Black, Rachel; Lester, Susan Elizabeth; Dunstan, Emma Victoria; Shahram, Farhad; Nadji, Abdolhadi; Bayat, Noushin; Saeedfar, Kayvan; Ziaei, Naghmeh; Hill, Catherine Louise; Rischmueller, Maureen; Davatchi, Fereydoun
Fc-gamma receptor 3B copy number variation is not a risk factor for Behçet's disease, International Journal of Rheumatology, 2012; 2012:167096

Hindawi Publishing Corporation
International Journal of Rheumatology
Volume 2012, Article ID 167096, 4 pages
doi:10.1155/2012/167096

*Research Article*

# Fc-Gamma Receptor 3B Copy Number Variation Is Not a Risk Factor for Behçet's Disease

**Rachel Black,[1] Sue Lester,[1] Emma Dunstan,[1] Farhad Shahram,[2] Abdolhadi Nadji,[2] Noushin Bayat,[2,3] Kayvan Saeedfar,[2,4] Naghmeh Ziaei,[2] Catherine Hill,[1,5] Maureen Rischmueller,[1,5] and Fereydoun Davatchi[2]**

[1] *Department of Rheumatology, The Queen Elizabeth Hospital, 28 Woodville Road, Woodville South, SA, 5011, Australia*
[2] *Rheumatology Research Centre, Tehran University of Medical Sciences, Shariati Hospital, Kargar Avenue, Tehran 14114, Iran*
[3] *Rheumatology Department, Baqyiatallah University of Medical Sciences, Baghiatallah hospital, Molla Sadra Street, Tehran 14359, Iran*
[4] *Chronic Respiratory Diseases Research Centre, Shahid Beheshti University of Medical Sciences, Massih Daneshvari Hospital, Shaid Bahonar Street, Tehran 19556, Iran*
[5] *Discipline of Medicine, The University of Adelaide, Adelaide, SA 5005, Australia*

Correspondence should be addressed to Rachel Black, rachel.black@health.sa.gov.au

Received 13 February 2012; Accepted 10 April 2012

Academic Editor: Lorenzo Beretta



Behçet's disease (BD) is an immune-mediated systemic vasculitis associated with HLAB51. Other gene associations are likely and may provide further insight into the pathogenesis of this disease. Fc-gamma receptors play an important role in regulating immune function. Copy number variation (CNV) of the Fc-gamma receptor 3B (FCGR3B) gene is associated with other inflammatory conditions and may also play a role in BD. The aim of this study was to determine whether CNV of the FCGR3B gene is associated with BD or its clinical features. FCGR3B copy number was determined for 187 Iranian patients and 178 ethnicity-matched controls using quantitative real-time PCR. The genotype frequencies were comparable in both BD patients and controls. The odds ratio for low copy number (<2CN) was 0.6 ($P = 0.16$) and the odds ratio for high copy number (>2CN) was 0.75 ($P = 0.50$). There was no association found between high or low CN of the FCGR3B gene and BD or its clinical features in this Iranian population. We are the first to report this finding which, when looked at in the context of other genetic studies, gives us further insight into the complex pathogenesis of BD.

## 1. Background

Behçet's disease (BD) is an immune-mediated, systemic vasculitis, in which blood vessels of all sizes (small, medium, and large) in both the venous and arterial circulation can be affected. Clinically it is characterised by recurrent aphthous ulcers of the mouth and/or genitals in combination with other systemic manifestations involving the skin, eyes, joints, vessels, gastrointestinal tract, or central nervous system [1].

The pathogenesis of BD is not understood. It lies at the crossroad between autoimmune and autoinflammatory disorders [2], may be triggered by infectious agents, and is characterised by a number of immunological aberrations, such as neutrophil hyperactivity (reviewed in [3, 4]). There is also clearly a genetic component. BD is most frequently observed in populations around the Mediterranean basin, the Middle East, and the Far East, and the clustering of BD in populations along the ancient Silk Route suggests that an inherited tendency to BD was spread by travellers along these trading routes. Multiple studies, in multiple populations, have confirmed a strong association between HLA-B51 and BD [4], but other genes are also likely to be involved. Copy number variation in the *FCGR3B* gene is one candidate gene of interest.

