NFKB2 polymorphisms associate with the risk of developing rheumatoid arthritis and response to TNF inhibitors: Results from the REPAIR consortium

Jose Manuel Sánchez-Maldonado[1,2], Manuel Martínez-Bueno PhD[3], Helena Canhão MD, PhD[4], Rob ter Horst[5], Sonia Muñoz-Peña[1], Ana Moñiz-Díez[1], Ana Rodríguez-Ramos[1], Alejandro Escudero MD, PhD[8], Signe B Sorensen PhD[9,10], Merete L Hetland PhD[11,12], Miguel A. Ferrer MD, PhD[13], Bente Glintborg PhD[11,12], Ileana Filipescu MD, PhD[14], Eva Pérez-Pampin MD, PhD[15], Pablo Conesa-Zamora PhD[16], Antonio García MD, PhD[13], Alfons den Broeder PhD[17], Salvatore De Vita MD, PhD[18], Svend Erik Hove Jacobsen PhD[19], Eduardo Collantes MD, PhD[8], Luca Quartuccio MD, PhD[18], Mihai G. Netea MD PhD[5,6], Yang Li PhD[5,7], João E. Fonseca MD, PhD[20,21], Manuel Jurado MD, PhD[1,2], Miguel Ángel López Nevot MD, PhD[2,22], Marieke J. H. Coenen PhD[17], Vibeke Andersen MD PhD[9,10], Rafael Cáliz MD, PhD[1,2,13], Juan Sainz PhD[1,2]

Supplementary Table 1. Demographic and clinical characteristics of anti-TNF patients.

	Anti-TNF patients (n=2041)		
Demographic characteristics	*Discovery Population (n=604)*	*DREAM Registry (n=882)**	*DANBIO Registry (n=555)*
Age (years)	58.75 ± 12.22	54.63 ± 12.80	53.86 ± 13.38
Sex ratio (female/male)	4.08 (485/119)	2.07 (477/230)	2.75 (407/148)
Clinical assessment			
Percentage of patients with RF positivity	420 (69.65)	534 (77.62)	175 (62.50)
*Percentage of ACPA-positive patients**	364 (69.07)	151 (62.14)	309 (71.69)
DAS28 at baseline	5.80 ± 1.25	5.33 ± 1.26	4.74 ± 1.27
Disease duration (years)	16.8 ± 9.78	9.70 ± 9.57	8.53 ± 9.12
Treatments			
First biologic agent			
Infliximab (%)	240 (39.74)	244 (34.46)	142 (25.59)
Etanercept (%)	179 (29.64)	130 (18.36)	175 (31.53)
Adalimumab (%)	163 (26.98)	334 (47.18)	137 (24.68)
Golimumab (%)	22 (03.65)	-	33 (05.95)
Certolizumab (%)	-	-	47 (09.37)
Biosimilar Infliximab (%)	-	-	16 (02.88)

Data are means ± standard deviation or n (%). Abbreviations: RF, rheumatoid factor; ACPA: anti-citrullinated protein antibodies; DAS28, disease activity score; DMARDs, disease-modifying anti-rheumatic drugs.

* Clinical data available in 708 patients (those used to genotype the most interesting markers).

RF and ACPA data were available in 598 and 527 patients in the discovery population.

RF and ACPA data were available in 688 and 243 patients in the DREAM population.

RF and ACPA data were available in 280 and 431 patients in the DANBIO population.

Supplementary Table 2. Meta-analysis for the association of NFKB- and inflammosome-related polymorphisms and RA risk.

