Letters to the Editors

Lack of association between *Toll-like receptor 4* gene polymorphism and Henoch-Schönlein purpura

Sirs,

Henoch-Schönlein purpura (HSP) is the most common type of primary small-sized blood vessel vasculitis in children and a rare entity in adults (1, 2). Infections are reported to be precipitating factors in at least 50% of the cases (1, 2). Infectious agents are also involved in the modulation the innate immune system, and low-grade chronic or recurrent infections and several infectious agents have been proposed to play a role in autoimmunity.

Genetic factors may determine the immunological and inflammatory response to unknown antigens in patients with HSP. Previous studies have shown that different genes may influence the phenotype and the outcome of HSP (3, 4). Human toll-like receptors (TLRs) participate in the innate response and signal the activation of adaptive immunity. TLR-4 recognises lipopolysaccharide (LPS) of gram-negative bacteria, products from fungal pathogens, a soluble component of *Mycobacterium* tuberculosis and endogenous ligands, such as fibronectin and several heat shock proteins (5).

The *TLR4* gene is involved in innate immune recognition with subsequent proinflammatory cytokine release (6). A single nucleotide polymorphism-SNP-(+896A/G) resulting in the amino acid substitution Aspartic acid/Glycine (Asp299Gly) (rs4986790), in high linkage disequilibrium with other non-synonymous polymorphisms of *TLR4* in Caucasian population, has been proposed to interrupt TLR-4 mediated signalling (6). The mutant allele (G) of this variant, which occurs with an allelic frequency of less than 6% in most European populations (7), was associated with decreased susceptibility to some autoimmune disorders such as rheumatoid arthritis (8).

Taking into account all these considerations, in the present study we sought to determine the potential implication of the *TLR4* (+896 A/G) gene polymorphism in the susceptibility to HSP.

Patients with cutaneous vasculitis that fulfilled classification criteria for HSP (1, 2) were recruited from the Divisions of Pediatrics and Rheumatology of the Hospital Xeral-Calde (Lugo, northwest Spain). Sixty HSP patients (47 children [≤20 years] and 13 adults; 30 males) with at least 2 year's follow-up were studied. All patients presented palpable purpura. Forty-two had arthralgias and/or arthritis, 48 suffered severe gastrointestinal manifestations (gastrointestinal bleeding and/or bowel angina) and 39 haematuria with or without proteinuria. After a minimum of 2 year's follow-up (median 8 years) only 12 (20%) had persistent renal involvement, mainly hematuria.

Age and sex and ethnically matched controls without history of cutaneous vasculitis or any other autoimmune diseases (n=234) were also studied.

DNA from patients and controls was obtained from peripheral blood. Samples were genotyped for the *TLR4-(+896 A/G)* (rs4986790) gene polymorphism by polymerase chain reaction, using a pre-designed TaqMan allele discrimination assay (7). Informed consent and ethical approval was obtained.

No evidence of departure from Hardy-Weinberg equilibrium was observed in controls.

No significant differences for this biallelic polymorphism were observed between HSP patients and controls (Table I). It was also the case when patients were stratified by the presence of severe gastrointestinal manifestations, nephritis or persistent renal involvement (data not shown).

Few studies have assessed the potential influence of the functional *TLR4* (+896 A/G) gene polymorphism in the susceptibility to primary systemic vasculitides. In this regard, we have recently observed an increased frequency of the mutant *TLR4*-allele G in biopsy-proven giant cell arteritis (GCA) patients compared to controls (7). However, no significant association was found in GCA patients from Reggio-Emilia, Italy (9).

The present report constitutes the first attempt to establish the potential influence of this functional biallelic polymorphism in the susceptibility to cutaneous vasculitis. However, as reported for other common gene polymorphisms (10, 11), the *TLR4* (+896 A/G) gene polymorphism does not appear to be a genetic risk factor for HSP. Although the frequency of the mutant allele G in controls (5.7%) was quite similar to that described in other reports assessing the Spanish population (5.7%, 5.4%) (7, 12), this study that was focused on a rare disease is limited by the low sample size (power <20%). Therefore, further studies are needed to fully exclude the contribution of the *TLR4* (+896 A/G) gene polymorphism in the pathogenesis of HSP.

Table I. Genotypic and allelic frequencies of TLR4 rs4986790 polymorphism in patients with HSP and controls.

