Published in final edited form as:

Arthritis Rheum. 2009 October 15; 61(10): 1305–1311. doi:10.1002/art.24460.

Immunogenetic Risk and Protective Factors for Development of L-tryptophan-associated Eosinophilia-Myalgia Syndrome and Associated Symptoms

Satoshi Okada¹, Mary L. Kamb², Janardan P. Pandey³, Rossanne M. Philen², Lori A. Love⁴, and Frederick W. Miller^{4,*}

¹Satoshi Okada, MD, Department of Internal Medicine, Ichikawa General Hospital, Tokyo Dental College, Ichikawa, Japan

²Mary L. Kamb, MD, Rossanne M. Philan, MD: Centers for Disease Control and Prevention, Atlanta, GA

³Janardan P. Pandey, Ph.D., Medical University of South Carolina, Charleston, SC

⁴Lori A. Love, MD, PhD, Frederick W. Miller, MD, PhD: Environmental Autoimmunity Group, National Institute of Environmental Health Sciences, HHS, Bethesda, MD

Abstract

Objective—To assess L-tryptophan (LT) dose, age, gender and immunogenetic markers as possible risk or protective factors for development of LT-associated eosinophilia myalgia syndrome (EMS) and related clinical findings.

Methods—HLA DRB1 and DQA1 allele typing and GM/KM phenotyping were performed on a cohort of 94 Caucasian subjects with documented LT ingestion and standardized evaluations. Multivariate analyses compared LT dose, age, gender and alleles among groups of subjects who ingested LT and subsequently developed surveillance criteria for EMS (EMS), or developed EMS or characteristic features of EMS (EMS spectrum disorder), or developed no features of EMS (unaffected).

Results—Considering all sources of LT, higher LT dose (odds ratio (OR) 1.4, 95% confidence interval (CI) 1.1–1.8), age >45 years (OR 3.0, CI 1.03–8.8) and HLA DRB1*03 (OR 3.9, CI 1.2–15.2), DRB1*04 (OR 3.9, CI 1.1–16.4) and DQA1*0601 (OR 13.7, CI 1.3–1874) were risk factors for the development of EMS, while DRB1*07 (OR 0.12, CI 0.02–0.48) and DQA1*0501 (OR 0.23, CI 0.05–0.85) were protective. Similar risk and protective factors were seen for developing EMS following ingestion of implicated LT, except DRB1*03 was not a risk factor and DQA1*0201 was an additional protective factor. EMS spectrum disorder also showed similar findings, but with DRB1*04 being a risk factor and DRB1*07 and DQA1*0201 being protective. There were no differences in gender distribution, GM/KM allotypes or GM/KM phenotypes among any groups.

Conclusion—In addition to the xenobiotic dose and subject age, polymorphisms in immune response genes may underlie the development of certain xenobiotic-induced immune-mediated disorders and these findings may have implications for future related epidemics.

*Correspondence to: Frederick W. Miller, M.D., Ph.D., Chief, Environmental Autoimmunity Group, National Institute of Environmental Health Sciences, NIH 10, Room 4-2352, 10 Center Drive, MSC 1301, Bethesda, MD 20892-1301, Phone: 301-451-6273, Fax: 301-451-5585, millerf@mail.nih.gov.

INTRODUCTION

An epidemic outbreak of an unusual condition, characterized by an acute onset of incapacitating myalgia and peripheral eosinophilia (eosinophil count $\geq 10^9/L$ without known causes for eosinophilia) and termed the eosinophilia-myalgia syndrome (EMS) was observed in 1989 (1). Several epidemiologic studies suggested that most patients who developed EMS in late 1989 had consumed L-tryptophan (LT) produced by a single manufacturer (1-3). Soon thereafter, an import alert by the United States Food and Drug Administration (FDA) effectively removed LT from the U.S. market and the epidemic resolved.

Previous studies indicated that in addition to the source of LT, increased dosage of LT, increased age, and use of LT as a sleeping aid were risk factors in development of EMS (3,4). Methodologic limitations in the epidemiologic studies and the inability to identify the exact pathogenic components of the implicated batches of LT have hampered understanding of disease pathogenesis (5,6). Moreover, sporadic cases meeting EMS surveillance criteria existed prior to 1989, continued after 1989 and have been reported in persons who did not take LT (7,8).

