FcγRIIa, IIIa and IIIb gene polymorphisms in Behçet's disease: do they have any clinical implications?

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ABSTRACT

Objective. *Behçet's disease (BD) is a unique systemic vasculitis involving both arteries and veins of all sizes. Since Fcγ receptors (FcγR) are important in mediating various immune effector functions, FcγR gene polymorphisms may affect the susceptibility to systemic inflammatory diseases such as BD. The aim of this study was to show the distribution of FcγRIIa, IIIa ve IIIb receptor gene polymorphisms in BD, and to investigate possible genotype-phenotype relationships.*

Methods. *In this cross-sectional study, FcγRIIa (H/H131, H/R131, R/R131), IIIa (F/F158, F/V158, V/V158) and IIIb (NA1/NA1, NA1/NA2, NA2/NA2) receptor gene polymorphisms were investigated in 216 unrelated Turkish BD patients (M/F: 130/86) and in 241 healthy subjects, using an allele-specific polymerase chain reaction.*

Results. *The FcγRIIa R/R131 (p=0.019) and FcγRIIIa F/F158 genotypes (p=0.001) were found to be significantly more frequent in BD compared with healthy controls, whereas the FcγRIIIb genotypes were not (p=0.108). Allele analysis showed that the FcγRIIIa F158 (p=0.001) and FcγRIIIb NA2 (p=0.016) alleles were more frequent in BD than in healthy controls. In BD patients the FcγRIIIa V/V158 genotype was significantly associated with the presence of arthritis (p=0.002) and with an earlier disease onset (p=0.008), while the FcγRIIIb NA2/NA2 genotype was significantly associated with disease severity (p=0.02), vascular involvement (p=0.014), and pathergy positivity (p=0.02).*

Conclusion. *We found that the genotype frequencies and allelic distributions of the FcγRIIa, FcγRIIIa and FcγRIIIb gene polymorphisms were significantly different between BD patients and healthy controls. In addition, certain FcγRIIIa and FcγRIIIb gene polymorphisms appear to be associated with an early disease onset, disease severity, the presence of arthritis, and vascular involvement in BD.*

Introduction

Behçet's disease (BD) is a systemic vasculitis characterized by recurrent oral, genital ulcerations and ocular inflammation. It may also involve the joints, skin, vessels, and central nervous and gastrointestinal systems (1). Although neutrophil hyperreactivity and endothelial cell dysfunction contribute to the pathogenesis (2-4), the exact etiology of BD is still unknown. However, the most widely accepted hypothesis is that of a profound inflammatory response triggered by an infectious agent in a genetically susceptible host (5).

There is some evidence that infectious agents such as *Streptococci* could be involved in the pathogenesis of BD (5, 6). Streptococcal antigens and *S. aureus* activate BD lymphocytes to release increased proinflammatory cytokines (7). In addition, oral streptococcal colonization is increased in BD patients and the effectiveness of antibacterial treatments supports the role of streptococci in BD (8).

Receptors for the Fc domain of IgG (FcγRs), which binds IgG containing immune complexes/IgG autoantibodies, provide a critical link between humoral and cellular immunity (9, 10). These receptors are found on diverse hematopoietic cells, including neutrophils, monocytes, lymphocytes, natural killer cells, and platelets. FcγRs have been shown to play critical roles in the initiation and regulation of many immunological and inflammatory processes and to intensify the immune response to infection (11). Activating the FcγRs (FcγRI, FcγRIIa, FcγRIIIa, FcγRIIIb) potentiates phagocytosis in response to IgG-containing immune complexes/opsonized microorganisms

and triggers degranulation, the oxidative burst, and the release of cytokines. Polymorphic variants of FcγRs could play an essential role in determining the inflammation mediated by IgG antibodies and IgG-containing immune complexes, as well as in inducing susceptibility to specific infections that may be associated with vascular/inflammatory diseases (11).

Gene polymorphisms and/or mutations may play a role in the pathogenesis of BD, leading to genetic susceptibility. For this reason various gene polymorphisms and/or mutations have been studied in BD (5). However, to date there have been no published reports on polymorphisms of the human FcγR in BD. We therefore conducted this study of FcγRIIa, FcγRIIIa and FcγRIIIb gene polymorphisms in Turkish BD patients in order to see whether there is an association between these polymorphisms and various clinical features of BD.

