

# Polymorphisms of nuclear factor- $\kappa$ B family genes are associated with development of multiple myeloma and treatment outcome in patients receiving bortezomib-based regimens

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Online Supplementary Table S1. PCR and extension primer sequences.

SNP ID*	Major/minor allele	PCR primer (5'-3')	Amplified (bp)	Extension primer (5'-3')	Expected molecular weight of non-extended allele (Da)	Extension allele	Expected molecular weight of extended allele (Da)
<b>IKB<math>\alpha</math></b>							
rs3138053	A/G	ACGTTGGATGTGGATACCTTGCAATAGCAG ACGTTGGATGTCACGATCCTTTTCTGCGG	119	GTTTATGCTATCTGACCTACA	6371.2	G A	6618.3 6698.3
rs2233406	C/T	ACGTTGGATGCACAATGTAGGTCAGATAGC ACGTTGGATGGTAGTGGTGGTTGTGGATAC	109	GAACACAATAGCTACTCTG	5780.8	C T	6028 6107.9
rs2233409	C/T	ACGTTGGATGTGCACCCTGTAATCCTGTCC ACGTTGGATGCGACGACCCCAATTCAAATC	101	CTGTAATCCTGTCCCTTGCAAGTGA7897.1		T C	8168.3 8184.3
rs1050851	C/T	ACGTTGGATGAAAGGCACTGACCATGGAAG ACGTTGGATGGCTGCAGGTTGTTCTGGAAG	97	AGGTGAAGGGAGACCTGGC	5942.9	C T	6190.1 6270
rs3138054	G/A	ACGTTGGATGTGCCTGGACTCCTTAAGTTG ACGTTGGATGACAATGGTATGTCTGCCTCC	115	CACCTGCCCTTCTCCA	5002.3	G A	5249.4 5349.4
rs2233419	C/T	ACGTTGGATGTGGAGCTTTTGGTGTCCCTTG ACGTTGGATGCACAACATAAGCAGGAGGAG	116	AGGTTGGTGTCTCCTGCC	5497.6	C T	5744.7 5824.7
rs8904	C/T	ACGTTGGATGTTACAGTGCCTTATGATGAC ACGTTGGATGAGTCCATGTTCTTTCAGCCC	101	GGCCAGCGTCTGACGTTATGAG	6791.4	C T	7038.6 7118.5
rs696	G/A	ACGTTGGATGGGTGTACTTATATCCACACTG ACGTTGGATGAAGCAACAAAATGAGGGCTG	99	CACACTGCACACTGCCT	5075.3	A G	5346.5 5362.5
rs2273650	C/T	ACGTTGGATGCCAACAATACATTATGTACAC ACGTTGGATGTAAGCGTTCAGTGATGTGGG	108	ACAATACATTATGTACACCATTTA	7278.8	C T	7526 7605.9
rs3138055	G/A	ACGTTGGATGCTCAACTGGCAATTAGGGTTC ACGTTGGATGTTTGGCCTGTGGTTGGAGAC	103	ACGGGATGACAGAATGACAA	6208.1	G A	6455.3 6535.2
<b>NFKB2</b>							
rs7076748	G/C	ACGTTGGATGGGAAACAGATGACGATTAGG ACGTTGGATGGTGGAGACTCCATCTCAAAAC	117	TGACGATTAGGATGAAAATTAGT	7150.7	C G	7397.9 7497.9
rs12769316	G/A	ACGTTGGATGTTACTTATAGTCCAGTGCAGG ACGTTGGATGAAGCTATCTCGGGTCTTTCC	100	GGACTGTGATTCTGTTCTTAAAAAC	7671	A G	7942.2 7958.2
rs12772374	A/G	ACGTTGGATGACCTCCATGAGCTTAGCATC ACGTTGGATGTGGAGTTTCTACTTAGCCCC	98	AGCATCTGACCAAGGGGAAAG	6513.3	A G	6784.5 6800.5
rs7897947	T/G	ACGTTGGATGCGGAGATTCTATGGAGTGAG ACGTTGGATGAGCCCCAGCTTCTTAAATG	97	GAGTGAGATATGGATTAGTTG	6580.3	G T	6827.5 6851.5
rs11574851	C/T	ACGTTGGATGTCCCAGGTGCTGACATCCAT ACGTTGGATGCGAGTCGCTATCAGAGGTAG	100	TGCTGACATCCATGCTGAAAA	6414.2	C T	6661.4 6741.3
rs7077329	C/T	ACGTTGGATGACTGTCTTCTTACCTCTG ACGTTGGATGAAGTGGATCTGGACCTGGA	114	AGGAATGGGATAGTCAAAGCCTC	7121.7	C T	7392.9 7408.9
rs1056890	C/T	ACGTTGGATGCCTCAGGAGCCTAGGCTA ACGTTGGATGCCACCCTGTACGAAAAAGTG	97	CCTAGGCTAGGGCACCTC	5789.8	C T	6036.9 6116.9