Since only a fraction of persons who ingested implicated batches of LT developed disease, additional factors likely played pathogenetic roles. Few investigations, however, have assessed genetic susceptibility for EMS (9,10). In hopes of understanding additional risk and protective factors that may have implications for future similar epidemics, we explored the possible role of LT dose, age, gender and immunogenetic markers for susceptibility in the development of LT-associated EMS and associated clinical findings in a unique cohort in which LT intake and clinical outcomes were carefully documented.

SUBJECTS AND METHODS

Subjects

Blood samples were available from 96 Caucasian subjects who were previously studied as part of a single physician's practice (3) and were enrolled into protocols approved by the CDC and FDA. All 96 subjects had ingested LT and 60 of them took implicated LT. Two subjects were excluded to avoid genetic confounding because they were blood relatives of other subjects. The remaining 94 unrelated LT users were divided into three groups based on outcomes (3): 1) EMS (n=28), defined as those who developed surveillance criteria for EMS (incapacitating myalgia and a peripheral eosinophil count $\geq 10^9/L$ without known causes for eosinophilia); 2) EMS spectrum disorder (EMSSD, n=57), defined as those with EMS, or those who had an eosinophil count $\geq 10^9/L$ but without incapacitating myalgias, or those who developed more than one symptom characteristic of EMS (myalgias, arthralgias, neuropathy, alopecia or skin thickening); and 3) unaffected (n=37), defined as those who took LT and did not develop any of the above. In extension studies, those who developed specific signs and symptoms found in EMS subjects, including incapacitating myalgia (N=34), muscle weakness (N=22), increased skin thickness (N=11) or numbness (N=28), after LT ingestion were assessed, and healthy Caucasian controls (n=872), enrolled in studies of the pathogenesis of autoimmune diseases at the National Institutes of Health, were also studied as another comparison group.

Methods

Genomic DNA was isolated from peripheral blood and amplified by polymerase chain reactions using primers and sequence-specific oligonucleotide probes as previously described (11) to identify 34 HLA DRB1 and eight DQA1 alleles. GM/KM allotypes and phenotypes, genetic markers of gamma and kappa-type light chains, respectively, which

regulate immunoglobulin production and are known risk factors for several immune-mediated disorders, were determined as previously described (12).

Statistical analyses

The primary study assessed variables potentially influencing susceptibility to develop EMS or EMS spectrum disorder following LT ingestion from any source and following ingestion of implicated LT. Each potential predictor variable (gender, LT dose, age, HLA alleles and GM/KM allotypes and phenotypes) was analyzed using Fisher's exact test, and odds ratios (ORs), 95% confidence intervals (95% CIs) were determined. Univariate analyses were performed for the each outcome to identify polymorphisms associated with the risk of the specific outcome. Results of the univariate analyses were used to develop a multivariate model of potential predictors of outcome following LT ingestion.

We performed multivariate analyses to minimize possible bias due to confounding effects by the dose of LT, age of subjects and gender (13). We used logistic regression models with a backward stepwise analysis in three ways. First, all variables including HLA alleles, dose of LT, age of subjects and gender were entered simultaneously into a model followed by a stepwise analysis. Second, HLA alleles were forcedly entered into a model and other variables were included for a stepwise analysis. Also, LT dose was forcedly entered into a model, followed by a stepwise analysis for other variables. In each analysis, conditional stepwise methods and stepwise methods based on likelihood ratios were performed. After identifying variables that remained in the model, the penalized maximum likelihood estimate was used for bias correction to determine OR and 95% C.I.(14,15)

Comparisons were also made between EMS or EMS spectrum disorder and normal controls without multivariate analysis. Whether homozygosity of any associated alleles altered risk was also studied. SAS (SAS Institute, Cary, NC), SPSS 16.0j (SPSS, Tokyo, Japan) and LogXact8 (Cytel Inc., Cambridge, MA) were used for these statistical analyses. Power analyses were performed using StatXact (Cytel Inc., Cambridge, MA) to determine the power to detect a significant difference ($p < 0.05$, two-tailed) between the groups being compared (unconditional difference).