Table I. Clinical characteristics of the patients with Behçet's disease.

Characteristic	N	(216)
Male/Female	130/86	
Mean (range) age, years	38.6 ± 9.7	(16-70)
Mean (range) disease duration, months	113.7 ± 84.5	(1-360)
Mean (range) disease onset time, years	29.6 ± 7.6	(6-53)
Recurrent oral ulcers, n (%)	216	(100 %)
Recurrent genital ulcers, n (%)	189	(87.5 %)
Eye involvement	89	(41.2 %)
Vascular involvement	91	(42.1 %)
Deep venous thrombophlebitis	78	(36.1 %)
Superficial thrombophlebitis	53	(24.5 %)
Large vessel involvement	38	(17.6%)
Arthritis	74	(34.3%)
Central nervous system involvement	20	(9.3 %)

Materials and methods

Patients

For this cross-sectional study, 216 consecutive BD patients (M/F: 130/86, mean age: 38.63±9.69 years, age range: 16-70 years) being followed at out-patient clinics of Ege University Hospital were enrolled; all fulfilled the International Study Group Criteria (12). The patients' characteristics are shown in Table 1. BD patients with any coexisting autoimmune disease such as type I diabetes mellitus were excluded. A group of 241 ethnically matched, unrelated healthy volunteers were investigated as normal controls.

In addition to comparing the genotypes and allele frequencies of FcγR genes between BD and healthy controls, we also investigated whether in BD patients particular genotypes and/or alleles were significantly associated with parameters such as gender, mean age at disease onset, disease severity and specific clinical manifestations, including eye involvement, vascular involvement and arthritis. The subgroup of BD patients with vascular involvement was diagnosed based on the presence of deep vein thrombosis or large vessel involvement or both.

In BD patients the total clinical severity score [as defined by Krause (13)] was calculated as follows: 1 point each for mild symptoms (oral ulcers, genital ulcers, typical skin ulcers, arthralgia, recurrent headaches, epididymitis, mild gastrointestinal symptoms, pleuritic pains, and superficial vein thrombosis); 2 points each for moderate symptoms (arthritis, deep venous thrombosis, anterior uveitis, and gastrointestinal bleeding); and 3 points each for severe disease manifestations (posterior/panuveitis, retinal vasculitis, arterial thrombosis or aneurysm, major vein thrombosis, neuro-Behçet's, and bowel perforation). Patients were sub-divided into severe (severity score ≥4) and mild (severity score <4) disease activity groups.

We also wanted to compare early and late onset disease. If the onset was before 30 years of age, the patient was designated as having early onset disease.

The Institutional Review Boards of Ege University Medical Center approved the study. Written informed consent was obtained from all study participants.

DNA purification

Genomic DNA from patients and healthy controls was extracted from peripheral blood leukocytes using the QIAmp DNA Blood Mini Kit 50 (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions.

FcγRIIa genotyping

FcγRIIa genotyping was performed using the allele-specific PCR described previously by Flesh *et al.* (14). Briefly, a 25-μl PCR mixture containing 2.5 μI of genomic DNA, 2.5 μI of 10X PCR buffer (Applied Biosystems, Foster City, CA, USA), 2 mM $MgCl_2$, 200 μmol/I of each dNTP (Promega, Madison, USA), and 0.5 U AmpliTag DNA Polymerase (Applied Biosystems) was used. In addition, 0.5 μmol/l of H131-specific sense primer (5'-ATCC-CAGAAATTCTC-CCA-3') from the second extracellular domain of FcγRIIa receptor or 0.5 μmol/l of R131-specific sense primer (5'-ATCCCAGAAATTCTCCCG-3') and 0.5 μmol/I of common anti-sense primer from an area downstream of the intron (5'-CAATTTTGCTGCTAT-GGGC-3') were employed. All primer oligonucleotides were synthesized by QIAGEN (Qiagen Operon Co.) The resulting fragment was 253 bp in length. As the internal PCR control, we used 0.125 μmol/I of human growth hormone (HGH)-1 forward primer (5'-CAGTGCCTTCCCAACCATTCCCT-TA-3') and 0.125 μmol/I of HGH-II reverse primer (5'-ATCCACTCACG-GATTTCTGTTGTGTTTC-3'), which resulted in a 439-bp fragment.

