To the editor:

Association between p47^{phox} pseudogenes and inflammatory bowel disease

We read with great interest the recent article by Heyworth et al,[1] which describes the ratio between the p47^{phox} pseudogenes (ψ*NCF1*) and the p47^{phox} gene (*NCF1*). Gene duplication has prevented elucidation of the genomic sequence at 7q11.23, although the ψ*NCF1/NCF1* ratio had been assumed[2-4] to be 2:1. Specifically, the location and quantity of ψ*NCF1* pseudogenes is unknown. Heyworth et al[1] demonstrated the ratio to be 1:1 and 1:2 in 13% and 4% of healthy individuals, respectively, the rest being 2:1. Using a family study, they elegantly showed that variability in the ratio probably occurred following DNA exchange by recombination or, conceivably, gene conversion between *NCF1* and ψ*NCF1*, to produce a gene hybrid (type II ψ*NCF1*). Similar to *NCF1*, type II ψ*NCF1* contains a GT repeat (GTGT) at the start of exon 2, and therefore its transcription product encodes a full-length protein that is homologous to *NCF1*. ψ*NCF1*, however, contains a dinucleotide deletion at this allele (ΔGT) that results in a premature stop codon. The functional significance of type II ψ*NCF1* remains unknown.

We adapted a Genescan method[5] to try to identify *NCF1* heterozygotes using the ΔGT/GTGT ratio in 138 patients with inflammatory bowel disease (IBD) and 37 healthy individuals. Several findings suggested that *NCF1* haploinsufficiency could be a susceptibility factor for IBD. First, chronic bowel inflammation is a feature of chronic granulomatous disease (CGD) caused by p47^{phox} deficiency. Second, as with CGD, defects in innate immunity are found in Crohn disease (CD).[6,7] Third, significant linkage has been demonstrated in IBD[8] to microsatellite markers spanning a 22-centimorgan (cM) region of chromosome 7, which encompasses the *NCF1* locus. Finally, p47^{phox} heterozygotes have reduced neutrophil oxygen consumption in response to phorbol myristate acetate but have normal neutrophil oxygen consumption in response to opsonized zymosan,[9] and we noted the same phenomenon in a minority of patients with CD (M.H., unpublished observation, March 2002).

We were surprised to find an excess of IBD patients with a ΔGT/GTGT ratio of approximately 1:1, which was greater in patients with CD (CD, 22.4%; control, 8.1%; Fisher exact test, $P < .05$, odds ratio 3.3) than in those with ulcerative colitis (UC) (UC, 14.1%; $P = .28$) (Figure 1). This suggested that type II ψ*NCF1* might be a susceptibility factor for CD. Therefore, we assessed the effect of the ΔGT/GTGT ratio on cellular migration into an acute inflammatory cantharidin blister.[10] In patients with a ratio lower than 1.2 (n = 10; 5 CD, 3 healthy subjects) there was a significant increase in blister cell number compared to those with a ratio higher than 1.2 (n = 62; 28 CD, 26 healthy subjects), comprising a geometric mean 3.27×10^6 cells/mL, compared with 1.33×10^6 cells/mL (*t* test, $P = .04$). This finding applied both to neutrophils (1.52×10^6 cells/mL compared with 0.60×10^6 cells/mL) and macrophages (0.32×10^6 cells/mL compared with 0.16×10^6 cells/mL). The mechanism is unknown.

Three parents of confirmed p47^{phox} CGD patients, presumed heterozygous for mutant *NCF1*, had ratios of 3.4, 3.2, and 1.6 (Figure 1). The ratio of 1.6 indicates either that the type II ψ*NCF1* pseudogene was present or that spontaneous mutation had occurred in *NCF1* to cause CGD.

We agree with the conclusion of Heyworth et al that determining the ΔGT/GTGT ratio cannot always detect *NCF1* heterozygosity. As such, we were unable to conclusively refute our original hypothesis, although it appears unlikely that p47^{phox} haploinsufficiency is a susceptibility factor for IBD. However, it is conceivable that the presence of type II ψ*NCF1* exaggerates the inflammatory response and so predisposes to IBD. This needs to be confirmed in a replicative association study.

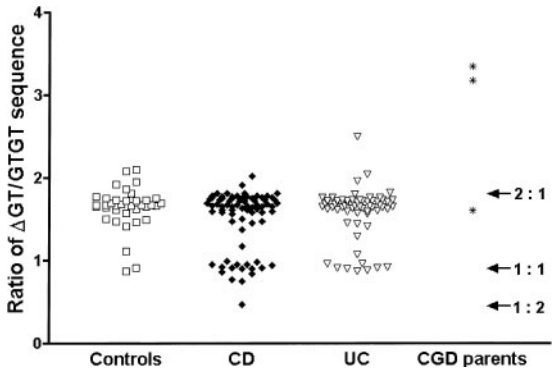

Figure 1. Ratio of ΔGT/GTGT sequence in patients with CD and UC and 3 parents of CGD patients. Three ratio populations were apparent, approximating to 2:1, 1:1, and 1:2. There was a significant excess of the 1:1 ratio in CD ($P < .05$), implying an excess of the type II ψ*NCF1* pseudogene. Each point represents the mean of triplicate measurements.

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