RESULTS

As described previously (3), the dose of LT ingested, regardless of the LT source, was a risk factor for the development of EMS and it was also found to be a risk factor for EMS spectrum disorder (Table 1). The dose of LT, however, was not significantly different in those who did or did not develop incapacitating myalgia (Table 1) or muscle weakness (data not shown). Although the overall ages were not significantly different among any groups, analysis of age distributions by decade showed that those who were older than 45 were more likely to develop EMS or EMS spectrum disorder, while those who were older than 29 were more likely to have incapacitating myalgia (Table 1). Gender was not found to be a risk factor in any analyses. Significant differences were noted in the frequencies of certain HLA alleles in LT users who developed EMS compared to those who were unaffected by multivariate analyses incorporating LT dose and age (Table 2). There were no significant differences noted in these associations whether conditional stepwise methods or stepwise methods based on likelihood ratios were used. Multivariate analyses showed that HLA DRB1*03, DRB1*04 and DQA1*0601 were risk factors as those alleles were more frequently seen in subjects who developed EMS than in unaffected subjects. HLA DRB1*07 and DQA1*0501, however, appeared to be protective factors as they were found more frequently in unaffected subjects compared to those who developed EMS. Analysis of a subset of EMS and unaffected subjects who took implicated LT, showed that DRB1*04 was again a risk factor and DRB1*07 and DQA1*0501 were protective for development of

EMS, but that DQA1*0201 was an additional protective allele (Table 2). Since the most common allele in the DRB1*07 supertype (DRB1*0701) is in linkage disequilibrium with DQA1*0201, we analyzed the frequencies of the presumptive haplotype DRB1*07-DQA1*0201 among study groups. The frequency of this presumptive haplotype was found to be significantly lower in EMS subjects (13.0%) compared to unaffected subjects (57.1%) (OR 0.11, CI 0.02-0.77) among those who took implicated LT.

Given concerns that the surveillance criteria for EMS may not have included all persons that had been affected by ingesting LT, we analyzed additional cases that had characteristic features of EMS, which together with EMS were defined as EMS spectrum disorder. As was the case with EMS, DRB1*04 was a risk factor and DRB1*07 and DQA1*0201 were protective for development of EMS spectrum disorder when implicated sources of LT were considered (Table 2). In contrast to EMS, however, DQA1*0501 was not a risk factor for EMS spectrum disorder in those who ingested implicated LT. Given the small samples sizes for some groups, however, the power to detect risk or protective factors is relatively low in some cases (Table 2) and this might explain some of the differences noted between risk factors for those who ingested any form of LT and those who took implicated LT. We analyzed the frequencies of the presumptive haplotype DRB1*07-DQA1*0201 among study groups and again found that the frequency of this haplotype was significantly less frequent in EMS spectrum disorder subjects (13.5 %) compared to unaffected subjects (57.1%) (OR 0.15, CI 0.02-0.93) among those who took implicated LT.

Because of the relatively small sample sizes in our primary study cohort, we next asked if there was bias in the frequencies of HLA alleles between Caucasians with EMS or EMS spectrum disorder and Caucasian healthy controls. DRB1*03 was significantly more frequent in those with EMS who took any source of LT than in healthy controls (Table 2). Regardless of the source of LT, DQA1*0601 was more frequent in EMS subjects than healthy controls. Moreover, regardless of the source of LT, DRB1*03 and DQA1*0601 were also significantly more frequent in those with EMS spectrum disorder than healthy controls. Significant differences in allele frequencies were not observed for DRB1*04, DRB1*07, DQA1*0201 and DQA1*0501 in those with EMS in comparison to healthy controls, although these alleles were observed in different frequencies among those with different outcomes following LT ingestion.

Since little is known about risk factors for the development of specific signs and symptoms found in EMS subjects, we next asked if there was any bias in the frequencies of HLA alleles between subjects with certain characteristic EMS symptoms, including incapacitating myalgia, muscle weakness, increased skin thickness or numbness. Among subjects who took LT from any source, DRB1*07 was protective for developing incapacitating myalgia, and among subjects who took implicated LT, DQA1*0501 was protective for developing incapacitating myalgia (Table 3). Furthermore, HLA DQA1*0101 was a risk factor for developing muscle weakness among subjects who took LT from any source, while DQA1*0501 was protective among subjects who took implicated LT. No DQA1 or DRB1 alleles, however, were found to be risk or protective factors for development of increased skin thickness or numbness (data not shown). The frequencies of DQA1*0601 were again significantly higher in those with incapacitating myalgia or muscle weakness who took implicated LT compared to normal controls.

