Lupus (2019) 28, 365–370
journals.sagepub.com/home/lup

PAPER

Anti–α-enolase antibody combined with β2 microglobulin evaluated the incidence of nephritis in systemic lupus erythematosus patients

Y Huang[1], L Chen[2], K Chen[1], F Huang[1], Y Feng[1], Z Xu[1] and W Wang[1]
[1]Department of Laboratory Medicine, Sun Yat-sen University affiliated Zhongshan Hospital, Zhongshan, China; and [2]Department of Pharmacy, Shenzhen Children's Hospital, Shenzhen, China

Objective: Anti–α-enolase antibody (Ab) combined with β2 microglobulin (β2-MG) were investigated to predict the incidence of nephritis in systemic lupus erythematosus (SLE) patients. **Methods:** Levels of serum anti–α-enolase Ab and urinary β2-MG were detected in 115 SLE patients, 29 SLE patients with nephritis and 70 healthy controls by ELISA and immunoturbidimetry, respectively. Furthermore, the correlation between anti–α-enolase Ab combined with β2-MG and the incidence of nephritis in SLE patients was evaluated by correlation analysis. **Results:** The optical density value of serum anti–α-enolase Ab in SLE patients with nephritis (0.84) was greatly increased compared with SLE patients (0.76) or healthy controls (0.54). Moreover, the levels of urinary β2-MG in SLE patients with nephritis (6.75 mg/L) were increased compared with SLE patients (3.45 mg/L) or healthy controls (1.48 mg/L). There was a positive correlation between the level of anti–α-enolase Ab and β2-MG ($r = 0.754$). Furthermore, anti–α-enolase Ab combined with β2-MG for evaluating the incidence of nephritis in SLE patients had the best assessment of the effectiveness (area under the receiver operating characteristic curve (AUC): 92.7%) compared with only anti–α-enolase Ab (AUC: 80.9%) or β2-MG (AUC: 84.5%). **Conclusion:** These data suggested that anti–α-enolase Ab may be a potential indicator for the prediction of nephritis in SLE patients. *Lupus* (2019) **28,** 365–370.

Key words: Systemic lupus erythematosus; nephritis; anti–α-enolase antibody; β2 microglobulin

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with a highly variable course and prognosis. The management of the disease is still a clinical challenge for the treating physicians in many aspects regarding the disease pathogenesis, clinical picture and outcomes.[1–3] Renal disease is a frequent complication of SLE which can lead to significant illness and even death.[4,5] Early diagnosis of renal disease can be helpful in the case of flares in SLE patients with an already established chronic renal insufficiency or in the case of patients who do not respond to treatment.[4] Therefore, development of optimal clinical diagnostic indicators for the nephritis in SLE patients is highly necessary.

β2 microglobulin (β2-MG), a light-chain molecule of the major histocompatibility complex class I antigens, is a low-molecular-weight (11 kDa) protein located on the surface membrane of almost all nucleated cells.[6] In clinical work, β2-MG is often used as a classic marker of renal tubule lesion. In Madureira et al.'s study, the authors suggested that serum β2-MG/cystatin C index could be a better indicator of renal activity in SLE.[7] However, Wakabayashi et al. found that serum β2-MG level is an indicator of disease activity and response to therapy in other diseases, such as adult-onset Still's disease (AOSD).[8] So it is necessary to find a more specific indicator to evaluate the activity of SLE complicated with nephritis.

Correspondence to: Wei-jia Wang, Sun Yat-sen University affiliated Zhongshan Hospital, 2 East of Sun Wen Road, Shi Qi District, Zhongshan 528403, Guangdong Province, China.
Email: wwj@163.com
Received 8 August 2018; accepted 11 January 2019

