Letters to the Editor

Am. J. Hum. Genet. 76:184–187, 2005

The R620W Polymorphism of the Protein Tyrosine Phosphatase PTPN22 Is Not Associated with Multiple Sclerosis

To the Editor:

We recently reported the association of the minor allele of a missense SNP (1858C→T [dbSNP accession number rs2476601]) in the gene for hematopoietic-specific intracellular protein tyrosine phosphatase (*PTPN22*) with susceptibility to both rheumatoid arthritis (RA [MIM 180300]) (Begovich et al. 2004) and systemic lupus erythematosus (SLE [MIM 152700]) (Kyogoku et al. 2004). Independently, Bottini et al. (2004) reported that the same risk allele is also strongly associated with type 1 diabetes mellitus (T1D [MIM 222100]). This SNP results in the substitution of a highly conserved arginine with tryptophan (R620W) in the proximal proline-rich SH3-binding domain of PTPN22, which is important for interaction with the c-Src tyrosine kinase, Csk (Cloutier and Veillette 1996; Gregorieff et al. 1998), and for down modulation of T cell receptor signaling via phosphorylation of regulatory tyrosines of another Src family kinase, Lck (Cloutier and Veillette 1999; Gjorloff-Wingren et al. 1999). In vitro experiments show that the W620 variant of PTPN22 binds less efficiently to Csk (Begovich et al. 2004; Bottini et al. 2004), suggesting that T cells expressing this allele may be hyperresponsive. Correspondingly, knockout mice deficient in the murine orthologue of PTPN22, PEP, show selective disregulation of the effector/memory T cell compartment, with enhanced activation of Lck, hyperproliferation, and exaggerated early signaling responses in restimulated T cells (Hasegawa et al. 2004). These mice also spontaneously develop germinal centers and increased serum levels of certain immunoglobulin isotypes; however, they do not display overt signs of autoimmunity.

Together, these genetic and functional data suggest that the W620 variant of this phosphatase is a significant genetic risk factor for both organ-specific (T1D) and systemic (RA and SLE) autoimmune syndromes, perhaps because of enhanced activation of memory/effector T cells. These findings support the hypothesis that there are common genetic variants contributing to general immune dysregulation and susceptibility to autoimmunity (Marrack et al. 2001; Wandstrat and Wakeland 2001) and suggest that this SNP should be studied in other autoimmune disorders.

Multiple sclerosis (MS [MIM 126200]) is a severe autoimmune disorder of the CNS characterized by chronic inflammation, myelin loss, gliosis, varying degrees of axonal pathology, and progressive neurological dysfunction (Hauser and Goodin 2001). A large body of research supports a multifactorial etiology for MS, with an underlying complex genetic component likely acting in concert with undefined environmental exposures. *HLA-DR,* within the MHC on chromosome 6p21, is the only locus that has consistently demonstrated linkage in studies of families with MS (Haines et al. 1996; Sawcer et al. 1996; Barcellos et al. 2002), and both family-based and case-control MS studies have convincingly shown a specific association between the *HLA-DR2* (*DRB1* 1501, DQB1*0602*) haplotype and disease susceptibility (Fogdell-Hahn et al. 2000; Rubio et al. 2002; Barcellos et al. 2003). *HLA* has been estimated to account for 17%–62% of the genetic etiology as calculated from sibling relative risk (Haines et al. 1998); hence, much of the genetic effect in MS remains to be explained.

To determine whether the R620W SNP in *PTPN22* also plays a role in susceptibility to MS, we genotyped two large, well-characterized, family-based data sets of 748 MS-prone families (563 single-case and 185 multicase families) comprising 3,251 individuals, including 1,086 affected individuals and 2,165 unaffected family members. All individuals and their known ancestors were non-Hispanic whites of European descent. Diagnostic criteria, ascertainment protocols, and clinical and demographic characteristics are summarized elsewhere (Barcellos et al. 2002, 2003). A third African American data set was ascertained using identical clinical criteria and consisted of 398 cases and 209 controls (primarily spouses of patients) (Oksenberg et al. 2004). The appropriate institutional review boards approved all studies, and written informed consent was obtained from all participants. Genotyping was performed using a Taqman Assay By Design (Applied Biosystems). Primer and probe sequences were as follows: forward primer, 5′-CAACTGCTCCAAGGATAGATGATGA-3′; reverse primer, 5′-CCAGCTTCCTCAACCACAATAAATG-3′; probe for C allele, 5′-FAM-TCAGGTGTCCGTACAG-

