interval; OR, odds ratio; RA, rheumatoid arthritis; SE, shared epitope.

Finally, we determined whether there was evidence of multiplicative interaction between SE and MMEL1-TNFRSF14. These data are shown in Table 6. As the minor allele is protective in a dominant manner, we tested the interaction between SE and the homozygous major allele state of the MMEL1-TNFRSF14 SNP (AA) as risk factors. The data indicate that, compared with the absence of both risk factors (OR = 1), having both SE and AA is associated with a substantial increase in risk, compared with having only one of these risk factors (OR 2.6, 95% CI 1.7-4.1, P< 0.0001). This interaction was further confirmed using a logistic regression model, where an interaction term SE*AA was added to a model that included both risk factors and gender. In this model, the interaction term was significantly associated with RA (OR 2.2, 95% CI 1.1-4.6, P = 0.03). An analysis of TRAF1-C5 as a risk factor showed a similar interaction with SE (OR 2.3, 95% CI 1.2-2.3, P<0.001).

Table 6. *Evidence for interaction between the homozygous major allele (AA) genotype of MMEL1-TNFRSF14 and SE*

MMEL1-TNFRSF14 AA genotype	SE allele	Cases/ controls	OR (95% CI)	P-value
0	0	35/87	1.0	
0	1	123/203	1.5 (1.0-2.4)	0.06
1	0	26/73	0.9 (0.5-1.6)	0.60
1	1	154/151	2.6 (1.7-4.1)	<0.0001

Abbreviations: CI, confidence interval; OR, odds ratio; SE, shared epitope. The reference genotype with both risk factors absent is shown in the first row. The presence of the individual risk factors alone and in combination is shown in the lower rows.

DISCUSSION

NAN populations have been shown to have a high prevalence of RA, compared with most other populations worldwide[4]. Moreover, as is the case with a number of the NAN populations that have been studied, we have shown that the Cree/Ojibway population in Central Canada has a high prevalence of SE alleles, specifically *0404 and *1402.8 Because of the high prevalence of SE in the population, we looked for SNP associations in non-HLA regions that may further predispose NAN individuals to RA, or alternatively, protect them from the risk associated with SE. In the current study, we show that several non-HLA SNP were marginally associated with RA risk in the NAN population, but that SNPs in the TRAF1-C5 and MMEL1-TNFRSF14 regions interacted significantly with SE, albeit in opposite directions with the former increasing risk and the latter being protective in the presence of the HLA-DRB1 risk.

In comparing the SNP allele frequencies in the NAN population with those reported in previous GWAS and candidate gene studies, some differences in SNP MAFs were apparent (Table 2). One potential confounder in such a comparison is that of ethnic admixture in the NAN population. However, to minimize admixture effects, inclusion in this study required each individual to have at least three of four grandparents of NAN background. Although this strategy does not eliminate the possibility of Caucasian (or other ethnic) admixture, our previously reported HLA data suggest that the frequency of the Caucasian HLA-A1 allele in this NAN population is <5%.7

The association with MMEL1-TNFRSF14 was initially demonstrated in a meta-analysis of three GWAS of approximately 4000 European patients and 12 000 European controls, whereas it had not been detected in any of the smaller individual GWAS[14]. In that study, the minor allele was also shown to have a protective effect on disease development (OR = 0.86), which is similar to what we detect in the current study. The modeling we performed suggested that the homozygous state for the major allele conferred the highest risk, and that the presence of the minor allele was indeed protective in a dominant manner. This finding is particularly relevant to a population, where most individuals carry one or more SE alleles, the primary risk factor for RA susceptibility. In such a population, it is likely that other genes have an important modulating effect on the HLA-DRB1 associated risk.