Homozygosity of any of the alleles noted above was not found to play a significant role in developing EMS or EMS spectrum disorder. Finally, there were no significant differences in GM/KM allotypes or phenotypes among any groups.

DISCUSSION

LT-associated EMS is a heterogeneous group of connective tissue disorders, which share the common features of eosinophilia and incapacitating myalgia, and that transiently reached epidemic proportions in late 1989. Despite intense research, the specific components of LT responsible for, and the mechanisms causing LT-associated EMS remain unknown. Nonetheless, clinical, serological, and pathological findings suggest that immune mechanisms play a role (16). Further evidence for the role of the immune system in the pathogenesis of EMS includes the presence of chronic inflammatory infiltrates in skin, muscle, and nerves, characterized by activated T-lymphocytes, macrophages, eosinophils, and fibroblasts (16) and the response of some patients to immunosuppressive therapies (17).

Because of the low attack rates, prior studies assessed LT-associated EMS for possible risk factors. Previous investigations did find that LT dose and age were risk factors but these could not explain the entire risk (3). Other investigations assessed immunogenetics and compared LT-associated EMS cases to normal controls that likely did not take LT as a specific agent and found that HLA DR4 (9) and certain cytochrome P450 polymorphisms (10) were possibly associated with EMS. In this study, we performed analyses including LT dose, age, gender and immunogenetics in comparisons of subjects who ingested LT and either developed features of EMS or did not. We did not find an influence of gender, but did find that LT dose, age and HLA DRB1*04 were risk factors for development of LT-associated EMS or EMS spectrum disorder. Nonetheless, we also identified other immunogenetic risk and protective factors. For example, DRB1*03 and DQA1*0601 were risk factors for the development of EMS following ingestion of LT from any source. This finding was further supported by comparison of the allele distributions in EMS to those in healthy controls. Not all associations of other alleles comparing LT-affected groups to LT-unaffected subjects, however, were replicated when comparing LT-affected groups to healthy controls, and when assessing groups of subjects who took implicated LT and those taking LT from any source. The reasons for these differences, as well as the variable findings between this study and other investigations, remain unclear. Nonetheless, the varying study designs, different populations investigated, variable numbers of subjects in the groups and the resulting different powers to detect risk or protective factors (see Tables 2 and 3), may account for some of the variable results. We also do not know why age appears to be a risk factor for the development of EMS and EMS spectrum disorder, although several lines of evidence suggest that age and aging of the immune system are implicated in the development of a number of autoimmune diseases (18,19).

The findings in our investigation (summarized in Table 4), and prior studies showing that LT dose, age and certain metabolizer and immune response genes are associated with development of EMS, support the hypothesis that the dose of LT, age of the subject, and both metabolic and immune-mediated mechanisms may be important for the pathogenesis of EMS. While the reasons for the genetic associations remain unknown, certain of the associated genes, such as DRB1*03, have been linked to many other immune-mediated conditions via multiple mechanisms including a favored TH2 profile (20). It is interesting that TH2 profiles also appear to predominate during *in vitro* responses to possible contaminants in implicated LT (21) and that lung specimens from a related disorder called toxic oil syndrome, which developed in Spain from 1981-1983 after ingestion of contaminated rapeseed oil, appear to support a TH2 mechanism in that disease (22). Additionally, genetic studies in toxic oil syndrome, implicated certain HLA-A, -B, -DR4 and -DQA alleles as possible risk and protective factors, implying that polymorphisms in immune response genes may underlie the development of multiple xenobiotic-induced immune-mediated disorders and that these findings may have implications for future related epidemics (23).

EMS may present in a variety of ways and with different symptoms including incapacitating myalgia, muscle weakness, skin thickening and peripheral neuropathy. Our findings suggest that different immune response genes in LT users appear to be associated with the development of myalgia and eosinophilia (EMS) compared to the development of the other manifestations of the LT-associated EMS spectrum disorder. Thus, an individual's genetic background may explain some of the variability in the development of specific manifestations following LT ingestion.