Copy number variation (CNV) is departure from the normal diploid number of genes ($n = 2$) which may arise from gene duplication and deletion events and which may contribute substantially to quantitative variation in expression. An increasing number of CNVs have been characterised in the human genome with implications for both evolution and disease susceptibility [5]. CNV has been well characterised in the FCGR gene cluster on chromosome 1q23. This cluster carries 5 highly homologous genes that encode for low affinity receptors for IgG-complexed antigens, which are expressed widely throughout the haematopoietic system. These low affinity Fc-gamma receptors are involved in the regulation of a multitude of innate and adaptive immune responses, with implications for both response to infection and susceptibility to autoimmunity [6].

CNV in the *FCGR3B* gene is of particular interest. FCGR3B is expressed almost exclusively on neutrophils [6], and there is a clear correlation between gene copy number and FCGR3B cell surface expression, neutrophil adherence to IgG-coated surfaces, and immune complex uptake [7]. Further, multiple studies have identified low FCGR3B CN (i.e., <2 copies) as a risk factor for systemic autoimmune diseases, such as systemic lupus erythematosus [8–10], rheumatoid arthritis [11, 12], and primary Sjögren's syndrome [9, 10]. We therefore examined the association between *FCGR3B* CNV and BD, in a cohort of Iranian patients.

## 2. Methods

*2.1. Subjects.* Ethics approval was obtained from the Rheumatology Research Centre's ethics committee of Tehran University of Medical sciences. A written informed consent was obtained from all participants. 187 Iranian BD patients were recruited from the Behçet's clinic at Shariati Hospital, Tehran, from early 2005 until late 2006. 178 ethnically-matched, unrelated healthy volunteers were also recruited as normal controls between 2005 and 2007. All the BD patients recruited for this study met the International Study Group (ISG) criteria for BD [13]. Baseline data was collected using a standardised questionnaire at the time of blood sampling. Information was obtained regarding their age, gender, ethnicity, clinical features, and family history. Serotyping for HLA-B51 status was carried out in selected general immunologic laboratories within Iran using routine commercial kits. Samples were then transported to Australia for further analysis.

*2.2. FCGR3B Copy Number (CN) Genotyping.* Genomic FCGR3B CN was determined using a quantitative real-time PCR method, as previously described [11]. Briefly, a duplex Taqman copy number assay was performed, using FCGR3B specific primers (Applied Biosystems, Hs04211858, FAM-MGB dual labeled probe) and RNase P (Applied Biosystems, product 4403326, VIC-TAMRA dual-labeled probe) as the reference assay. The assay was performed according to the manufacturer's instructions and PCR reactions were run on an Applied Biosystems 7300 Real-Time PCR machine. All samples were tested in triplicate, and fluorescence signals were normalised to ROX. Copy number was determined using Copy Caller software (v.1.0, Applied Biosystems, USA), and results were accepted only when calling confidence was >80%, and ΔCq standard deviation between replicates was <0.20.

*2.3. Statistical Analysis.* Analysis of *FCGR3B* CN in BD patients compared to controls, and with clinical manifestations within BD patients, was performed using logistic regression. Analysis was performed using Statistica v6 (Statsoft, Tulsa OK, USA).