Recently, a study of Spanish RA patients and controls demonstrated an epistatic interaction between MMEL1-TNFRSF14 (as identified by rs6684865) and another TNFR superfamily member, TNFRSF6B (as identified by rs4809330), which increased the risk of RA[24]. TNFRSF6B alone did not affect RA risk. Moreover, although the genotypic distribution of MMEL1-TNFRSF14 was similar to that of NAN controls in our study, the association with MMEL1-TNFRSF14 alone was marginally demonstrable only in the replication study and not in the primary study, even after stratification for SE. The reasons for these differences remain unclear, but may relate to the distribution of the SE alleles in the Spanish population. The causal gene in the MMEL1-TNFRSF14 association has not been identified, as the SNP studied is not located in a genomic region where they would have a clear impact

on gene function. It is appealing to speculate that TNFRSF14 is indeed the RA associated gene as it is a key homeostatic molecule involved in T-cell activation[25]. Also known as herpes virus entry mediator for its role as a herpes virus receptor, TNFRSF14 is expressed on the surface of T cells and synovial fibroblasts and activates the canonical nuclear factor-kB (NF-KB) pathway upon ligation of LIGHT, a TNF super family member[26-28].

TRAF1-C5 was previously reported to be associated with RA in primarily Caucasian populations[29]. The OR for association with the minor allele of the rs3761847 SNP was reported at 1.32, which is comparable to an OR of 1.23 we report in the NAN population, using the same SNP. We further demonstrate that the OR is increased to 1.48 when analyzing SE positive cases and controls, and as with MMEL1-TNFRSF14, there is an indication of a significant interaction with SE. In both cases, the minor allele appeared to act in a dominant manner, albeit in opposite directions. This conclusion has to be interpreted cautiously however, in view of the challenges inherent to analysis of epistasis between gene loci in complex disorders such as RA[30].

TRAF1 and TNFRSF14 are both involved in the activation of NF-KB, a central pathway in the pathogenesis of RA. Indeed, many of the non-HLA SNPs that have been associated with RA are centered around the NF-KB pathway, and include TNFAIP3, CD40 and REL in addition to TRAF1 and TNFRSF14. The NF-KB pathway is activated by TNFa and IL-1P, two key cytokines in RA pathogenesis, and in turn, regulates the transcription of a gene signature that promotes both the chronic inflammatory response and the matrix degrading mechanisms that are a characteristic feature of the disease. SE encoding HLA-DRB1 alleles have been hypothesized to predispose to RA by efficiently presenting citrullinated antigens to T cells[31], and in turn, generating immune responses that target endogenous citrullinated antigens such as cit-vimentin, cit-fibrinogen and cit-enolase[32]. It is thus possible that in the context of such facilitated antigen presentation by SE encoding alleles, the subsequent downstream activation of immune effector cells such as T cells and macrophages is modulated by polymorphic variants of molecules in the NF-KB pathway, such as TRAF1 and TNFRSF14. To date, the functional consequences of these polymorphisms have not been demonstrated, nor is it clear that they are indeed the disease causal genes.

In addition to MMEL1-TNFRSF14 and TRAF1-C5, a number of other marginal associations were detected that perhaps would be more significant if the study had greater power. Perhaps, most surprising was the absence of a clear association with the R620W allele of PTPN22, this having been shown to predispose to RA in multiple populations. We found a frequency of 3% in controls for the R620W genotypes in the NAN population, as identified by rs2476601, and an almost identical genotypic distribution for a second SNP found in the PTPN22 gene, rs6679677. Although this frequency is somewhat lower than the 12-15% reported in Caucasian populations, it contrasts with that in several present-day East Asian populations in which this polymorphism is absent[15-17]. We were also unable to confirm a previously reported protective effect of an SNP in the IL10 promoter[33].

CONCLUSION

In summary, we have evaluated genomic RA risk in a NAN population with a high prevalence of RA. A spectrum of SNPs previously identified using GWAS and candidate gene approaches were tested. The data indicate that, in this population where RA-associated SE alleles are prevalent, there is a modulatory effect on disease risk from non-HLA genes, particularly MMEL1-TNFRSF14 and TRAF1-C5.

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