A thermal cycler (GeneAmp 9700, Applied Biosystems) was employed to perform a hot-start PCR as follows: 5 min at 95ºC, 10 cycles of 1 min at 95ºC, 2 min at 57ºC, and 1 min at 72ºC; thereafter, to enhance the sensitivity we used 22 cycles of 1 min at 95ºC, 2 min at 54ºC, and 1 min at 72ºC and a final extension step for 5 min at 72ºC. The PCR amplification products were separated on a 2% agarose gel and visualized by ethidium bromide staining.

FcγRIIIa genotyping
Genotyping was performed by allele-specific PCR modified from Wu *et al*. (15). Five oligonucleotide primers were used, three of which were designed specifically for typing genomic DNA at position 559 in FcγRIIIA. The FcγRIIIA-specific forward primer (5'-TCA CAT ATT TAC AGA ATG GCA ATG G-3') corresponding to the FcγRIIIA sequence between positions 449 and 473 was used in both T allele-specific and G allele-specific PCR assays. The reverse primers corresponding to nt 586–559 provided allele specificity. The nt 559 G-specific reverse primer (5'-TCT CTG AAG ACA CAT TTC TAC TCC CTA C-3') differed in one nucleotide from the T-specific reverse primer (5'-TCT CTG AAG ACA CAT TTC TACTCC CTA A-3') at the 3' end. Two primers from the T-cell receptor Va22 gene (Ctrl-1: 5'-GAT TCA GTG ACC CAG ATG GAA GGG-3') and (Ctrl-2: 5'-AGC ACA GAA GTA CAC CGC TGA GTC-3') amplified a fragment of 270 bp and were used as the internal positive control.
The PCR reaction was performed in a GeneAmp 9700 PCR System with 100 ng of DNA, 200 nM of each primer, 200 mM of dNTPs (Promega, Madison, USA), 1.5 mM of $MgCl_2$, and 1.0 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems) in a 25-reaction volume starting with a sequence at 95ºC 10 min, 10 cycles of 1 min at 95ºC, 2 min at 57ºC, and 1 min at 72ºC. Thereafter, to enhance the sensitivity we used 22 cycles of 1 min at 95ºC, 2 min at 54ºC, and 1 min at 72ºC, with a final extension step for 5 min at 72ºC. The allele-specific PCR product of 138 bp was assayed on a 2% agarose gel and visualized by ethidium bromide staining. Again, two PCR procedures were required for the genotyping of a subject.

FcγRIIIb genotyping
A PCR modified from Bux *et al.* (16) was used for the FcγRIIIb-NA1/NA2 genotyping. The NA1-specific primer (5'-CAGTGGTTTCACAATGTGAA-3') yielded a 141-bp fragment, and the NA2-specific primer (5'-CAATGGTACAGCGTGCTT-3') amplified a 219-bp fragment. The reverse primer (5'-ATGGACTTCTACCTGCAC-3') did not discriminate between the two allotypes. Because there was a substantial difference in length between the NA1-specific and NA2-specific reaction products, both alleles could be detected in the same reaction. The 25 μl reaction mixture contained 100 ng of genomic DNA, 2.5 μI of 10X PCR buffer (Applied Biosystems), 2.5 nM of $MgCl_2$, 1 nmol/I of each dNTP (Promega), 4 pmol of each of the control primers, 0.012 nmol of the NA1 and NA2 primers, 0.025 nmol of the reverse primer, and 0.5 U of AmpliTag DNA polymerase (Applied Biosystems). After denaturation for 3 min at 94ºC, 35 PCR cycles consisting of 94ºC for 1 min, 57ºC for 2 min, and 72ºC for 1 min were run. After a final extension of 7 min at 72ºC, the samples were resolved in 2% agarose gels and stained with ethidium bromide.
Before sequencing, the PCR products were purified using a Millipore Montage PCR purification kit (Millipore, Bedford, MA, USA). Big D terminator chemistry (v3.1) (Applied Biosystems, Foster City, CA) was employed for these reactions, and sequences were resolved using the ABI 310 Genetic Analyser system. For the sequence evaluation, ABI Prism 230 DNA sequencing analysis software was used.