G-3′; and probe for T allele, 5′-VIC-TCAGGTGTCCATACAGG-3′. This assay was validated using samples of known genotype. All family genotypes were examined for Mendelian inconsistencies by use of PEDCHECK (O'Connell and Weeks 1998), and any discrepancies were addressed. Family-based association analysis was performed using the pedigree disequilibrium test (PDT), version 4.0 (Martin et al. 2000). *P* values for χ^2 or Fisher's exact tests of allele or genotype case-control comparisons were derived using SAS, version 9.0 (SAS Institute). No deviations from Hardy-Weinberg equilibrium were observed for *PTPN22* R620W genotypes in any of the patient groups, controls, or unrelated family founders.

We performed an extensive evaluation of this polymorphism, using large family-based and case-control comparisons. First, we compared the W620 allele frequencies of the white probands with MS from each of the two family-based studies with the results generated from 1,961 white controls (Begovich et al. 2004). The frequency of the T allele in controls was 8.7%, compared with 9.8% in the simplex probands ($P = .22$), 8% in the multiplex probands ($P = .65$), and 9.4% in the combined patient group ($P = .40$). The frequency of this SNP allele in 398 African American patients with MS was 2.9%, compared with 1.4% in 209 spousal controls ($P = .11$) and 2.4% in 409 randomly chosen African American controls ($P = .58$) (Begovich et al. 2004).

Results from PDT analyses for both the single-case and multicase families are shown in table 1. There was no evidence of transmission distortion in either data set considered individually ($P = .48$ and .28, respectively) or when combined for analysis ($P = .69$). Similar results were also observed for PDT analyses of *PTPN22* genotypes in families ($P = .56$; data not shown) (Martin et al. 2003). Families were stratified by the presence or absence of *HLA-DR2* in affected individuals, to identify potential genetic interactions with the *PTPN22* R620W SNP. No difference in transmission of either allele was observed in either *DR2*-positive or *DR2*-negative families ($P = .91$ and .22, respectively). To identify a genetic relationship between *PTPN22* R620W and clinical presentation of MS, families were also categorized by whether cases had an initial relapsing-remitting (RR) course or had a primary progressive or progressive relapsing (PP or PR) course. Significant results for *PTPN22* R620W were not observed in either clinical subset ($P = .59$ in one and .69 in the other). Furthermore, no evidence of association was present in the African American data set when cases were stratified by disease course (RR at onset vs. PP or PR at onset) and then compared ($P = .18$; data not shown). Overall, we observed no evidence of genetic association between the *PTPN22* R620W polymorphism and MS susceptibility or disease course in both the white and African American data sets.

Table 1

***P* Values from PDT Analysis of White Families with MS**

Families	*n*	*P*
All	748	.69
Single-case	563	.48
Multicase	185	.28
With disease phenotype[a]:		
RR course at onset	652	.59
Progressive course at onset	36	.69
With *HLA-DR2* status[b]:		
DR2 positive	324	.91
DR2 negative	231	.22

[a] For multicase families, only those families in which all members were concordant for either phenotype (RR course at onset or progressive course at onset) were used for analysis; phenotypically discordant families were not used.

[b] *HLA* data were available for 555 families. Multicase families in which all affected members were not concordant for *DR2*-positive or *DR2*-negative classifications were not used in the subgroup analyses.