TLR4		HSP patients n (%)	Controls n (%)	*p*-value	Odds ratio (95% CI)
rs4986790		n=60	n=234		
Genotype					
	A/A	54 (90.0)	208 (88.9)	0.81	1.02 (0.4- 2.5)
	A/G	5 (8.3)	25 (10.7)	0.60	0.90 (0.3- 2.3)
	G/G	1 (1.7)	1 (0.4)	0.30	3.90 (0.4- 38.2)
Allele					
	A	113 (94.2)	441 (94.3)	0.98	0.90 (0.4- 2.1)
	G	7 (5.8)	27 (5.7)	0.98	1.11 (0.5- 2.5)

O. TORRES, *PhD*[1]
R. PALOMINO-MORALES, *PhD*[1]
J.A. MIRANDA-FILLOY, *MD*[2]
T.R. VAZQUEZ-RODRIGUEZ, *MD*[2]
J. MARTIN, *MD, PhD*[1*]
M.A. GONZALEZ-GAY, *MD, PhD*[2*]

[*]Drs Martin and Gonzalez-Gay share senior authorship in this study.

[1]Instituto de Parasitología y Biomedicina López Neyra, CSIC, Granada, [2]Division of Rheumatology, Hospital Xeral-Calde, Lugo, Spain.

Address correspondence to:
Miguel A. Gonzalez-Gay, MD, PhD,
Rheumatology Division, Hospital Xeral-Calde,
c) Dr Ochoa s/n, 27004 Lugo, Spain.
E-mail: miguelaggay@hotmail.com

Competing interests: none declared.

References

1. CALVIÑO MC, LLORCA J, GARCÍA-PORRÚA C, FERNÁNDEZ-IGLESIAS JL, RODRIGUEZ-LEDO P, GONZÁLEZ-GAY MA: Henoch-Schönlein purpura in children from northwestern Spain: a 20-year epidemiologic and clinical study. *Medicine* (Baltimore) 2001; 80: 279-90.
2. GARCÍA-PORRÚA C, CALVIÑO MC, LLORCA J, COUSELO JM, GONZÁLEZ-GAY MA: Henoch-Schönlein purpura in children and adults: clinical differences in a defined population. *Semin Arthritis Rheum* 2002; 32: 149-56.
3. AMOLI MM, THOMSON W, HAJEER AH *et al*.: HLA-DRB1*01 association with Henoch-Schönlein purpura in patients from Northwest Spain. *J Rheumatol* 2001; 28: 1266-70.
4. AMOLI MM, THOMSON W, HAJEER AH *et al*.: Interleukin-8 gene polymorphism is associated with increased risk of nephritis in cutaneous vasculitis. *J Rheumatol* 2002; 29:2367-70.
5. FERWERDA B, MCCALL MB, VERHEIJEN K *et al*.: Functional consequences of toll-like receptor 4 polymorphisms. *Mol Med* 2008; 14:346-52.
6. ARBOUR NC, LORENZ E, SCHUTTE BC *et al*.: TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000; 25: 187-91.
7. PALOMINO-MORALES R, TORRES O, VAZQUEZ-RODRIGUEZ TR *et al*.: Association between *Toll-like receptor 4* gene polymorphism and biopsy-proven giant cell arteritis. *J Rheumatol* 2009; 36: 1501-6.
8. RADSTAKE TR, FRANKE B, HANSSEN S *et al*.: The Toll-like receptor 4 Asp299Gly functional variant is associated with decreased rheumatoid arthritis disease susceptibility. *Arthritis Rheum* 2004; 50: 999-1001.
9. BOIARDI L, CASALI B, FARNETTI E *et al*.: Toll-like receptor 4 (TLR4) gene polymorphism in giant cell arteritis. *Clin Exp Rheumatol* 2009; 27 (Suppl. 52): S40-4.
10. AMOLI MM, MARTIN J, MIRANDA-FILLOY JA, GARCIA-PORRUA C, OLLIER WE, GONZALEZ-GAY MA: Lack of association between interleukin-6 promoter polymorphism at position -174 and Henoch-Schönlein purpura. *Clin Exp Rheumatol* 2007; 25(Suppl. 44): S6-9.
11. OROZCO G, MIRANDA-FILLOY JA, MARTIN J, GONZALEZ-GAY MA: Lack of association of a functional single nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with susceptibility to Henoch-Schönlein purpura. *Clin Exp Rheumatol* 2007; 25: 750-3.
12. URCELAY E, BLANCO-KELLY F, DE LAS HERAS V, DE LA CONCHA EG, ARROYO R, MARTÍNEZ A: TLR4 haplotypes in multiple sclerosis: a case-control study in the Spanish population. *J Neuroimmunol* 2007; 192: 215-8.