10.1177/0961203319828822

α-enolase catalyzed the dehydration from 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway, as a multifunctional protein encoded by a single copy gene localized on chromosome 1.[9] Meanwhile, α-enolase is a component of the complex neutrophil extracellular trappings (NETs) which are released by neutrophils via an active process coined NETosis.[10] NETosis is a major trigger of autoimmunity in lupus externalization of α-enolase during lupus flares leading to formation of circulating α-enolase antibody (Ab).[11] Li et al. supported that anti–α-enolase Ab associates with active renal disease in SLE and might reflect a state of active autoimmunity and fibrinolysis inhibition.[10] Especially, Bruschi et al. found that serum anti–α-enolase and/or anti-annexin AI were detected in most patients with lupus nephritis (LN) but not patients with other glomerulonephritides, and serum levels of both autoantibodies decreased significantly after 12 months of therapy for LN. Isotype analysis of anti–α-enolase in glomerular eluates showed maximal amount of IgG2 (11/20 patients), minimal amount of IgG1 and negative IgG3 and IgG4.[12] However, others have found the link of anti–α-enolase Ab with inflammatory diseases such as inflammatory bowel disease, SLE, rheumatoid arthritis, Alzheimer's disease or psoriasis.[13,14] Therefore, applying anti–α-enolase alone has certain limitations in responding to kidney damage.

In this study, we investigated the clinical significance of serum anti–α-enolase Ab and β2-MG level to predict the incidence of nephritis in SLE by reviewing medical records and the results of laboratory tests.

Materials and methods

Participants

This study enrolled 115 SLE patients, 29 SLE patients with LN and 70 healthy controls in Sun Yat-sen University affiliated Zhongshan Hospital hospitalized from June 2016 to February 2018. Informed consents were obtained from all individual participants. Clinical and laboratory data which were required to assess disease activity were recorded. Serum from 144 SLE patients and 70 healthy individuals were immediately frozen at –80°C. All of the SLE patients or healthy controls displayed no evidences of infection, including past or current infection.

Data extraction

Clinical characteristics and laboratory test results of all enrolled subjects were extracted from the medical records. In addition, SLE Disease Activity Index 2000 (SLEDAI-2K), a global index based on the symptoms and laboratory findings,[15] was calculated according to medical records from each patient.

ELISA

The serum obtained from 144 SLE patients and 70 healthy controls were subjected to ELISA (#JL46123, Jianglai Biological, China), according to the manufacturer's instructions. Briefly, the Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of anti–α-enolase Ab in the sample, this anti–α-enolase Ab ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of optical density (OD) versus anti–α-enolase Ab concentration. The concentration of anti–α-enolase Ab in the samples is then determined by comparing the OD of the samples to the standard curve.

Ethics

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Board of the Sun Yat-sen University affiliated Zhongshan Hospital. This study had no influence on the subsequent management of patients.

Statistical analysis

Continuous variables were displayed as mean ± standard deviation and compared by Student's t test or Mann–Whitney U test. The Spearman approach was used to analyze the correlation between two continuous variables. All the statistical analyses were performed in SPSS 17.0 and GraphPad Prism 5.0. A p value < 0.05 was determined as significant.

Results

Clinical characteristics of the participants

Clinical characteristics of the 144 patients (115 SLE patients and 29 LN patients) and 70 healthy controls used in this study are shown in Table 1.

Levels of serum anti–α-enolase Ab and urinary β2-MG were increased in SLE with nephritis patients

Serum uric acid, serum cystatin C, creatinine and urea can reflect the function of the kidney. Compared with the control group, levels of serum uric acid ($308.62 \pm 67.60\,\mu mol/L$) and cystatin C ($0.97 \pm 0.28\,mg/L$) were increased in the SLE group. Furthermore, levels of serum uric acid ($365.90 \pm 125.45\,\mu mol/L$, Figure 1(a)) and cystatin C ($1.40 \pm 0.86\,mg/L$, Figure 1(b)) were increased significantly in LN patients. However, levels of serum creatinine (Figure 1(c)) and urea (Figure 1(d)) showed no significant differences between the three groups. β2-MG is a useful indicator of disease activity and response to therapy in SLE.[8] Levels of urinary β2-MG were $1.48 \pm 0.38\,mg/L$ in the control group, $3.45 \pm 3.27\,mg/L$ in the SLE group, $6.75 \pm 6.75\,mg/L$ in LN patients. Increased urinary β2-MG was observed in SLE patients with nephritis (Figure 2(a)). Anti–α-enolase Ab was associated with active renal disease in SLE.[10] As shown in Figure 2(b), patients with nephritis displayed significantly higher levels of anti–α-enolase Ab (OD value: 0.84 ± 0.02) compared with SLE patients (0.54 ± 0.17, $p = 0.01$).