				Discovery population (n=2521)		Replication DANBIO Registry (n=1740)		Meta-analysis (n=4261)		
Gene	**SNP ID**	**Chr.**	**Effect allele**	**OR (95% CI)**[∂]	***P***	**OR (95% CI)**[∂]	***P***	**OR (95% CI)**[∂]	***P***	I^2
GBP6	rs928655	1	A	0.94 (0.81-1.08)	0.37	1.12 (0.92-1.37)	0.25	0.93 (0.82-1.06)	0.29	0.76
IKBKB	rs11986055	8	A	0.93 (0.71-1.21)	0.59	-	-	-	-	-
IRF4	rs1050975	6	A	**1.30 (1.04-1.62)[§]**	**0.019**	0.93 (0.70-1.25)[§]	0.64	1.12 (0.80-1.55)[§]	0.52	0.072
IRF4	rs12203592	6	T	0.97 (0.81-1.18)	0.79	-	-	-	-	-
IRF4	rs1877175	6	T	1.13 (0.76-1.69)[§]	0.54	1.40 (0.86-2.27)[§]	0.17	1.23 (0.91-1.68)[§]	0.19	0.50
IRF4	rs7768807	6	T	0.95 (0.83-1.10)	0.51	-	-	-	-	-
KLRC1	rs7301582	12	T	**1.15 (1.00-1.34)**	**0.050**	0.87 (0.71-1.07)	0.18	1.01 (0.77-1.33)	0.94	**0.030**
KLRK1|KLRC4	rs1049174	12	C	1.11 (0.84-1.46)[§]	0.47	**1.56 (1.03-2.36)[§]**	**0.036**	1.27 (0.92-1.76)[§]	0.15	0.18
KLRK1|KLRC4	rs1154831	12	A	1.00 (0.86-1.16)	0.99	-	-	-	-	-
KLRK1|KLRC4	rs2255336	12	A	1.10 (0.94-1.27)	0.22	-	-	-	-	-
LOC105376246	rs2722824	9	A	0.96 (0.83-1.10)	0.53	-	-	-	-	-
NFKB1	rs4648110	4	A	1.10 (0.95-1.29)	0.20	-	-	-	-	-
NFKB2	rs11574851	10	T	1.18 (0.91-1.52)[†]	0.21	**1.52 (1.05-2.21)[†]**	**0.026**	**1.29 (1.02-1.64)[†]**	**0.035**	0.27
NFKB2	rs12769316	10	T	**1.70 (1.04-2.78)[§]**	**0.034**	**1.93 (1.01-3.66)[§]**	**0.042**	**1.78 (1.21-2.63)[§]**	**0.0037**	0.76
NFKB2|PSD	rs1056890	10	T	0.96 (0.84-1.09)	0.54	-	-	-	-	-
NFKBIB	rs3136645	19	C	1.07 (0.91-1.24)	0.42	-	-	-	-	-
NLRP3	rs4612666	1	T	**1.25 (1.05-1.49)[†]**	**0.013**	1.05 (0.83-1.32)[†]	0.69	1.17 (0.99-1.38)[†]	0.073	0.24
REL	rs13031237	2	T	1.16 (0.90-1.48)[§]	0.24	1.14 (0.82-1.58)[§]	0.44	1.15 (0.95-1.41)[§]	0.16	0.93
REL	rs842647	2	A	1.08 (0.94-1.24)	0.30	-	-	-	-	-
REL	rs13017599	2	A	1.10 (0.86-1.40)[§]	0.44	1.33 (0.96-1.85)[§]	0.090	1.18 (0.97-1.43)[§]	0.10	0.36
RELA	rs11820062	11	T	0.93 (0.82-1.06)	0.29	-	-	-	-	-
RELA	rs2306365	11	A	1.07 (0.89-1.29)	0.48	-	-	-	-	-
RELA	rs7119750	11	T	1.09 (0.91-1.32)	0.34	-	-	-	-	-
TLR10	rs11096957	4	A	1.12 (0.99-1.27)	0.066	**0.80 (0.67-0.96)**	**0.016**	0.95 (0.69-1.33)	0.77	**0.003**
TLR4	rs4986791	9	T	1.17 (0.89-1.54)	0.25	-	-	-	-	-
TLR5	rs5744174	1	C	0.99 (0.87-1.13)	0.86	-	-	-	-	-
TLR9||TWF2	rs187084	3	T	0.97 (0.85-1.10)	0.61	-	-	-	-	-
TRAF1||C5	rs3761847	9	A	0.97 (0.85-1.10)	0.61	-	-	-	-	-

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.
A random effect model was assumed for the meta-analysis of both cohorts.
[∂] Estimates calculated according to an additive model of inheritance and adjusted for age and sex.
[†] Estimates calculated according to a dominant model of inheritance and adjusted for age and sex.
[§] Estimates calculated according to a recessive model of inheritance and adjusted for age and sex.
P<0.05 in boldface.

Supplementary Table 3. Minor allele frequencies (MAF) of the NFKB2 SNPs in healthy controls stratified by country (discovery population) and their comparison with the ones reported in the 1000 genomes.

			Minor allele frequency (MAF) in healthy controls							
SNP ID	**Minor allele**	**CEU 1000 Genomes**	**SPAIN**	**P**	**PORTUGAL**	**P**	**ITALY**	**P**	**ROMANIA**	**P**
rs11574851	T	0.056	0.068	0.117	0.057	0.964	0.040	0.623	0.052	0.959
rs12769316	A	0.180	0.178	0.869	0.152	0.211	0.173	0.899	0.179	0.963
rs1056890	A	0.355	0.350	0.756	0.331	0.368	0.380	0.701	0.394	0.260

P values calculated considering the 1000 genomes population as reference.

* Frequencies found in the RA populations used for replication of the associations with anti-TNF response.

Supplementary Table 4. Meta-analysis for the association of NFKB- and inflammosome-related polymorphisms and RA risk in ACPA$^-$ patients.