Table 1: Baseline characteristics of patients with Behçet's Disease.

| Characteristic | *N* (187) |
|---|---|
| Age at diagnosis (yrs), mean (±SD) | 32.7 (8.2) |
| Female, *n* (%) | 89 (48%) |
| Duration of symptoms at diagnosis (yrs), mean (±SD) | 6.7 (6.1) |
| HLA testing | |
| HLA B51 positive | 41/80 (51%) |
| HLA B5 positive | 89/175 (51%) |
| Other tests | |
| Skin pathergy | 101/107 (94%) |
| Symptoms | |
| Mucous membrane symptoms | 187 (100%) |
| Skin symptoms | 116 (62%) |
| Ocular symptoms | 112 (60%) |
| Neurological symptoms | 15 (8%) |
| Joint symptoms | 58 (31%) |
| Gastrointestinal symptoms | 13 (7%) |
| Vascular lesions | 14 (7%) |
| ESR | |
| <20 | 83 (48%) |
| 20–49 | 58 (34%) |
| ≥50 | 32 (18%) |

Table 2: Distribution of *FCGR3B* copy number (CN) variants in Behçet's (BD, $N = 187$) patients and controls ($N = 178$). When compared to 2 CN, there was no evidence that <2 CN or >2 CN genotypes were different in frequency between BD patients and controls ($P = 0.16,\ 0.50$, resp.).

| *FCGR3B* CN | BD | Controls |
|---|---|---|
| 1 | 15 (8.0%) | 22 (12.4%) |
| 2 | 161 (86.1%) | 143 (80.3%) |
| 3 | 10 (5.3%) | 12 (6.7%) |
| 4 | 1 (0.5%) | 1 (0.6%) |
| Total | 187 | 178 |

## 3. Results

187 BD patients were included in the study (mean age 32.7 ± 8.2, 48% female). Baseline characteristics are summarised in Table 1. Of those who were tested for HLA status, 51% (89

Table 3: Codistribution of FCGR3B copy number variation with clinical features within Behçet's patients.

| Clinical Parameter | | *FCGR3B* CNV <2 | =2 | >2 | *N* | *P*[1] |
|---|---|---|---|---|---|---|
| Onset age: median (interquartile range) | | 26 (21,36) | 26 (21,31) | 25 (22,30) | 187 | 0.67 |
| Gender | Male: count (%) | 8 (8.2%) | 87 (88.8%) | 3 (3.1%) | 98 | 0.26 |
| | Female: count (%) | 7 (7.9%) | 74 (83.1%) | 8 (9.0%) | 89 | |
| HLA-B5 | Neg: count (%) | 8 (9.3%) | 71 (82.6%) | 7 (8.1%) | 86 | 0.71 |
| | Pos: count (%) | 7 (7.9%) | 78 (87.6%) | 4 (4.5%) | 89 | |
| ESR | Normal: count (%) | 9 (10.8) | 70 (84.3%) | 4 (4.8%) | 83 | 0.40 |
| | Elevated: count (%) | 4 (4.4%) | 80 (88.9%) | 6 (6.7%) | 90 | |
| Genital apthosis[2] | No: count (%) | 7 (10.8%) | 53 (81.5%) | 5 (7.7%) | 65 | 0.79 |
| | Yes: count (%) | 8 (6.6%) | 108 (88.5%) | 6 (4.9%) | 122 | |
| Pseudofolliculitis | No: count (%) | 10 (10.0%) | 86 (86.0%) | 4 (4.0%) | 100 | 0.13 |
| | Yes: count (%) | 5 (5.8%) | 75 (86.2%) | 7 (8.1%) | 87 | |
| Erythema nodosum | No: count (%) | 13 (9.4%) | 116 (83.5%) | 10 (7.2%) | 139 | 0.98 |
| | Yes: count (%) | 2 (4.2%) | 45 (93.8%) | 1 (2.1%) | 48 | |
| Arthritis | No: count (%) | 13 (8.9%) | 126 (86.3%) | 7 (4.8%) | 146 | 0.17 |
| | Yes: count (%) | 2 (4.9%) | 35 (85.4%) | 4 (9.8%) | 41 | |
| Uveitis | No: count (%) | 9 (8.3%) | 91 (84.3%) | 8 (7.4%) | 108 | 0.61 |
| | Yes: count (%) | 6 (7.6%) | 70 (88.6%) | 3 (3.8%) | 79 | |
| Retinal vasculitis | No: count (%) | 9 (7.5%) | 104 (86.7%) | 7 (5.8%) | 120 | 0.81 |
| | Yes: count (%) | 6 (9.0%) | 57 (85.1%) | 4 (6.0%) | 67 | |
| Venous thrombosis | No: count (%) | 15 (8.6%) | 149 (85.6%) | 10 (5.8%) | 174 | 0.33 |
| | Yes: count (%) | 0 (0%) | 12 (92.3%) | 1 (7.7%) | 13 | |