Table 1 Characteristics of participants

	SLE patients	*Healthy controls*	p *value*
Age (y)	38 ± 13	45 ± 15	NS
Gender (male/female)	10/134	20/50	NS
RBC	4.22 ± 0.71	4.58 ± 0.46	0.001
HGB	119.26 ± 20.86	136.99 ± 13.24	0.001
D-dimer	1.67 ± 2.17	0.34 ± 0.08	0.001
C3	0.81 ± 0.27	1.18 ± 0.16	0.001
C4	0.17 ± 0.08	0.28 ± 0.06	0.001
IgA	2.83 ± 1.37	2.30 ± 0.74	0.091
IgG	12.55 ± 5.43	11.40 ± 1.95	0.009
IgM	1.01 ± 0.54	1.19 ± 0.50	0.176
ASO	52.66 ± 57.15	—	—
RF	32.66 ± 131.85	—	—
SLEDAI-2K score	9.5 ± 4.6		

ASO: anti-streptolysin O; C3: complement 3; C4: complement 4; HGB: hemoglobin; RBC: red blood cell; RF: rheumatoid factor; SLE: systemic lupus erythematosus; SLEDAI: SLE Disease Activity Index 2000.

Correlation between anti–α-enolase Ab and SLE complicated with nephritis

Firstly, we analyzed the correlation between serum anti–α-enolase Ab level and the above renal function indicators. Table 2 shows the *r* and