Statistical analysis
Statistical analyses were performed using the SPSS 12.0 for Windows statistical package (SPSS, Chicago, Illinois, USA).
Allele and genotype frequencies were compared by means of 2x2 and 3x2 contingency tables, respectively, and the χ^2 test was used for the statistical comparisons. Two-sided *p*-values less than 0.05 were considered statistically significant. Odds ratios (ORs) and their 95% confidence intervals (95%CIs) were calculated to quantify the magnitude of the association between alleles and phenotypes.
The distribution of the control genotypes was checked for Hardy-Weinberg equilibrium. The overall distribution of alleles and genotypes for each polymorphism was compared between cases and controls using chi-square analysis. ORs and 95%CIs were calculated to assess the risk associated with particular alleles and genotypes.
A power calculation was conducted to determine the number of samples for both patients and controls in order to achieve 99% power to detect an OR of 2.5 at the 5% significance level, assuming both autosomal dominant and autosomal recessive models of inheritance. This was carried out using the EPI Info software package, version 6.
Single nucleotide polymorphism spectral decomposition (SNPSpD) was employed to calculate the Meff value and correct for multiple testing.

Results
The demographic data and clinical characteristics of the BD patients are delineated in Table I. The median age at disease onset and the median disease duration were 29.04±7.09 (range 6-44) years and 113.7±84.5 months, respectively. There was no significant difference between male and female BD patients with regard to these parameters. The distributions of all the alleles were in Hardy-Weinberg equilibrium in the healthy controls.

FcγRIIa genotype and allele frequencies
In the BD group, the FcγRIIa R/R131 genotype was significantly more frequent than in the healthy controls (p=0.019). The distribution of FcγR genotypes and FcγR alleles in the BD and control groups is shown in Table II. The FcγRIIa R131 allele was also more frequent in BD than in healthy controls, but the difference was not significant (p=0.157).

FcγRIIIa genotype and allele frequencies
In the BD group, the FcγRIIIa F/F158 genotype was significantly more frequent than in the healthy controls (p=0.001). The frequency of the FcγRIIIa F158 allele in the BD group was also significantly higher than in the healthy controls (Fisher's exact test, p=0.001).

FcγRIIIb genotype and allele frequencies
In the BD group, the FcγRIIIb NA2/

Table II. (a) Distribution of FcγR genotypes in the patients with BD and healthy controls.

FcγRIIa	HH		HR		RR		Statistics	
	n	%	n	%	n	%	χ^2	*P*
Behçet's disease	100	46.3	80	37	36	16.7	7.88	0.019*
Healthy controls	114	47.5	106	44.2	20	8.3		
FcγRIIIa	**FF**		**FV**		**VV**			
	n	%	n	%	n	%	χ^2	*p*
Behçet's disease	129	59.7	47	21.8	40	18.5	42.581	0.001
Healthy controls	76	31.5	116	48.1	49	20.3		
FcγRIIIb	**NA1/NA1**		**NA2/NA2**		**NA1/NA2**			
	n	%	n	%	n	%	χ^2	*p*
Behçet's disease	32	14.8	109	50.5	75	34.7	4.452	0.108
Healthy controls	50	20.8	100	41.7	90	37.5		

χ^2: chi square test.

(**b**) Distribution of FcγR alleles in BD patients and healthy controls.

FcγRIIa	H		R		Statistics	Odds ratio
	n	%	n	%	*p*	
Behçet's disease	280	64.8	152	35.2	0.157*	1.147 (0.94-1.38)
Healthy controls	330	69.3	146	30.7		
FcγRIIIa	**F**		**V**			
		n	%	n	%	*p*
Behçet's disease	283	65.5	149	34.5	0.001*	1.514 (1.34-1.713)
Healthy controls	206	43.3	270	56.7		
FcγRIIIb	**NA1**		**NA2**			
	n	%	n	%	*p*	
Behçet's disease	139	32.2	293	67.8	0.016*	1.13 (1.02-1.25)
Healthy controls	190	39.9	286	60.1		

*Fisher's exact test.