[1] Ordinal *P* value (proportional odds ratio).
[2] All patients had oral apthosis.

of 175) were HLA B5 positive and 51% (41 of 80) were HLA B51 positive.

The frequency of *FCGR3B* CN variants in both BD patients and controls is presented in Table 2. Copy numbers ranged from 1 to 4, and no null genotypes were observed in this cohort. The genotype frequencies were comparable in both BD patients and controls. The odds ratio for low copy number (<2 CN) was 0.6 (95% CI 0.30, 1.21, $P = 0.16$) and the odds ratio for high copy number (>2 CN) was 0.75 (96% CI 0.33, 1.73, $P = 0.50$). Therefore there was no evidence that either low or high *FCGR3B* was associated with BD, and in fact, both low CN and high CN were slightly decreased in BD patients relative to controls. Further, there was no evidence of associations between *FCGR3B* CN variants and clinical manifestations within BD patients as shown in Table 3.

## 4. Discussion

This is the first study to examine the relationship between *FCGR3B* CN variants and Behçet's disease, and we report no evidence of an association in terms of either disease susceptibility or clinical manifestations in this cohort of patients from Iran. However, a previous study of Turkish BD patients has reported associations between *FCGR2A*, *FCGR3A,* and *FCGR3B* SNPs and BD, in terms of both disease susceptibility and clinical manifestations [14], but this remains unconfirmed.

Other studies have reported intriguing but conflicting relationships between *FCGR3B* CN and vasculitis in the context of different diseases. For example, *FCGR3B* low CN (<2) is associated with Granulomatosis with Polyangiitis (Wegener's Granulomatosis) and Microscopic Polyangiitis (antineutrophil cytoplasmic antibody-associated systemic vasculitidies) in one study [8], and high *FCGR3B* CN (>2) in another [7], and no association has been observed with vasculitis in conjunction with systemic lupus erythematosus [15]. Further, similar to BD, susceptibility to other systemic vasculitidies including Giant Cell Arteritis and Kawasaki's disease has also been linked to SNPs within the FCGR gene cluster [16, 17].

The FCGR gene cluster is a complex genomic region, with both SNP and CNV polymorphism. While we were unable to demonstrate an association between *FCGR3B* CN and BD in this study, there are undoubted links to polymorphism in this region with vasculitic conditions. In future, the challenge will be to integrate CNV and SNP data into haplotypes in order to systematically evaluate and contrast associations with different vasculitidies. Such studies will provide valuable insight into the underlying pathogenetic mechanisms involved.

## Conflict of Interests

The authors declare that they have no conflict of interests.

## Acknowledgments

A health services grant, code no. 85-132/8485, from the Research Deputy of Tehran University of Medical Sciences was received. In Australia, funding for this study was obtained through Arthritis Australia and The Queen Elizabeth Hospital Research Foundation.

## References

[1] T. Sakane, M. Takeno, N. Suzuki, and G. Inaba, "Behçet's disease," *The New England Journal of Medicine*, vol. 341, no. 17, pp. 1284–1291, 1999.

[2] D. McGonagle and M. F. McDermott, "A proposed classification of the immunological diseases," *PLoS Medicine*, vol. 3, no. 8, article e297, 2006.