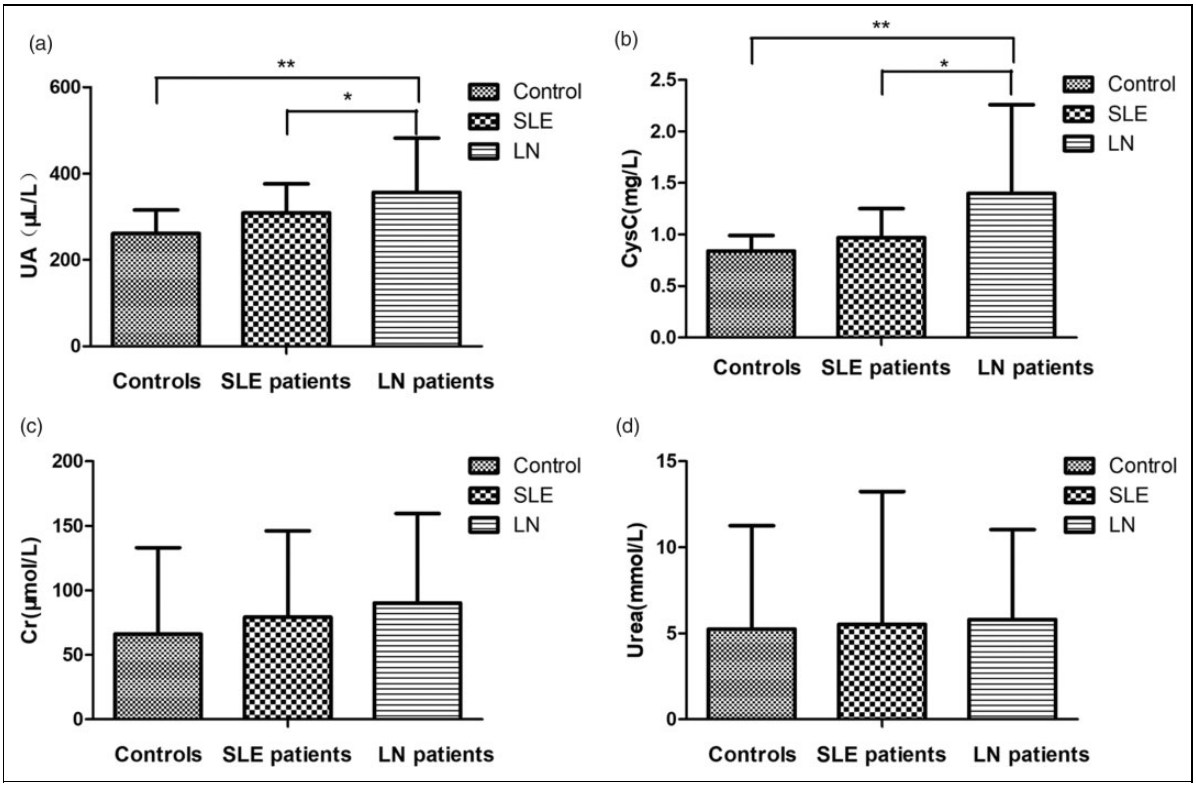

Figure 1 Renal function indicators serum uric acid (UA) (a), cystatin C (CysC) (b), creatinine (Cr) (c) and urea (d) in healthy controls, systemic lupus erythematosus (SLE) patients and lupus nephritis (LN) patients.

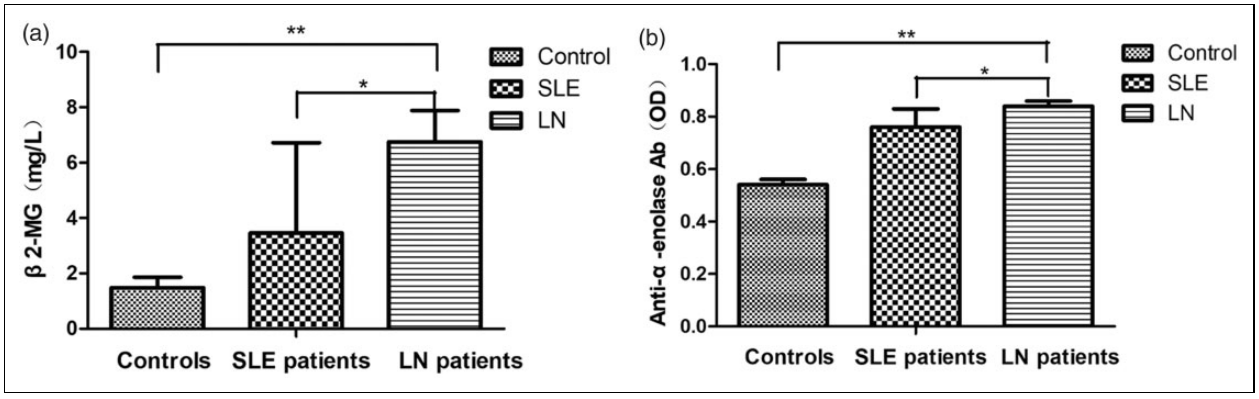

Figure 2 Urinary β2 microglobulin and serum anti–α-enolase antibody in health controls, systemic lupus erythematosus (SLE) patients and lupus nephritis (LN) patients.

Table 2 Analysis of the correlations between serum anti–α-enolase antibody level and β2 microglobulin (β2-MG), creatinine, uric acid and urea.

	β2-MG	*Creatinine*	*Uric acid*	*Urea*
r	0.754	0.608	0.728	0.491
p value	0.028	0.001	0.011	0.001

p values from the correlation analysis. We found β2-MG had the highest correlation with anti–α-enolase Ab ($r = 0.754$).

Then, we utilized anti–α-enolase Ab combined with β2-MG to predict the incidence of nephritis in SLE patients. We found that anti–α-enolase Ab combined with β2-MG for evaluating SLE complicated with nephritis had the best assessment of the effectiveness (area under the receiver operating characteristic curve (AUC): 92.7%) compared with only anti–α-enolase Ab (AUC: 80.9%) or β2-MG (AUC: 84.5%), respectively (Figure 3 and Table 3). These data suggested that anti–α-enolase Ab may be a potential indicator for SLE combined with nephritis disease.