NA2 genotype was more frequent than in healthy controls, but the difference was not significant (*p*=0.108). The frequency of the FcγRIIIb NA2 allele in the BD group was significantly higher than in healthy controls (*p*=0.016).

Association of FcγR genotypes with clinical characteristics of BD

We found no association between the FcγRIIa gene and the clinical characteristics of BD (data not shown). In contrast, FcγRIIIa gene analysis showed the V/V158 genotype to be significantly associated with both earlier disease onset (*p*=0.008) and the presence of arthritis in BD (*p*=0.002). Likewise, FcγRIIIa V158 allele frequency was significantly higher in BD patients with arthritis compared to those without (*p*=0.002, OR:1.613, 95% 1.21-2.14). On the other hand, the F/F158 and F/V158 genotypes of FcγRIIIa were significantly associated with central nervous system (CNS) involvement in BD (*p*=0.028). The correlations between FcγRIIIa genotypes and the clinical manifestations of BD are shown in Table III.

FcγRIIIb gene analysis showed the NA2/NA2 genotype to be significantly associated with pathergy positivity, pure deep vein thrombosis, and vascular involvement. Even more importantly, the NA2/NA2 genotype was significantly associated with disease severity (Krause clinical severity score) (*p*=0.004). Clinical associations with the FcγRIIIb genotypes in BD are shown in Table IV.

Allele analysis revealed that the NA2 allele was significantly associated with pathergy positivity (*p*=0.020) and vascular involvement (*p*=0.014). There was no significant association between large vessel involvement and the FcγRIIIb genotypes or alleles (*p*>0.05).

Discussion

To our knowledge, this is the first study investigating FcγRs gene polymorphisms in BD. We found that the FcγRIIa R/R131 and FcγRIIIa F/F158 genotypes, as well as the FcγRIIIa F158 and FcγRIIIb NA2 alleles, were significantly more frequent in BD than in healthy controls. Furthermore, the FcγRIIIa V/V158 genotype was significantly associated with the presence of arthritis and an earlier disease onset, while the FcγRIIIb NA2/NA2 genotype was significantly associated with disease severity and vascular involvement.

Since the immunological abnormalities in BD may possibly be induced by microbial pathogens in genetically susceptible individuals (5), gene polymorphisms and/or mutations could also play a role in the pathogenesis of BD. In the light of previous polymorphism studies (5), we investigated BD pathogenesis from the perspective of FcγR gene polymorphism. FcγR gene polymorphisms potentially have the ability to inhibit or stimulate the immune response. In other words, some genotypes and allelic variants of the FcγRs are considered to be susceptibility factors and disease accelerants in autoimmune diseases. Supporting this view, in earlier studies many autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (RA) were reported to be associated with FcγR gene polymorphisms (17). Even if the role of autoimmunity in BD is controversial (6), we believe that – independently of the autoimmunity aspect – the possible role of FcγR gene polymorphisms in genetic susceptibility to BD deserves investigation.

Uncontrolled, innately related inflammation causing secondary adaptive immune system activation has also been suggested to play a role in BD pathogenesis (6, 18). Since an increase in the expression of activating FcγRs (namely

Table III. Clinical associations with FcγRIIIa genotypes in BD.

	FF		FV		VV		Total		Statistics	
	n	%	n	%	n	%	n	%	χ^2	*p*
Gender Male	77	59.2	29	22.3	24	18.5	130	100	0.059	0.971
Female	52	60.3	18	20.9	16	18.6	86	100		
Vascular involvement (+)	55	60.4	19	20.9	17	18.7	91	100	0.072	0.965
Vascular involvement (-)	74	59.2	28	22.4	23	18.4	125	100		
Eye involvement (+)	55	61.8	22	24.7	12	13.5	89	100	2.791	0.248
Eye involvement (-)	74	58.3	25	19.7	28	22	127	100		
Arthritis (+)	38	51.4	14	18.9	22	29.7	74	100	9.378	0.009
Arthritis (-)	91	64.1	33	23.2	18	12.7	142	100		
Skin involvement (+)	126	59.7	46	21.8	39	18.5	211	100	0.013	0.993
Skin involvement (-)	3	60.0	1	20	1	20	5	100		
CNS* involvement (+)	9	45.0	9	45	2	10	20	100	7.135	0.028
CNS* involvement (-)	120	61.2	38	19.4	38	19.4	196	100		
Recurrent genital ulcer (+)	113	59.8	42	22.2	34	18	189	100	0.379	0.828
Recurrent genital ulcer (-)	16	59.3	5	18.5	6	22.2	27	100		
Deep venous thrombosis (+)	47	60.3	17	21.8	14	17.9	78	100	0.027	0.986
Deep venous thrombosis (-)	82	59.4	30	21.7	26	18.8	138	100		
Large vessel involvement (+)	26	68.4	4	10.5	8	21.1	38	100	3.418	0.181
Large vessel involvement (-)	103	57.9	43	24.2	32	18	178	100		
Pathergy positive	59	62.8	15	16	20	21.3	94	100	3.516	0.172
Pathergy negative	70	57.4	32	26.2	20	16.4	122	100		