[3] V. D. Kapsimali, M. A. Kanakis, G. A. Vaiopoulos, and P. G. Kaklamanis, "Etiopathogenesis of Behçet's disease with emphasison the role of immunological aberrations," *Clinical Rheumatology*, vol. 29, no. 11, pp. 1211–1216, 2010.

[4] M. Pineton de Chambrun, B. Wechsler, G. Geri, P. Cacoub, and D. Saadoun, "New insights into the pathogenesis ofBehçet's disease," *Autoimmunity Reviews*. In press.

[5] P. Stankiewicz and J. R. Lupski, "Structural variation in the human genome and its role in disease," *Annual Review of Medicine*, vol. 61, pp. 437–455, 2010.

[6] F. Nimmerjahn and J. V. Ravetch, "Fc$\gamma$ receptors as regulators of immune responses," *Nature Reviews Immunology*, vol. 8, no. 1, pp. 34–47, 2008.

[7] L. C. Willcocks, P. A. Lyons, M. R. Clatworthy et al., "Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake," *Journal of Experimental Medicine*, vol. 205, no. 7, pp. 1573–1582, 2008.

[8] M. Fanciulli, P. J. Norsworthy, E. Petretto et al., "FCGR3B copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity," *Nature Genetics*, vol. 39, no. 6, pp. 721–723, 2007.

[9] M. Mamtani, J. M. Anaya, W. He, and S. K. Ahuja, "Association of copy number variation in the FCGR3B gene with risk of autoimmune diseases," *Genes and Immunity*, vol. 11, no. 2, pp. 155–160, 2010.

[10] J. C. Nossent, M. Rischmueller, A. Becker-Merok, and S. Lester, "Low copy number of Fcgamma receptor 3B gene is a disease susceptibility an severity factor in primay Sjögren's syndrome," *Arthritis & Rheumatism*, vol. 63, article S389, 2011.

[11] S. W. Graf, S. Lester, J. C. Nossent, C. L. Hill, S. Proudman, and A. Lee, "Low copy number of the FCGR3B gene and rheumatoid arthritis: a case control study and meta-analysis," *Arthritis Research & Therapy*, vol. 14, no. 1, article R28, 2012.

[12] C. McKinney, M. Fanciulli, M. E. Merriman et al., "Association of variation in Fc$\gamma$ receptor 3B gene copy number with rheumatoid arthritis in Caucasian samples," *Annals of the Rheumatic Diseases*, vol. 69, no. 9, pp. 1711–1716, 2010.

[13] International Study Group for Behçet's disease, "Criteria for diagnosis of Behçet's disease," *The Lancet*, vol. 335, pp. 1078–1080, 1990.

[14] K. Aksu, G. Kitapcioglu, G. Keser et al., "Fc$\gamma$RIIa, IIIa and IIIb gene polymorphisms in Behçet's disease: do they have any clinical implications?" *Clinical and Experimental Rheumatology*, vol. 26, no. 4, supplement 50, pp. S77–S83, 2008.

[15] H. A. Niederer, M. R. Clatworthy, L. C. Willcocks, and K. G. C. Smith, "Fc$\gamma$RIIB, Fc$\gamma$RIIIB, and systemic lupus erythematosus," *Annals of the New York Academy of Sciences*, vol. 1183, pp. 69–88, 2010.

[16] A. W. Morgan, J. I. Robinson, J. H. Barrett et al., "Association of FCGR2A and FCGR2A-FCGR3A haplotypes with susceptibility to giant cell arteritis," *Arthritis Research and Therapy*, vol. 8, no. 4, article R109, 2006.

[17] C. C. Khor, S. Davila, W. B. Breunis, Y. C. Lee, C. Shimizu, V. J. Wright et al., "Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease," *Nature Genetics*, vol. 43, no. 12, pp. 1241–1246, 2011.