

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Remmers EF, Plenge RM, Lee AT, et al. *STAT4* and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med* 2007;357:977-86.

Supplementary Table S1: **Chromosome 2q RA Linkage Peak Candidate Genes.**

<i>Gene Symbol</i>	<i>Gene Identification</i>
<i>NOSTRIN</i>	Nitric oxide synthase traffic inducer
<i>WASPIP</i>	Wiskott-Aldrich syndrome protein interacting protein
<i>FRZB</i>	Frizzled-related protein
<i>STAT1</i>	Signal transducer and activator of transcription 1
<i>STAT4</i>	Signal transducer and activator of transcription 4
<i>HSPD1</i>	Heat shock 60kDa protein 1 (chaperonin)
<i>CFLAR</i>	CASP8 and FADD-like apoptosis regulator
<i>CASP10</i>	Caspase 10, apoptosis-related cysteine peptidase
<i>CASP8</i>	Caspase 8, apoptosis-related cysteine peptidase
<i>BMPR2</i>	Bone morphogenetic protein receptor, type II (serine/threonine kinase)
<i>CD28</i>	CD28 antigen
<i>CTLA4</i>	Cytotoxic T-lymphocyte-associated protein 4
<i>ICOS</i>	Inducible T-cell co-stimulator

Supplementary Table S2: Association of *STAT1/STAT4* Region SNPs with Disease Susceptibility in 607 Independent NARAC RA Cases and 1309 Unrelated Controls.

#	Name	Assoc Allele	Freq in cases	Freq in controls	Chi square	P value
1	rs3088307	G	0.48	0.45	2.73	0.099
2	rs16824035	C	0.86	0.85	0.30	0.587
3	rs6718902	C	0.74	0.74	0.31	0.581
4	rs13395505	G	0.60	0.59	0.54	0.464
5	rs17749316	C	0.93	0.93	0.00	0.984
6	rs1547550	G	0.66	0.65	0.26	0.612
7	rs2280234	G	0.61	0.59	0.87	0.350
8	rs2280233	C	0.48	0.46	1.90	0.168
9	rs2280232	T	0.78	0.76	1.59	0.208
10	rs13005843	C	0.93	0.93	0.48	0.490
11	rs11887698	A	0.85	0.84	0.21	0.645
12	rs10199181	A	0.60	0.60	0.02	0.877
13	rs13029532	A	0.93	0.93	0.10	0.752
14	rs10208033	T	0.58	0.57	0.03	0.869
15	rs1467199	C	0.75	0.75	0.02	0.899
16	rs16833177	T	0.79	0.79	0.24	0.623
17	rs4853456	A	0.83	0.82	0.81	0.368
18	rs3024904	T	0.11	0.10	0.11	0.740
19	rs3024936	G	0.04	0.03	1.53	0.216
20	rs925847	C	0.73	0.72	0.23	0.634
21	rs6749371	T	0.07	0.07	0.00	1.000
22	rs6715106	G	0.05	0.05	0.63	0.428
23	rs16833215	G	0.34	0.30	5.10	0.024
24	rs16833220	G	0.16	0.15	0.08	0.780
25	rs3024866	C	0.28	0.25	3.62	0.057
26	rs932169	C	0.09	0.08	0.15	0.702
27	rs1517352	A	0.43	0.38	6.10	0.014
28	rs13017460	A	0.44	0.40	4.83	0.028
29	rs2459611	T	0.90	0.90	0.13	0.715
30	rs7601754	A	0.83	0.82	0.17	0.683
31	rs11889341	T	0.28	0.22	13.08	3.00E-04
32	rs13010752	T	0.91	0.91	0.19	0.662
33	rs12998748	G	0.91	0.91	0.12	0.733
34	rs6434435	G	0.83	0.83	0.23	0.630
35	rs10931481	G	0.36	0.31	7.98	0.005
36	rs13011805	C	0.91	0.91	0.02	0.882
37	rs7574865	T	0.28	0.22	15.49	8.29E-05
38	rs8179673	C	0.28	0.22	12.41	4.00E-04
39	rs10181656	G	0.28	0.22	13.36	3.00E-04
40	rs13401064	C	0.92	0.92	0.11	0.745
41	rs16833260	G	0.36	0.32	6.91	0.009
42	rs6752770	G	0.31	0.29	1.92	0.166
43	rs7599504	T	0.84	0.84	0.37	0.545
44	rs4341966	T	0.23	0.21	2.14	0.144
45	rs17769459	T	0.06	0.05	0.31	0.579
46	rs2356350	G	0.41	0.40	0.06	0.811
47	rs11685878	T	0.42	0.41	0.55	0.457
48	rs4853546	G	0.65	0.65	0.00	0.976
49	rs1031509	C	0.73	0.73	0.22	0.639
50	rs12327969	C	0.23	0.22	0.04	0.834
51	rs10497711	G	0.09	0.07	4.73	0.030
52	rs7572482	A	0.35	0.35	0.26	0.614
53	rs2278940	A	0.09	0.07	3.85	0.050
54	rs897200	A	0.37	0.36	0.21	0.646
55	rs16833437	T	0.37	0.36	0.30	0.587
56	rs1031507	T	0.35	0.34	0.21	0.645
57	rs13001658	G	0.72	0.71	0.53	0.468
58	rs1869624	A	0.31	0.30	0.08	0.779
59	rs4853550	G	0.21	0.19	1.07	0.302
60	rs4853551	T	0.09	0.08	0.25	0.618
61	rs12467660	A	0.13	0.11	2.10	0.147
62	rs2054090	A	0.25	0.25	0.23	0.636
63	rs7595886	T	0.13	0.12	0.65	0.420