We calculated power for PDT analyses in the families with MS under a multiplicative model (Risch and Merikangas 1996), assuming an allele frequency of 9.0% (derived from white controls used in this study) and a type 1 error rate of 5%. On the basis of these assumptions, we estimate that we have close to 90% power to detect a very modest genotype relative risk of 1.5 for disease susceptibility in the family data set. This estimate is very conservative, in that a combined total of 475 independent trios and 375 independent discordant sib pairs (DSPs) were used for calculation of power. A total of 582 trios and 1,467 DSPs were actually available for the analyses, and, although this larger number of trios and DSPs were not derived from independent families, their inclusion is expected to increase statistical power substantially (Martin et al. 2000). It is important to note that, owing to the significantly lower *PTPN22* SNP allele frequency in the African American controls, compared with that in white controls (1.4%–2.4% vs. 8.7%), there is less power to detect modest genetic effects in the African American case-control analysis. Under the assumption of a type 1 error rate of 5%, there is 80% power to detect a relative risk of ~3.0–4.0. To rule out a smaller genetic contribution to disease susceptibility for *PTPN22* R620W in this population, a larger data set is needed.

The observation that MS is not associated with an increased frequency of the *PTPN22* W620 variant, whereas RA, T1D, and SLE are, suggests that the latter three diseases share a common underlying mechanism that may not be integral to MS pathogenesis. All three diseases associated with the *PTPN22* W620 variant are

characterized by the presence of autoantibodies (anti-citrulline antibodies and rheumatoid factor in RA, anti-GAD (anti–glutamate decarboxylase) antibodies in T1D, and an array of autoantibodies in SLE) that, more often than not, predate clinical disease. This has led to the proposal that the *PTPN22* W620 variant may predispose individuals to autoimmunity by facilitating the generation of certain disease-associated autoantibodies, thereby contributing to disease onset and progression (Kyogoku et al. 2004). This hypothesis is supported by the observation that the W620 variant is strongly associated with rheumatoid factor–positive RA, whereas there is no significant association with rheumatoid factor–negative RA (Begovich et al. 2004; Lee et al., in press).

Interestingly, MS is thought to be primarily a T cell–mediated disease, and, although there are reports of anti-myelin antibodies in the sera and cerebrospinal fluid (CSF) of patients with MS, these antibodies appear to be of low affinity, and their role in disease pathogenesis and progression is not well understood. Genain et al. (1999) have observed myelin-specific infiltrating B cells in the brains of patients with MS, and the CSF and brain of an affected individual can show an elevated frequency of clonally expanded B cells with properties of postgerminal center memory or antibody-forming lymphocytes (Baranzini et al. 1999). This apparent dichotomy can be explained by the observation that MS has distinctive patterns of presentation; this heterogeneity is also reflected in the neuropathology of MS. Recent work has demonstrated the existence of multiple histologic phenotypes in which the core components of the lesion (inflammation, demyelination, and oligodendrocyte pathology) are present in different degrees (Lucchinetti et al. 2000). For example, some hyperacute forms are characterized by substantial CNS inflammation, which suggests cellular mechanisms of disease, whereas immunoglobulin deposition is prominent in other demyelinating forms. The well-documented effect of locus heterogeneity in MS raises the question of whether distinct phenotypes are associated with distinct underlying genotypes. Hence, although the experimental tools to accurately assess humoral endophenotypes in MS are not yet available, the possibility of *PTPN22* variation being a susceptibility factor in a subset of patients with a dominant antibody component warrants further investigation.

In summary, the W620 variant of *PTPN22* does not appear to play a major role in MS pathogenesis in our data sets. Analyses of the role of this SNP in additional autoimmune disorders should provide hints about the underlying mechanisms of these complex diseases as well as a framework to begin to understand how they are related and why certain autoimmune diseases can cosegregate in the same families.

Acknowledgments

We are grateful to the patients with MS and their families, for participating in this study. We also thank V. Carlton, S. Schrodi, and T. White, for helpful comments. This work was funded by National Multiple Sclerosis Society grant RG2901 and National Institutes of Health grant NS46297.