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**TRAF3**

rs10131139	C/T	ACGTTGGATGGGGATGCATAGTCAATGTT ACGTTGGATGGGAAATATTGGGTCCAAA	119	TTTTCAAACATTTTGTTCATTA	7281.8	C T	7529 7608.9
rs7143468	G/A	ACGTTGGATGAAAACACGAAGCCCACTG ACGTTGGATGACACTGATGAGACAGAGCGG	103	ATGCCTTCCTTAAATCCA	5995.9	G A	6243.1 6323
rs12147254	G/A	ACGTTGGATGGAACGAATGGAATCTGCAC ACGTTGGATGCTGCACTACCACTCATGTTC	116	GTGTATTCTGAATTATGCTCA	6426.2	G A	6673.4 6753.3
rs8023164	A/G	ACGTTGGATGGGAAAGAATGCTCTACCTG ACGTTGGATGTAGCTGAGGGAATGCATGAC	98	TCTACCTGGCTACGTTTTTCCT	6314.1	A G	6585.3 6601.3
rs11846158	A/G	ACGTTGGATGTACTGGTCACTCCCAATTC ACGTTGGATGGGCCATTTTCATCAGAGAAC	104	ATGACATGTCAGACCTC	5154.4	G A	5401.6 5481.5
rs12435483	C/T	ACGTTGGATGTGGCCACCTCATTGATATAG ACGTTGGATGGAATGACACGAACCTGAG	89	TGATATAGAAGGTCACGGGGCTGC7457.9		C T	7705 7784.9
rs3783384	C/T	ACGTTGGATGCGACTATTAAGCCCTATCCC ACGTTGGATGTGCAGTTGATGTCCTTGTGG	111	GTCCATGAGCAGAGCAAG	5557.6	T C	5828.8 5844.8
rs12588538	A/G	ACGTTGGATGACACAGTGCAGCAGTTTCTC ACGTTGGATGTTATGCATCCTGCACTCTCG	117	CAGTTTCTCACTCTGTCA	5400.5	A G	5671.7 5687.7
rs11160707	G/A	ACGTTGGATGTGAGAGGAAGACACTCAG ACGTTGGATGTGTCACGCGCTTGTCTTTG	86	GAACAGAGACCCCGATA	5197.4	G A	5444.6 5524.5

\* According to National Center for Biotechnology Information SNP database rs number. 'bp: base pair. 'Da: Dalton.

**Online Supplementary Table S2. Characteristics of the patients and their diseases.**

Characteristic	All patients n = 252	Patients treated with a bortezomib-based regimen n = 83
Median age, years (range)	58 (28-82)	58 (40-78)
Sex, n (%)		
Male	161 (64)	51 (61)
Female	91 (36)	32 (39)
Type of M protein, n (%)		
IgG	157 (62)	47 (57)
IgA	50 (20)	19 (23)
IgM	2 (1)	1 (1)
Light-chain	40 (16)	15 (18)
Non-secretory	3 (1)	1 (1)
Durie Salmon staging, n (%)		
I	2 (1)	0
II	16 (6)	0
III	234 (93)	83 (100)
Bone marrow plasmacytosis, %, median (range)	38 (5-98)	35 (5-94.5)
$\beta$ 2-microglobulin level, mg/L, median (range)	3.49 (0.71-49.43)	3.76 (1.04-46.78)
Albumin level, g/L, median (range)	35 (18-96)	36 (20-51)
Hemoglobin level, g/L, median (range)	102 (32.7-151)	92 (40-146)
C-reactive protein level, mg/L, median (range)	4.03 (1.63-156)	4.14 (2-156)
Lactate dehydrogenase level, U/L, median (range)	169 (79-821)	174 (81-804)
Previous therapy, n (%)		
MP	-	23 (28)
VAD	-	24 (29)
M2	-	15 (18)
TD	-	16 (19)
SCT	-	5 (6)

“-” indicates that the number of patients was not calculated.