*Central nervous system.

Table IV. Clinical associations with FcγRIIIb genotypes in BD.

	NA1/NA1		NA2/NA2		NA1/NA2		Total		Statistics	
	n	%	n	%	n	%	n	%	χ^2	*p*
Gender Male	19	14.6	66	50.8	45	34.6	130	100	0.02	0.992
Female	13	15.1	43	50	30	34.9	86	100		
Vascular involvement (+)	5	5.5	57	62.6	29	31.9	91	100	14.21	0.001
Vascular involvement (-)	27	21.6	52	41.6	46	36.8	125	100		
Eye involvement (+)	13	14.6	39	43.8	37	41.6	89	100	3.37	0.185
Eye involvement (-)	19	15.0	70	55.1	38	29.9	127	100		
Arthritis (+)	11	14.9	43	58.1	20	27.0	74	100	3.22	0.200
Arthritis (-)	21	14.8	66	46.5	55	38.7	142	100		
Skin involvement(+)	31	14.7	107	50.7	73	34.6	211	100	0.25	0.885
Skin involvement (-)	1	20.0	2	40	2	40	5	100		
CNS* involvement (+)	3	15.0	10	50	7	35	20	100	0.01	0.999
CNS* involvement (-)	29	14.8	99	50.5	68	34.7	196	100		
Recurrent genital ulcer (+)	28	14.8	97	51.3	64	33.9	189	100	0.54	0.762
Recurrent genital ulcer (-)	4	14.8	12	44.4	11	40.7	27	100		
Deep venous thrombosis (+)	5	6.4	48	61.5	25	32.1	78	100	9.04	0.011
Deep venous thrombosis (-)	27	19.6	61	44.2	50	36.2	138	100		
Large vessel involvement (+)	4	10.5	23	60.5	11	28.9	38	100	1.94	0.379
Large vessel involvement (-)	28	15.7	86	48.3	64	36	178	100		
Pathergy Positive	6	6.4	58	61.7	30	31.9	94	100	12.53	0.002
Pathergy Negative	26	21.3	51	41.8	45	36.9	122	100		

*Central nervous system.

FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb, as well as their polymorphisms) can affect the degree of innately related inflammation, we may speculate that the FcγRs play a role in BD pathogenesis by affecting the innate immunity. Bacterial antigens such as lipotheicoic acid could stimulate the production of proinflammatory cytokines [including interleukin-6 and interferon-γ (IFN-γ)] by the peripheral blood mononuclear cells, which in turn increase the expression of activating FcγRs on innate immune cells. These activating FcγRs play critical roles in augmenting the inflammatory processes against infectious agents by stimulating phagocytosis and triggering degranulation, the oxidative burst, and the release of cytokines (19).

Before discussing the effect of FcγR gene polymorphism on BD, the cellular expression of FcγRs in BD needs to be summarized. In the literature we found only two studies of neutrophil FcγR expression in BD and these reported different results. Using flow cytometry, Eksioglu-Demiralp *et al.* (20) found decreased neutrophil FcγRIII expression in active BD, whereas Ureten *et al.* (21) reported increased neutrophil FcγRI expression in active BD.

Our finding of a significant association between the FcγRIIIa V158 allele and arthritis in BD deserves attention, because this can be linked to the report of Morgan *et al.*, who showed in a large series of patients that this same allele was associated with RA (22). Although the arthritis in RA and BD are different in terms of pathogenesis and severity, the association of the same allele in RA and the subgroup of BD with arthritis is of interest.