Supplementary Table S3: Association of *STAT1/STAT4* Region SNPs with Disease Susceptibility in 1013 anti-CCP Positive Singleton RA Cases and 1326 Controls.

#	Name	Assoc Allele	Freq in cases	Freq in ctrls	Chi square	P value
1	rs3088307	G	0.44	0.43	0.83	0.362
2	rs16824035	C	0.85	0.85	0.07	0.791
3	rs6718902	T	0.26	0.25	1.75	0.186
4	rs13395505	A	0.42	0.41	0.35	0.556
5	rs17749316	G	0.08	0.07	2.63	0.105
6	rs1547550	C	0.37	0.36	0.79	0.375
7	rs2280234	G	0.58	0.58	0.06	0.811
8	rs2280233	C	0.46	0.45	0.76	0.384
9	rs2280232	T	0.76	0.74	3.95	0.047
10	rs13005843	C	0.94	0.92	6.81	0.009
11	rs11887698	A	0.83	0.83	0.57	0.452
12	rs10199181	T	0.41	0.41	0.00	0.963
13	rs13029532	A	0.94	0.92	6.62	0.010
14	rs10208033	T	0.56	0.54	0.83	0.361
15	rs1467199	C	0.75	0.75	0.00	0.949
16	rs16833177	T	0.79	0.78	0.07	0.793
17	rs4853456	A	0.83	0.80	8.18	0.004
18	rs3024904	A	0.91	0.89	3.49	0.062
19	rs3024936	G	0.03	0.03	1.30	0.254
20	rs925847	C	0.73	0.71	2.49	0.114
21	rs6749371	A	0.94	0.93	2.61	0.107
22	rs6715106	A	0.95	0.94	0.50	0.480
23	rs16833215	G	0.32	0.31	0.52	0.470
24	rs16833220	G	0.15	0.15	0.01	0.924
25	rs3024866	C	0.27	0.26	0.11	0.737
26	rs932169	C	0.08	0.08	0.00	0.975
27	rs1517352	A	0.42	0.40	1.46	0.227
28	rs13017460	A	0.44	0.42	3.77	0.052
29	rs2459611	T	0.90	0.88	4.38	0.036
30	rs7601754	A	0.84	0.81	7.13	0.008
31	rs11889341	T	0.27	0.22	16.88	3.98E-05
32	rs13010752	T	0.91	0.90	3.76	0.053
33	rs12998748	G	0.91	0.90	2.19	0.139
34	rs6434435	G	0.84	0.81	8.09	0.004
35	rs10931481	G	0.35	0.31	5.01	0.025
36	rs13011805	C	0.92	0.89	6.95	0.008
37	rs7574865	T	0.27	0.22	11.70	6.00E-04
38	rs8179673	C	0.27	0.22	13.16	3.00E-04
39	rs10181656	G	0.27	0.22	15.64	7.65E-05
40	rs13401064	C	0.93	0.92	2.88	0.090
41	rs16833260	G	0.35	0.32	2.80	0.094
42	rs6752770	G	0.34	0.27	23.70	1.13E-06
43	rs7599504	T	0.86	0.84	3.77	0.052
44	rs4341966	T	0.23	0.21	2.30	0.130
45	rs17769459	T	0.06	0.05	0.54	0.464
46	rs2356350	G	0.42	0.41	0.33	0.565
47	rs11685878	C	0.59	0.58	0.63	0.428
48	rs4853546	G	0.66	0.64	1.69	0.193
49	rs1031509	C	0.74	0.71	3.61	0.058
50	rs12327969	G	0.78	0.77	0.26	0.610
51	rs10497711	G	0.07	0.06	1.12	0.290
52	rs7572482	G	0.65	0.64	0.80	0.371
53	rs2278940	A	0.07	0.06	0.55	0.457
54	rs897200	G	0.64	0.63	0.83	0.364
55	rs16833437	G	0.64	0.63	1.31	0.252
56	rs1031507	G	0.66	0.64	1.14	0.286
57	rs13001658	G	0.73	0.70	4.34	0.037
58	rs1869624	A	0.30	0.30	0.02	0.902
59	rs4853550	T	0.82	0.80	1.07	0.301
60	rs4853551	C	0.92	0.91	0.02	0.882
61	rs12467660	C	0.89	0.89	0.03	0.855
62	rs2054090	T	0.76	0.74	1.03	0.309
63	rs7595886	C	0.89	0.87	2.56	0.109