				Discovery population ACPA$^-$ RA vs. controls (n=1971)		Replication DANBIO Registry ACPA$^-$ RA vs. controls (n=1136)		Meta-analysis ACPA$^-$ RA vs. Controls (n=3107)		
Gene	**SNP ID**	**Chr.**	**Effect allele**	**OR (95% CI)$^\partial$**	***P***	**OR (95% CI)$^\partial$**	***P***	**OR (95% CI)$^\partial$**	***P***	***I^2***
GBP6	rs928655	1	A	1.08 (0.84-1.38)	0.54	0.89 (0.65-1.22)	0.47	1.00 (0.83-1.22)	0.98	0.34
IKBKB	rs11986055	8	A	0.99 (0.65-1.53)	0.98	-	-	-	-	-
IRF4	rs1050975	6	A	1.28 (0.92-1.79)	0.14	0.82 (0.54-1.25)	0.36	1.04 (0.68-1.61)	0.85	0.10
IRF4	rs12203592	6	T	0.83 (0.60-1.17)	0.29	-	-	-	-	-
IRF4	rs1877175	6	T	1.04 (0.82-1.32)	0.74	0.96 (0.70-1.32)	0.79	1.01 (0.84-1.22)	0.92	0.69
IRF4	rs7768807	6	T	1.03 (0.82-1.30)	0.78	-	-	-	-	-
KLRC1	rs7301582	12	T	**1.56 (1.18-2.09)**†	**0.002**	0.76 (0.50-1.15)†	0.20	1.11 (0.55-2.24)†	0.78	**0.005**
KLRK1|KLRC4	rs1049174	12	C	1.21 (0.79-1.85)§	0.37	**2.57 (1.40-4.71)**§	**0.0036**	1.71 (0.82-3.56)§	0.35	0.050
KLRK1|KLRC4	rs1154831	12	A	0.92 (0.71-1.17)	0.48	-	-	-	-	-
KLRK1|KLRC4	rs2255336	12	A	1.33 (0.99-1.77)†	0.055	-	-	-	-	-
LOC105376246	rs2722824	9	A	1.08 (0.86-1.36)	0.50	-	-	-	-	-
NFKB1	rs4648110	4	A	0.86 (0.39-1.90)§	0.90	-	-	-	-	-
NFKB2	rs11574851	10	T	1.02 (0.68-1.52)	0.93	1.63 (0.95-2.80)	0.084	1.24 (0.79-1.96)	0.35	0.18
NFKB2	rs12769316	10	T	**2.53 (1.24-5.14)**§	**0.011**	1.91 (0.74-4.92)§	0.20	**2.28 (1.30-4.04)**§	**0.004**	0.64
NFKB2|PSD	rs1056890	10	T	1.01 (0.82-1.25)	0.90	-	-	-	-	-
NFKBIB	rs3136645	19	C	0.81 (0.62-1.04)	0.10	-	-	-	-	-
NLRP3	rs4612666	1	T	1.15 (0.93-1.44)	0.19	1.05 (0.76-1.44)	0.77	1.12 (0.93-1.34)	0.23	0.65
REL	rs13031237	2	T	**1.48 (1.02-2.15)**§	**0.040**	0.82 (0.46-1.45)§	0.48	1.15 (0.65-2.04)§	0.64	0.091
REL	rs842647	2	A	1.05 (0.83-1.33)	0.68	-	-	-	-	-
REL	rs13017599	2	A	1.17 (0.95-1.43)	0.13	0.90 (0.68-1.19)	0.45	1.05 (0.81-1.35)	0.74	0.14
RELA	rs11820062	11	T	1.07 (0.88-1.31)	0.49	-	-	-	-	-
RELA	rs2306365	11	A	1.16 (0.86-1.57)	0.32	-	-	-	-	-
RELA	rs7119750	11	T	1.24 (0.93-1.65)	0.15	-	-	-	-	-
TLR10	rs11096957	4	A	1.08 (0.89-1.33)	0.43	0.87 (0.64-1.19)	0.38	1.00 (0.82-1.23)	0.98	0.25
TLR4	rs4986791	9	T	1.00 (0.63-1.58)	0.99	-	-	-	-	-
TLR5	rs5744174	1	C	0.89 (0.72-1.10)	0.27	-	-	-	-	-
TLR9||TWF2	rs187084	3	T	1.02 (0.83-1.25)	0.88	-	-	-	-	-
TRAF1||C5	rs3761847	9	A	0.91 (0.74-1.13)	0.39	-	-	-	-	-

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.
A random effect model was assumed for the meta-analysis of both cohorts.
$^\partial$ Estimates calculated according to an additive model of inheritance and adjusted for age and sex.
† Estimates calculated according to a dominant model of inheritance and adjusted for age and sex.
§ Estimates calculated according to a recessive model of inheritance and adjusted for age and sex.
P<0.05 in boldface.