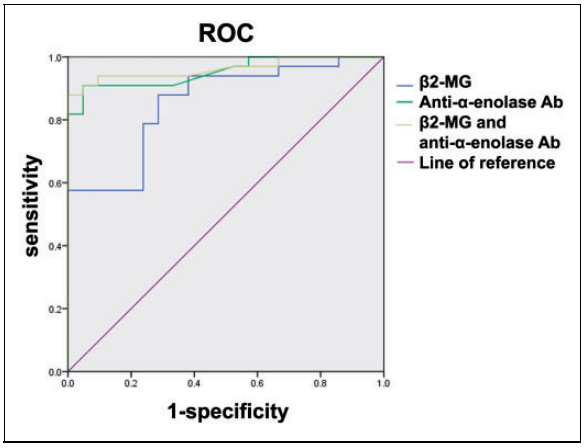

Figure 3 Receiver operating characteristic (ROC) curve for evaluating systemic lupus erythematosus complicated with nephritis by anti–α-enolase antibody (Ab) and β2 microglobulin (β2-MG).

Table 3 Evaluating SLE complicated with nephritis by anti–α-enolase antibody and β2 microglobulin.

	AUC	p *value*	*Sensitivity (%)*	*Specificity (%)*	*Youden index*
β2-MG	0.845	0.004	81.8%	90.0%	0.718
Anti–α-enolase Ab	0.809	0.001	82.2%	90.5%	0.727
β2-MG and anti–α-enolase Ab	0.927	0.001	91.9%	93.3%	0.852

Ab: antibody; AUC: area under the curve; β2-MG; β2 microglobulin.

Discussion

In the present study, we observed elevated levels of serum anti–α-enolase Ab in SLE patients. The serum level of anti–α-enolase Ab was relatively higher in the LN patients, and positively correlated with β2-MG. This is in line with previous studies detecting anti–α-enolase Abs in patients with SLE involving renal damage.[10,12] Moreover, our results also showed that serum level of anti–α-enolase Ab positively correlated with the level of uric acid, cystatin C, creatinine and urea. This means that an elevated level of anti–α-enolase Ab may be a marker reflecting overproduction in active SLE patients with renal involvement. The major finding

of this work demonstrated that anti–α-enolase Ab combined with β2-MG could evaluate the incidence of nephritis in SLE patients more effectively.

Bruschi et al. have done great work on anti–α-enolase in LN. The authors showed that the major isotype of anti-ENO is IgG2, which is a crucial point. However, the results of anti-ENO isotype were paradoxical. The result from the sample of renal biopsies showed maximal IgG2 (11/20 patients) and a minimal amount of IgG1; anti–α-enolase IgG3 and IgG4 were negative. But the result from the sample of serum showed that IgG2 was higher; IgG1 and IgG3 serum levels were undetectable in all patient categories; IgG4 was negative in LN and SLE patients.[12] Actually, in 2011, Bruschi et al. found that specific IgG1 and IgG4 reacting with podocyte α-enolase were eluted from microdissected glomeruli.[16] Therefore, circulating anti–α-enolase (a target antigen of autoimmunity in humans) autoantibodies can be detected, but the total anti-ENO or the anti–α-enolase IgG2 in serum should be chosen needs more research. It has been suggested that serum β2-MG level would be a useful indicator of disease activity and response to therapy, and a much higher level would be an indicator of either hepatopulmonary syndrome development or an unfavorable prognosis in patients with SLE and AOSD.[8,17] However, urinary β2-MG level for evaluating SLE complicated with nephritis is not well understood. In this study, we observed elevated levels of urine β2-MG level in SLE patients compared with healthy controls. The serum level of urinary β2-MG was relatively higher in the LN patients group than in the SLE patients group. When anti-α-enolase Ab combined with β2-MG were used to evaluate the incidence of nephritis in SLE patients, the area under the receiver operating characteristic curve (AUC) is 0.927. It is higher than anti-α-enolase Ab (0.809) or β2-MG (0.845) alone. Previous studies showed that β2-MG was a disease activity marker in SLE.[17–19] On the other hand, we also found that both the levels of serum anti–α-enolase Ab and urine β2-MG were higher in active SLE patients, and positively correlated with SLEDAI scores (data not show). In future studies, we aim to further investigate the relationship between serum anti–α-enolase Ab and markers for SLE activity including C3, C4, CRP and dsDNA.

In conclusion, anti–α-enolase Ab combined with urinary β2-MG for evaluating SLE complicated with nephritis had the best assessment of the effectiveness. We hope that its usefulness will be further investigated in a larger patient population.