Supplementary Table S4: Linkage Disequilibrium Among Disease-Associated *STAT4* SNPs.

SNP ident.	LD with rs7574865*
rs11889341	1.00
rs7574865	--
rs7568275	1.00
rs8179673	0.95
rs10181656	0.95
rs7582694	1.00
rs10174238	0.90

* r^2 determined in HapMap CEU population.

SUPPLEMENTARY METHODS

GENOTYPING

The bead-based array method, the Illumina Golden Gate assay (Illumina, Inc.) was used to analyze 1536 SNPs in a multiplexed assay. Briefly, 250 ng of DNA was biotinylated and immobilized on streptavidin coated paramagnetic beads. Allele specific and locus specific primers were used to interrogate each SNP locus. Following primer hybridization, extension and ligation, the resulting product served as a template for PCR amplification. Using Cy3 and Cy5 labeled universal primers each allele could be specifically identified. The PCR products were then hybridized overnight to an appropriate Sentrix Array Matrix, which allows for the separation of the assay products in solution onto a solid surface for individual SNP genotype readout. A Bead Array Reader analyzed the fluorescent intensities. Genotype clusters and allele calls were generated with the BeadStudio genotyping software.

In the primer extension method (Sequenom, Inc.), multiplex PCR was used to amplify DNA products containing up to 28 SNPs in one reaction from 5 ng genomic DNA. Synthetic oligonucleotides that bind adjacent to the SNP site were then hybridized and extended with nucleotides complementary to the template SNP site using modified nucleotides that terminate the extension reaction at the interrogated SNP, thus generating alternate products of sufficiently different masses to be separated by mass spectrometry. The extended products were separated by MALDI-TOF mass spectrometry and the genotypes determined with SpectroTyper software (Sequenom). Calls were evaluated and edited by cluster analysis performed with the SpectroTyper software. Genotype data will be made available on the NARAC web site (<http://www.naracdata.org/>).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1: **Imputing SNPs.**

The genotypes at two SNPs, rs1517352 and rs7601754 (red asterisks), are used to “impute” the genotype at rs7574865 with an $r^2 = 0.91$ (based on CEU HapMap). That is, when a chromosome contains an “A” allele at both rs1517352 and rs7601754, this accurately predicts a “G” allele at rs7574865. Any other combination predicts a “T” allele at rs7574865. The number of imputed SNPs at a locus depends upon the size and linkage disequilibrium structure. Additional tutorial material is available through the HapMap website at <http://www.hapmap.org/downloads/presentations/> (download the presentation file “2_Daly.ppt”).