Ann B. Begovich,[1] Stacy J. Caillier,[2] Heather C. Alexander,[1] Joanne M. Penko,[3] Stephen L. Hauser,[2] Lisa F. Barcellos,[2,3] and Jorge R. Oksenberg[2]

[1]*Celera Diagnostics, Alameda, CA;* [2]*Department of Neurology, University of California–San Francisco, San Francisco; and* [3]*Division of Epidemiology, School of Public Health, University of California–Berkeley, Berkeley*

Electronic-Database Information

The accession number and URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ (for *PTPN22* R620W [accession number rs2476601])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for RA, SLE, T1D, and MS)

References

Baranzini S, Jeong MC, Butunoi C, Murray RS, Bernard CCA, Oksenberg JR (1999) B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions. J Immunol 163: 5133–5144

Barcellos LF, Oksenberg JR, Begovich AB, Martin ER, Schmidt S, Vittinghoff E, Goodin DS, Pelletier D, Lincoln RR, Bucher P, Swerdlin A, Pericak-Vance MA, Haines JL, Hauser SL (2003) *HLA-DR2* dose effect on susceptibility to multiple sclerosis and influence on disease course. Am J Hum Genet 72:710–716

Barcellos LF, Oksenberg JR, Green AJ, Bucher P, Rimmler JB, Schmidt S, Garcia ME, Lincoln RR, Pericak-Vance MA, Haines JL, Hauser SL (2002) Genetic basis for clinical expression in multiple sclerosis. Brain 125:150–158

Begovich AB, Carlton VEH, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG, et al (2004) A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (*PTPN22*) is associated with rheumatoid arthritis. Am J Hum Genet 75:330–337

Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, Eisenbarth GS, Comings D, Mustelin T (2004) A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet 36:337–338

Cloutier JF, Veillette A (1996) Association of inhibitory tyrosine protein kinase p50csk with protein tyrosine phosphaste PEP in T cells and other hemopoietic cells. EMBO J 15:4909–4918