Supplementary Table 5. NFKB2 SNPs in strong LD with the rs1056890 variant and showing correlation with IL10 levels.

Variant	Location	Distance (bp)	r2	D'	Consequence Type	Located in gene(s)	Association with IL10 (PBMCs stimulated with LPS, 1ng/ul)
rs3740418	10:102399176	3837	1.000	1.000	non coding transcript exon variant	NFKB2	P=0.0025
rs72845694	10:102409976	6963	1.000	1.000	intron variant	PSD	P=0.0025
rs1005044	10:102392386	10627	1.000	1.000	regulatory region variant	-	P=0.0025
rs2145308	10:102391242	11771	1.000	1.000	regulatory region variant	-	P=0.0025
rs56238324	10:102390057	12956	1.000	1.000	intergenic variant	-	P=0.0025
rs7077329	10:102402210	803	0.957	1.000	intron variant	NFKB2	P=0.0043
rs6584499	10:102428502	25489	0.935	1.000	intron variant	CUEDC2	P=0.0090

Measurements of steroid hormones

Cortisol, 11-deoxycortisol, androstenedione and 17-hydroxyprogesterone were analyzed by Liquid Chromatography Tandem-Mass Spectrometry (LCMSMS) after protein precipitation and solid-phase extraction as described in Ter Horst et al. (2016)[35] with the following additional compound specific configurations and characteristics. Internal standard [$^{13}C_3$]-cortisol (Isoscience, King of Prussia, PA), $^{2}H_5$-11-deoxycortisol (Isoscience, King of Prussia, PA), [$^{13}C_3$]- androstenedione (Isoscience, King of Prussia, PA) and [$^{13}C_3$]-17-hydroxyprogesterone (Isoscience, King of Prussia, PA) were used. Retention time was 1.46 min, 2.68 min, 3.70 min and 4.78 min for cortisol, 11-deoxycortisol, androstenedione and 17-hydroxyprogesterone respectively. An 9-point calibration curve was used cortisol (Sigma); 11-deoxycortisol (Sigma); androstenedione (Sigma) and 17-hydroxyprogesterone (Sigma). Two transitions (qualitative and quantitative) were monitored. Transitions (Q1>Q3) were m/z 363.4 > 121.1 (25kEV) and m/z 363.4 > 97.1 (34 kEV) for cortisol; m/z 366.4 > 124.1 (25 kEV) and m/z 366.4 > 100.1 (35 kEV) for $^{13}C_3$-cortisol; m/z 347.2 > 97.1 (29 kEV) and m/z 347.2 > 109.1 (31 kEV) for 11-deoxycortisol; m/z 352.3 > 100.1 (31 kEV) and m/z 352.3 > 113.1 (29 kEV) for $^{2}H_5$-11-deoxycortisol; m/z 387.2 > 97.1 (23 kEV) and m/z 387.2 > 109.1 (26 kEV) for androstenedione; m/z 290.2 > 100.1 (21 kEV) and m/z 290.2 > 112.1 (26 kEV) for $^{13}C_3$-androstenedione; m/z 331.3 > 97.1 (31 kEV) and m/z 331.3 > 109.1 (31 kEV) for 17-hydroxyprogesterone; m/z 334.3 > 100.1 (30 kEV) and m/z 334.3 > 112.1 (33 kEV) for $^{13}C_3$-17-hydroxyprogesterone. Dwell time was 100 ms, 40 ms, 100 ms and 60 ms for cortisol, 11-deoxycortisol, androstenedione and 17-hydroxyprogesterone, respectively. The method was linear assessed by CLSI EP6 protocol. Recovery was within 96.5 – 102% cortisol, 98.4 – 104% 11-deoxycortisol, 99.4 – 99.8% androstenedione and 98.6 – 102% 17-hydroxyprogesterone. Total CV for cortisol is 3,6% at 301 nmol/L and 3,1% at 1092 nmol/L. Total CV for 11-deoxycortisol is 5,9% at 2,1 nmol/L and 5,1% at 27 nmol/L. Total CV for androstenedione is 4,7% at 3,2 nmol/L and 4,1% at 23 nmol/L. Total CV for 17-hydroxyprogesterone is 5,6% at 2,6 nmol/L and 5,1% at 95 nmol/L. LOQ was 1.91 nmol/L (13.4% CV), 0.10 nmol/L (10% CV), 0.05 nmol/L (10% CV) and 0.10 nmol/L (10% CV) for cortisol, 11-deoxycortisol, androstenedione and 17-hydroxyprogesterone respectively.

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