Acknowledgment

We would like to thank all the patients and controls included in this study.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: National Nature Science Foundation of China (grant no. 81301492) and Medical research funds of Sun Yat−sen University affiliated Zhongshan Hospital (grant no. B2018093).

ORCID iD

Y Huang http://orcid.org/0000-0002-4843-7426

References

1 Adinolfi A, Valentini E, Calabresi E, *et al*. One year in review 2016: systemic lupus erythematosus. *Clin Exp Rheumatol* 2016; 34: 569–574.
2 Tsokos GC. Systemic lupus erythematosus. *New Eng J Med* 2011; 365: 2110–2121.
3 Xiong W, Lahita RG. Novel treatments for systemic lupus erythematosus. *Ther Adv Musculoskelet Dis* 2011; 3: 255–266.
4 Moroni G, Depetri F, Ponticelli C. Lupus nephritis: when and how often to biopsy and what does it mean? *J Autoimmun* 2016; 74: 27–40.
5 Komolafe OO. Rapidly progressive glomerulonephritis: a wild card manifestation of lupus nephritis. *Saudi J Kidney Dis Transpl* 2018; 29: 443–451.
6 Cooper EH, Plesner T. Beta-2-microglobulin review: its relevance in clinical oncology. *Med Pediatr Oncol* 1980; 8: 323–334.
7 Madureira Silva MV, Moscoso-Solorzano GT, Nishida SK, Mastroianni-Kirsztajn G. Serum Beta 2-microglobulin/cystatin C index: a useful biomarker in lupus nephritis? *Nephron Extra* 2012; 2: 169–176.
8 Wakabayashi K, Inokuma S, Matsubara E, *et al*. Serum beta2-microglobulin level is a useful indicator of disease activity and hemophagocytic syndrome complication in systemic lupus erythematosus and adult-onset Still's disease. *Clin Rheumatol* 2013; 32: 999–1005.
9 Giallongo A, Oliva D, Cali L, Barba G, Barbieri G, Feo S. Structure of the human gene for alpha-enolase. *Eur J Biochem* 1990; 190: 567–573.
10 Li M, Li J, Wang J, Li Y, Yang P. Serum level of anti-alpha-enolase antibody in untreated systemic lupus erythematosus patients correlates with 24-hour urine protein and D-dimer. *Lupus* 2018; 27: 139–142.

11 Bonanni A, Vaglio A, Bruschi M, *et al*. Multi-antibody composition in lupus nephritis: isotype and antigen specificity make the difference. *Autoimmun Rev* 2015; 14: 692–702.
12 Bruschi M, Sinico RA, Moroni G, *et al*. Glomerular autoimmune multicomponents of human lupus nephritis in vivo: alpha-enolase and annexin AI. *J Am Soc Nephrol* 2014; 25: 2483–2498.
13 Kang HJ, Jung SK, Kim SJ, Chung SJ. Structure of human alpha-enolase (hENO1), a multifunctional glycolytic enzyme. *Acta Crystallogr D Biol Crystallogr* 2008; 64: 651–657.
14 Fujii A, Yoneda M, Ito T, *et al*. Autoantibodies against the amino terminal of alpha-enolase are a useful diagnostic marker of Hashimoto's encephalopathy. *J Neuroimmunol* 2005; 162: 130–136.
15 Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002; 29: 288–291.
16 Bruschi M, Carnevali ML, Murtas C, *et al*. Direct characterization of target podocyte antigens and auto-antibodies in human membranous glomerulonephritis: alfa-enolase and borderline antigens. *J Proteomics* 2011; 74: 2008–2017.
17 Kim HA, Jeon JY, Yoon JM, Suh CH. Beta 2-microglobulin can be a disease activity marker in systemic lupus erythematosus. *Am J Med Sci* 2010; 339: 337–340.
18 Plesner T, Wiik A. Demonstration of electrophoretic heterogeneity of serum beta 2-microglobulin in systemic lupus erythematosus and rheumatoid arthritis: evidence against autoantibodies to beta 2-microglobulin. *Scand J Immunol* 1979; 9: 247–254.
19 Evrin PE, Strom T. Beta 2-microglobulin and its binding activity in serum from patients with SLE. *Ann Rheum Dis* 1984; 43: 267–274.