Our data also appeared to show a significant association between the FcγRIIIa F/F158 and F/V158 genotypes and CNS involvement, but this result could have been due to a type 1 statistical error, since the number of BD patients with CNS involvement in our study was very small.

A gene polymorphism in BD can only be considered significant if it causes an altered functioning of the gene. However, a plausible link between the pathogenesis of BD and FcγR gene polymorphisms has not yet been found. For example, in our study the FcγRIIIb NA2/NA2 genotype was significantly associated with disease severity and vascular involvement in BD, but it is difficult for us to find a scientific explanation for this. Since the expression of FcγRIIIb is limited to neutrophils and eosinophils (23), one would expect to see a link between the NA2 allele and neutrophilic hyperactivity in BD. However, in contrast to the NA1 allele, the NA2 allele confers a decreased oxidative burst and degranulation response in neutrophils (24, 25). Likewise, in the normal population, individuals with the FcγRIIIb NA2/NA2 homozygous genotype are reported to have a lower capacity to mediate neutrophilic phagocytosis than individuals bearing the NA1 alleles (23, 24). This means that if there is actually a causal relationship between the FcγRIIIb NA2/NA2 genotype and disease severity in BD, it does not appear to be related to the neutrophilic hyperactivity commonly reported in BD. The possible explanation for this conflicting finding may be that the pathogenesis of BD is complicated and cannot be solely attributed to neutrophilic hyperactivity. In other words, the relationship between the NA2/NA2 homozygous genotype and disease severity in BD could be independent of the neutrophilic phagocytic capacity. It should be kept in mind that, in addition to the NA2 allele, there are several other factors, including HLA-B51 positivity, which might affect neutrophilic activity and the oxidative burst in BD (5).

Apart from BD, the FcγRIIIb NA2 and NA1 alleles have also been studied in Guillain-Barré syndrome and multiple sclerosis (MS). In Guillain-Barré syndrome, an association between the NA2/NA2 homozygote genotype and disease activity was reported (26). In MS, patients with the FcγRIIIb NA1/NA1 homozygote genotype were found to have a significantly more benign course that those with the FcγRIIIb NA2 allele (27).

In conclusion, the genotype frequencies and allelic distribution of FcγRIIa, IIIa and IIIb gene polymorphisms are significantly different in BD compared with healthy controls. Although it is difficult at present to suggest a plausible explanation for this finding, certain FcγRIIIa and FcγRIIIb gene polymorphisms appear to be associated with an early disease onset, disease severity, the presence of arthritis, and vascular involvement in BD. Future studies are clearly needed to elucidate the relationship between FcγR gene polymorphisms and the pathogenesis of BD.

References

1. YAZICI H, YURDAKUL S, HAMURYUDAN V, FRESKO I: Behçet's syndrome. In: *Textbook of Rheumatology*, 3rd ed., New York, Mosby, 2003: 1665-9.
2. MIZUSHIMA Y: Chemotaxis and phagocytosis of leucocytes in Behçet's disease: an overview. *In* LEHNER T and BARNES CG (Eds.): *Recent Advances in Behçet's Disease*. London, Royal Society of Medicine Services, 1986: 85-7.
3. KANSU E, SIVRI B, SAHIN G *et al.*: Endothelial cell dysfunction in Behcet's disease. *In*: O'DUFFY JD and KOKMEN E (Eds.): *Behçet's Disease – Basic and Clinical Aspects*. New York, Marcel Dekker, 1991: 523-30.
4. SAHIN S, AKOGLU T, DIRESKENELI H, SEN LS, LAWRENCE R: Neutrophil adhesion to endothelial cells and factors affecting adhesion in patients with Behçet's disease. *Ann Rheum Dis* 1996; 55: 128-33.
5. GÜL A: Behçet's disease: An update on the pathogenesis. *Clin Exp Rheumatol* 2001: 24 (Suppl. 24): S6-12.
6. DIRESKENELI H: Autoimmunity vs autoinflammation in Behçet's disease: do we oversimplify a complex disorder? *Rheumatology (Oxford)* 2006; 45: 1461-5.
7. HIROHATA S, HASHIMATO T: Abnormal T cell responses to bacterial superantigens in Behçet's disease. *Clin Exp Immunol* 1998; 112: 17-24.
8. MUMCU G, ERGUN T, ELBIR Y *et al.*: Clinical and immunological effects of azithromycin in Behçet's disease. *J Oral Pathol Med* 2005; 34: 13-6.
9. SALMON JE, PRICOP L: Human receptors for immunoglobulin G. *Arthritis Rheum* 2001; 44: 739-50.
10. KIMBERLY RP, WU J, GIBSON AW *et al.*: Diversity and duplicity. *Immunologic Research* 2002; 26: 55-67.
11. MORGAN AW, ROBINSON JI, BARRETT JH *et al.*: Association of FCGR2A and FCGR2A-FCGR3A haplotypes with susceptibility to giant cell arteritis. *Arthritis Res Ther* 2006; 8: R109.
12. INTERNATIONAL STUDY GROUP FOR BEHÇET'S DISEASE: Evaluation of diagnostics ('classification') criteria in Behçet's disease – towards internationally agreed criteria. *Br J Rheumatol* 1992; 31: 299-308.
13. KRAUSE I, MADER R, SULKES J *et al.*: Behçet's disease in Israel: the influence of ethnic origin on disease expression and severity. *J Rheumatol* 2001; 28: 1033-6.