——— (1999) Cooperative inhibition of T-cell antigen recep-

tor signaling by a complex between a kinase and a phosphatase. J Exp Med 189:111–121
Fogdell-Hahn A, Ligers A, Gronning M, Hiller J, Olerup O (2000) Multiple sclerosis: a modifying influence of HLA class I genes in an HLA class II associated autoimmune disease. Tissue Antigens 55:140–148
Genain CP, Cannella B, Hauser SL, Raine CS (1999) Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med 5:170–175
Gjorloff-Wingren A, Saxena M, Williams S, Hammi D, Mustelin T (1999) Characterization of TCR-induced receptor-proximal signaling events negatively regulated by the protein tyrosine phosphatase PEP. Eur J Immunol 29:3845–3854
Gregorieff A, Cloutier JF, Veillette A (1998) Sequence requirements for association of protein-tyrosine phosphatase PEP with the Src homology 3 domain of inhibitory tyrosine protein kinase p50(csk). J Biol Chem 273:13217–13222
Haines JL, Ter-Minassian M, Bazyk A, Gusella JF, Kim DJ, Terwedow H, Pericak-Vance MA, et al (1996) A complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. Nat Genet 13:469–471
Haines JL, Terwedow HA, Burgess K, Pericak-Vance MA, Rimmler JB, Martin ER, Oksenberg JR, Lincoln R, Zhang DY, Banatao DR, Gatto N, Goodkin DE, Hauser SL (1998) Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. Hum Mol Genet 7:1229–1234
Hasegawa K, Martin F, Huang G, Tumas D, Diehl L, Chan AC (2004) PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. Science 303: 685–689
Hauser SL, Goodin D (2001) Multiple sclerosis and other demyelinating diseases. In: Braunwald E, Fauci AD, Kasper DL, Hauser SL, Longo DL, Jameson JL, eds. Harrison's principle of internal medicine, 15th ed. McGraw Hill, New York, pp 2452–2461
Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VEH, Chang M, Ramos P, Baechler EC, Batliwalla FM, Novitzke J, Williams AH, Gillett C, Rodine P, Graham RR, Ardlie KG, Gaffney PM, Moser KL, Petri M, Begovich AB, Gregersen PK, Behrens TW (2004) Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. Am J Hum Genet 75:504–507
Lee AT, Li W, Liew A, Bombardier C, Weisman M, Massarotti EM, Kent J, Wolfe F, Begovich AB, Gregersen PK. The PTPN22 R620W polymorphism associates with RF positive rheumatoid arthritis in a dose-dependent manner but not with HLA-SE status. Genes Immun (in press)
Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000) Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 47:707–717
Marrack P, Kappler J, Kotzin BL (2001) Autoimmune disease: why and where it occurs. Nat Med 7:899–905
Martin ER, Bass MP, Gilbert JR, Pericak-Vance MA, Hauser ER (2003) Genotype-based association test for general pedigrees: the genotype-PDT. Genet Epidemiol 25:203–213
Martin ER, Monks SA, Warren LL, Kaplan NL (2000) A test for linkage and association in general pedigrees: the pedigree disequilibrium test. Am J Hum Genet 67:146–154
O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63:259–266
Oksenberg JR, Barcellos LF, Cree BA, Baranzini SE, Bugawan TL, Khan O, Lincoln RR, Swerdlin A, Mignot E, Lin L, Goodin D, Erlich HA, Schmidt S, Thomson G, Reich DE, Pericak-Vance MA, Haines JL, Hauser SL (2004) Mapping multiple sclerosis susceptibility to the *HLA-DR* locus in African Americans. Am J Hum Genet 74:160–167
Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517
Rubio JP, Bahlo M, Butzkueven H, van der Mei IAF, Sale MM, Dickinson JL, Groom P, Johnson LJ, Simmons RD, Tait B, Varney M, Taylor B, Dwyer T, Williamson R, Gough NM, Kilpatrick TJ, Speed TP, Foote SJ (2002) Genetic dissection of the human leukocyte antigen region by use of haplotypes of Tasmanians with multiple sclerosis. Am J Hum Genet 70: 1125–1137
Sawcer S, Jones HB, Feakes R, Gray J, Smaldon N, Chataway J, Robertson N, Clayton D, Goodfellow PN, Compston A (1996) A genome screen in multiple sclerosis reveals susceptibility loci on chromosomes 6p21 and 17q22. Nat Genet 13:464–468
Wandstrat A, Wakeland E (2001) The genetics of complex autoimmune diseases: non-MHC susceptibility genes. Nat Immunol 2:802–809

Address for correspondence and reprints: Dr. Ann B. Begovich, Celera Diagnostics, 1401 Harbor Bay Parkway, Alameda, CA 94502. E-mail: Ann.Begovich@celeradiagnostics.com

0002-9297/2005/7601-0020$15.00

Am. J. Hum. Genet. 76:187–189, 2005

Corrections to the Parameterization of Constraints on Allele Sharing in Sibling Pairs Alter Covariate-Parameter Estimates but Not Sharing-Probability Estimates or Power of Tests for Linkage

To the Editor:

Errors in appendix B of our 1999 article in the *Journal* (Greenwood and Bull 1999) were recently pointed out to us (D. Weeks and H.-J. Tsai, personal communication). The simultaneous-boundary–constrained estimates for $z_0(x_i)$ presented in 1999 do not give the correct values for the covariate parameters under the null hypothesis. The correct expression for the expected proportion of sibling pairs sharing zero alleles identical by

Table 1

Original and Corrected Results of Simulation Models

Model, Constraint Method, and Covariate(s)	df	Results Reported in 1999		Corrected Results	
		Mean LOD Score	5% Empirical Power	Mean LOD Score	5% Empirical Power
Single-gene model (model 1; original table 2)[a]:					
Unconstrained model:					
No covariates	2	2.20	.70	2.19	.67
Constrained model:					
No covariates, no dominance variance	1	1.97	.74	1.96	.73
No covariates, no additive variance	1	1.26	.53	1.25	.53
No covariates, minmax-optimal constraint	1	1.78	.71	1.77	.71
Mean age at onset, no dominance variance, simultaneous-boundary constraint	2	3.08	.87	3.08	.85
Mean age at onset, no additive variance, simultaneous-boundary constraint	2	1.88	.58	1.88	.54
Mean age at onset, minmax-optimal simultaneous-boundary constraint	2	2.61	.82	2.80	.77
Gene-environment–interaction model (model 2b; original table 3)[b]:					
Unconstrained model:					
No covariates	2	1.47	.42	1.50	.44
Constrained model:					
No covariates, minmax-optimal constraint	1	1.23	.54	1.24	.51
Two covariates, minmax-optimal simultaneous-boundary constraint	2	1.72	.44	1.78	.41