**Online Supplementary Table S3. Response to therapy and survival of according to *IKBα*, *NFKB2*, and *TRAF3* genotype in patients who received bortezomib-based regimens.**

SNP ID*	Geno- type	Frequency		Overall response <sup>†</sup> (%)	P	Progression-free survival <sup>‡</sup>				Overall survival <sup>‡</sup>				
		n.	(%)			n.	Median (mo)	P	Hazard ratio (95% CI)	P	Median (mo) <sup>§</sup>	P	Hazard ratio (95% CI)	
<b><i>IKBα</i></b>														
rs3138053	AA	69	(83.1)	52	(75.4)	1.000	15	0.274	1.452(0.725-2.906)	0.292	-	0.798	0.854 (0.252-2.896)	0.800
	AG+GG	14	(16.9)	11	(78.6)		10				-			
rs2233406	CC	64	(77.1)	49	(76.6)	1.000	15	0.601	1.182 (0.618-2.260)	0.614	-	0.661	0.787 (0.265-2.335)	0.666
	CT+TT	19	(22.9)	14	(73.7)		12				-			
rs2233409	CC	69	(83.1)	53	(76.8)	0.931	15	0.438	1.302 (0.651-2.603)	0.455	-	0.742	0.817 (0.241-2.768)	0.745
	CT+TT	14	(16.9)	10	(71.4)		10				-			
rs1050851	CC	70	(93.3)	54	(77.1)	0.745	12	0.890	0.924 (0.287-2.974)	0.894	-	0.699	0.677(0.091-5.060)	0.704
	CT+TT	5	(6.7)	3	(60.0)		7				-			
rs3138054	GG	82	(98.9)	62	(75.6)	1.000	15	0.216	NA	0.261	NA	0.521	NA	0.672
	GA+AA	1	(1.1)	1	(100.0)		4				-			
rs2233419	CC	76	(91.6)	57	(75.0)	0.863	15	0.060	2.192 (0.919-5.226)	0.077	-	0.233	2.052 (0.606-6.945)	0.248
	CT+TT	7	(8.4)	6	(85.7)		6				-			
rs8904	CC	29	(35.8)	21	(72.4)	0.855	12	0.518	0.837 (0.479-1.464)	0.534	-	0.961	0.978 (0.405-2.362)	0.963
	CT+TT	52	(64.2)	40	(76.9)		12				-			
rs696	GG	29	(34.9)	21	(72.4)	0.783	12	0.722	0.908 (0.520-1.584)	0.733	-	0.689	0.842 (0.360-2.972)	0.693
	GA+AA	54	(65.1)	42	(77.8)		12				-			
rs2273650	CC	50	(60.2)	36	(72.0)	0.446	8	0.275	0.745 (0.428-1.295)	0.297	-	0.315	0.637 (0.428-1.295)	0.324
	CT+TT	33	(39.8)	27	(81.8)		15				-			
rs3138055	GG	24	(28.1)	17	(70.8)	0.685	15	0.959	0.985 (0.552-1.760)	0.960	-	0.955	0.975(0.397-2.394)	0.956
	GA+AA	59	(71.1)	46	(78.0)		12				-			
<b><i>NFKB2</i></b>														
rs7076748	GG	33	(40.2)	26	(78.8)	0.774	16	0.417	1.243(0.718-2.155)	0.437	-	0.158	1.928 (0.754-4.932)	0.171
	GC+CC	49	(59.8)	36	(73.5)		8				-			
rs12769316	GG	55	(66.3)	38	(69.1)	<b>0.042</b>	9	0.584	0.859 (0.487-1.515)	0.599	-	<b>0.020</b>	0.264 (0.078-0.893)	<b>0.032</b>
	GA+AA	28	(33.7)	25	(89.3)		15				-			
rs12772374	AA	54	(65.1)	38	(70.4)	0.180	9	0.728	0.909(0.519-1.591)	0.738	-	0.115	0.270 (0.063-0.921)	0.138
	AG+GG	29	(34.9)	25	(86.2)		15				-			
rs7897947	TT	34	(41.5)	27	(79.4)	0.814	15	0.667	1.122 (0.648-1.943)	0.680	-	0.162	1.931 (0.749-4.981)	0.173
	TT+GG	48	(58.5)	36	(75.0)		8				-			
rs11574851	CC	76	(92.7)	56	(73.7)	3.28	10	0.109	0.345 (0.084-1.422)	0.141	NA	0.158	NA	0.363
	CT+TT	6	(7.3)	6	(100.0)		24				-			
rs7077329	CC	48	(59.3)	39	(81.3)	0.348	10	0.666	0.886 (0.501-1.568)	0.678	-	0.207	1.716 (0.728-4.044)	0.217
	CT+TT	33	(40.7)	23	(69.7)		15				-			
rs1056890	CC	53	(63.9)	44	(83.0)	<b>0.047</b>	15	0.643	1.137 (0.647-1.997)	0.656	-	<b>0.037</b>	2.355 (1.016-5.460)	<b>0.046</b>
	CT+TT	30	(36.1)	19	(63.3)		10				-			
<b><i>TRAF3</i></b>														
rs7143468	GG	17	(20.7)	14	(82.4)	0.682	24	0.467	1.290 (0.628-2.647)	0.488	-	0.452	1.583(0.466-5.377)	0.461
	GA+AA	65	(79.3)	48	(73.8)		15				-			
rs12147254	GG	34	(42.5)	26	(76.5)	1.000	16	0.992	0.997 (0.569-1.749)	0.992	-	0.319	1.565(0.638-3.839)	0.328
	GA+AA	46	(57.5)	34	(73.9)		10				-			
rs11846158	AA	32	(38.6)	23	(71.9)	0.677	7	0.254	0.740 (0.431-1.272)	0.276	-	0.433	0.717 (0.310-1.663)	0.440
	AG+GG	51	(61.4)	40	(78.4)		16				-			
rs3783384	CC	27	(33.3)	19	(70.4)	0.649	7	0.105	0.642 (0.367-1.125)	0.122	-	0.570	0.777 (0.322-1.857)	0.575
	CT+TT	54	(66.7)	42	(77.8)		15				-			
rs12588538	AA	43	(52.4)	32	(74.4)	0.995	16	0.955	1.015 (0.595-1.732)	0.957	-	0.826	1.097 (0.475-2.531)	0.828
	AG+GG	39	(47.6)	30	(76.9)		12				-			
rs11160707	GG	65	(78.3)	47	(72.3)	0.253	8	0.018	0.428 (0.201-0.912)	0.028	-	0.117	0.334 (0.078-1.431)	0.140
	GA+AA	18	(21.7)	16	(88.9)		17				-			

