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Inducible nitric oxide synthase (NOS2) gene polymorphism and parasitic diseases

Sir—David Burgner and colleagues (Oct 10, p 1193)[1] report an association between a polymorphism in the promoter region of the inducible nitric oxide synthase (*NOS2*) gene and the risk of fatal cerebral malaria. This result accords with the findings of Kun and co-workers early this year.[2] Altogether these results suggest that susceptibility to severe malaria seems to be determined by complex genetic factors at the *NOS2* locus

We investigated whether the single nucleotide polymorphism (G-C) in the *NOS2* promoter region,[2] which is located at position –954 (not at –969 as previously reported[2]), is associated with susceptibility to or severe forms of Chagas' disease. We included 85 patients who were serologically positive for Chagas' disease and 87 healthy controls from Arequipa, Peru, South America.[3] Patients were grouped according to the presence (n=33) or absence (n=52) of cardiomyopathy.[3] The *NOS2* promoter mutation was analysed by PCR-RFLP with amplification-created restriction site (ACRS) for *Xho* I enzyme. A 60 bp fragment was amplified with 5' primer: 5'-CACTTGAGCTTCAGAGCTC and 3' primer: 5'-TGGTAGAGACTGGGTTTCAC. The mutated 5' primer introduces a restriction site for *Xho* I that recognises the wild type allele. There is a natural restriction site for *Bsa* I that directly detects the mutation,[2] however, *Xho* I is readily available and cheaper. Analysis of all the samples in the Peruvian population showed that only the wild type occurred.

Although studies show association between *NOS2* promoter polymorphisms and severe malaria, the functional consequences of these genetic variations in the *NOS2* expression is not known. Given the absence of *NOS2* promoter mutation in our population where *Trypanasoma cruzi* infection is endemic and the important role of nitric oxide in the defence against the parasite,[4] we believe that *NOS2* promoter mutation at –954 may have no functional relevance. On the other hand, Kremsner's group[2] reported that this mutation was not found in 100 controls. Therefore, the *NOS2* polymorphisms may be restricted to populations subjected to selective pressure of *Plasmodium falciparum*. Further studies of distribution of the *NOS2* promoter polymorphisms in other areas or ethnic groups where malaria is also endemic will help to elucidate this topic.

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