[a] For the single-gene model, 34/500 linked data sets were excluded from the results in the 1999 article (Greenwood and Bull 1999) because of lack of convergence. After correction, no data sets were excluded.

[b] For the gene-environment–interaction model, 82/500 data sets were excluded from the results in the 1999 article (Greenwood and Bull 1999) because of lack of convergence. After correction, 13/500 data sets were excluded.

descent (IBD) under the simultaneous-boundary constraint with no additive variance should be

$$z_0(x_i) = \frac{\exp(\beta_0' x_i)}{1 + 3\exp(\beta_0' x_i)} .$$

The relationships between this proportion and those for sharing one or two alleles IBD do not change from the 1999 article: $z_1(x_i) = 2z_0(x_i)$ and $z_2(x_i) = 1 - 3z_0(x_i)$.

For the minmax-optimal simultaneous-boundary constraint, the correct expression should be

$$z_0(x_i) = \frac{0.645\exp(\beta_0' x_i)}{1 + 1.58\exp(\beta_0' x_i)} .$$

As in the 1999 article, the constraints on the other sharing proportions are $z_1(x_i) = 0.355 + 0.58z_0(x_i)$ and $z_2(x_i) = 0.645 - 1.58z_0(x_i)$. Note that both the original (see appendix B in Greenwood and Bull 1999) and the corrected expressions for $z_0(x_i)$ satisfy the specified constraints on the expected sharing proportion $z_j(x_i)$ and differ only in those values of the covariate-associated parameter vector β_0 that correspond to specific null or alternative hypotheses.

When written in terms of the sharing proportion $z_0(x_i)$, the score equations—based on the LOD$^{**}(\beta_0)$ expressions given in appendix B (Greenwood and Bull 1999) and used in the M step of the estimation algorithm—are identical for the original and corrected expressions. Provided that the z_{ij}s in the E step are updated using the $z_0(x_i)$ estimates, the final $z_0(x_i)$ estimates from the expectation-maximization algorithm, and hence the LOD scores for the test of linkage with covariates, will also be identical.

This can be considered as a special case of the invariance of the likelihood to reparameterization. Therefore, the original conclusions concerning relative power and effects of constraints are unchanged.

We have rerun our simulations to confirm these theoretical conclusions, and we show here in table 1 some corrected results and original results from tables 2 and 3 of the 1999 article (Greenwood and Bull 1999). The corrected algorithms almost always converge, whereas we previously had more difficulty in obtaining convergence with these two constraint methods. Thus, we conclude that the small differences in mean LOD scores or in significance levels are a result of the fact that we no longer had to exclude as many data sets from our summaries. Therefore, although estimates of the parameter β_0 would not be correct if the expressions in the 1999 article were used, our conclusions about the power of the various approaches have not changed.

Celia M. T. Greenwood[1,2] and Shelley B. Bull[2,3]
[1]*Program in Genetics and Genomic Biology, Hospital for Sick Children,* [2]*Department of Public Health Sciences, University of Toronto, and* [3]*Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto*

Reference

Greenwood CMT, Bull SB (1999) Analysis of affected sib pairs, with covariates—with and without constraints. Am J Hum Genet 64:871–885

Address for correspondence and reprints: Dr. Celia M. T. Greenwood, Genetics and Genomic Biology, Elm 11-101, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada. E-mail: celia.greenwood@utoronto.ca

0002-9297/2005/7601-0021$15.00