14. FLESCH BK, BAUER F, NEPPERT J: Rapid typing of the human Fcγ receptor IIA polymorphism by polymerase chain reaction amplification with allele-specific primers. *Transfusion* 1998; 38: 174-6.
15. WU J, EDBERG JC, REDECHA PB *et al*.: A novel polymorphism of FcγRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J Clin Invest* 1997; 100: 1059-70.
16. BUX J, STEIN EL, SANTOSO S, MUELLER-ECKHARDT C: NA gene frequencies in the German population, determined by the polymerase chain reaction with sequence-specific primers. *Transfusion* 1995; 35: 54-7.
17. TAKAI T: Fc receptors and their role in immune regulation and autoimmunity. *J Clin Immunol* 2005; 1: 1-18.
18. GÜL A: Behçet's disease as an autoinflammatory disorder. *Curr Drug Target Inflam & Allerg* 2005; 4: 81-3.
19. NIMMERJAHN F, RAVETCH JV: Fc receptors as regulators of immunity. *Adv Immunol* 2007; 96: 179-204.
20. DEMIRALP-EKSIOGLU E, DIRESKENELI H, KIBAROGLU A, YAVUZ S, ERGUN T, AKOGLU T: Neutrophil activation in Behçet's disease. *Clin Exp Rheumatol* 2001; 19 (Suppl. 24): S19-S24.
21. URETEN K, ERTENLI I, OZTURK MA *et al*.: Neutrophil CD64 expression in Behçet's disease. *J Rheumatol* 2005; 32: 849-52.
22. MORGAN AW, KEYTE VH, BABBAGE SJ *et al*.: FcγRIIIA-158V and rheumatoid arthritis: a confirmation study. *Rheumatology* 2003; 42: 528-33.
23. HULETT MD, HOGARTH PM: Molecular basis of Fc receptor function. *Adv Immunol* 1994; 57: 1-127.
24. SALMON JE, MILLARD SS, BROGLE NL, KIMBERLY RP: Fc gamma receptor IIIb enhances Fc gamma receptor IIa function in an oxidant-dependent and allele-sensitive manner. *J Clin Invest* 1995; 95: 2877-85.
25. VAN SORGE NM, VAN DER POL W-M, VAN DE WINKEL: FcγR polymorphisms: implications for function, disease susceptibility and immunotherapy. *Tissue Antigens* 2003; 61: 189-202.
26. VEDELER CA, MYHR KM, NYLAND H: Fc receptors for immunoglobulin G: a role in the pathogenesis of Guillain-Barré syndrome and multiple sclerosis. *J Neuroimmunol* 2001; 118: 187-93.
27. MYHR KM, RAKNES G, NYLAND H, VEDELER C: Immunoglobulin G Fc-receptor (FcγR) IIA and IIIB polymorphisms related to disability in multiple sclerosis. *Neurology* 1999; 52: 1771-6.