\* According to National Center for Biotechnology Information SNP database rs number. <sup>†</sup>Overall response: complete response+very good partial response+partial response. <sup>‡</sup>Wild-type homozygote as reference; heterozygote+ polymorphic homozygote compared with wild-type homozygote. <sup>§</sup> "-" indicates that median overall survival was not reached. "NA" indicates that a current assessment was not available. Statistically significant values are marked in bold and italics.

**Online Supplementary Table S4. Multivariate analysis for overall response, progression-free survival and overall survival.**

Variable	Overall response*			Progression free survival <sup>†‡</sup>			Overall survival <sup>†‡</sup>		
	Odds ratio	95% CI	P	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
<i>NFKB2</i> rs12769316 G>A	0.329	0.083-1.1314	0.116	0.868	0.476-1.580	0.642	0.311	0.087-1.112	0.072
<i>NFKB2</i> rs1056890 C>T	2.187	0.732-6.530	0.161	1.073	0.591-1.949	0.817	1.649	0.683-3.982	0.266
<i>TRAF3</i> rs11160707 G>A	0.325	0.066-1.612	0.169	0.428	0.201-0.911	<b>0.028</b>	0.342	0.079-1.474	0.150

\* A multivariate logistic-regression analysis was performed. † A multivariate Cox regression analysis was performed. ‡ Adjusted for  $\beta$ 2-microglobulin level. Statistically significant values are marked in bold and italics.

**Online Supplementary Table S5. Clinical characteristics based on *NFKB2* rs12769316, rs1056890, and *TRAF3* rs11160707 genotypes in 83 patients treated with a bortezomib-based regimen.**

Parameter	<i>NFKB2</i> rs12769316			<i>NFKB2</i> rs1056890			<i>TRAF3</i> rs11160707		
	GG n = 55	GA+AA n = 28	P	CC n = 53	CT+TT n = 30	P	GG n = 65	GA+AA n = 18	P
Age, median (range)	59 (40-78)	55 (42-75)	0.789	58 (42-78)	57 (40-75)	0.346	58 (40-78)	56 (42-73)	0.417
Sex, n.									
Male	36	15	0.293	30	21	0.228	40	11	0.974
Female	19	13		23	9		25	7	
$\beta$ 2-microglobulin level, mg/L, median (range)	4.78 (1.06-46.78)	3.49 (1.21-39.86)	0.843	4.4 (1.21-39.86)	3.74 (1.04-46.78)	0.312	3.85 (1.04-46.78)	4.69 (1.21-22.87)	0.528
Albumin level, g/L, median (range)	34.5 (20-51)	37 (28-51)	0.157	34 (20-51)	36 (23-47)	0.732	36.5 (20-51)	32.5 (22-48)	0.260
Hemoglobin level, g/L, median (range)	92 (40-143)	93.5 (52-146)	0.633	94 (43-146)	87.5 (40-135)	0.642	94 (40-146)	83 (43-139)	0.320
CRP level, mg/L, median (range)	5.49 (3-156)	3.41 (2-67)	0.081	3.87 (2-156)	3.62 (3-115)	0.600	4.14 (2-156)	4.99 (3-112)	0.926
LDH level, U/L, median (range)	205 (81-804)	174 (110-534)	0.111	176.5 (87-534)	183 (81-804)	0.231	172 (81-804)	174.5 (96-687)	0.258

CRP: C-reactive protein; LDH: lactate dehydrogenase.