Why differences in genetic risk and protective factors were found in EMS cases that developed after ingestion of LT from any source compared to cases developing after exposure to implicated batches of LT remains unknown, although the relatively small sample sizes available for this study and the lack of any additional subjects to study limit our capacity to more fully assess these data at this time. Given the limited understanding of the pathogenesis of EMS, and since EMS has developed in persons who did not take LT as well as in persons taking LT from different manufacturers, it is possible that there are many potential mechanisms for the development of this syndrome. It is not clear whether the genetic risk and protective factors described in this investigation also play a role in EMS cases that have developed without LT exposure. Further studies of such cases are needed to understand if a variety of gene-environment interactions may underlie the evolution of eosinophilia and myalgia in different individuals.

Acknowledgments

This work was supported in part by the intramural research programs of the National Institute of Environmental Health Sciences, NIH, the Centers for Disease Control and the Food and Drug Administration. The authors thank Drs. Ejaz Shamim and Terrance O'Hanlon for technical support, Dr. Elizabeth Sullivan for clinical assistance and Drs. Richard Calvert and Sharon Adams for helpful discussions regarding the manuscript.

References

1. Clauw DJ, Pincus T. The eosinophilia-myalgia syndrome: what we know, what we think we know, and what we need to know. *J Rheumatol Suppl.* 1996; 46:2–6. [PubMed: 8895175]
2. Belongia EA, Hedberg CW, Gleich GJ, White KE, Mayeno AN, Loegering DA, et al. An investigation of the cause of the eosinophilia-myalgia syndrome associated with tryptophan use. *N Engl J Med.* 1990; 323(6):357–65. [PubMed: 2370887]
3. Kamb ML, Murphy JJ, Jones JL, Caston JC, Nederlof K, Horney LF, et al. Eosinophilia-myalgia syndrome in L-tryptophan-exposed patients. *JAMA.* 1992; 267(1):77–82. [PubMed: 1727200]
4. Back EE, Henning KJ, Kallenbach LR, Brix KA, Gunn RA, Melius JM. Risk factors for developing eosinophilia myalgia syndrome among L-tryptophan users in New York. *J Rheumatol.* 1993; 20(4): 666–72. [PubMed: 8496862]
5. Horwitz RI, Daniels SR. Bias or biology: evaluating the epidemiologic studies of L-tryptophan and the eosinophilia-myalgia syndrome. *J Rheumatol Suppl.* 1996; 46:60–72. [PubMed: 8895182]
6. Shapiro S. Epidemiologic studies of the association of L-tryptophan with the eosinophilia-myalgia syndrome: a critique. *J Rheumatol Suppl.* 1996; 46:44–58. discussion 58-9. [PubMed: 8895181]
7. Clauw DJ, Flockhart DA, Mullins W, Katz P, Medsger TA Jr. Eosinophilia-myalgia syndrome not associated with the ingestion of nutritional supplements. *J Rheumatol.* 1994; 21(12):2385–7. [PubMed: 7699648]
8. Sullivan EA, Staehling N, Philen RM. Eosinophilia-myalgia syndrome among the non-L-tryptophan users and pre-epidemic cases. *J Rheumatol.* 1996; 23(10):1784–7. [PubMed: 8895159]
9. Kaufman LD, Gruber BL, Gregersen PK. Clinical follow-up and immunogenetic studies of 32 patients with eosinophilia-myalgia syndrome. *Lancet.* 1991; 337(8749):1071–4. [PubMed: 1673503]
10. Flockhart DA, Clauw DJ, Sale EB, Hewett J, Woosley RL. Pharmacogenetic characteristics of the eosinophilia-myalgia syndrome. *Clin Pharmacol Ther.* 1994; 56(4):398–405. [PubMed: 7955801]

11. Begovich AB, McClure GR, Suraj VC, Helmuth RC, Fildes N, Bugawan TL, et al. Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. *J Immunol.* 1992; 148(1):249–58. [PubMed: 1727870]
12. Shamim EA, Rider LG, Pandey JP, O'Hanlon TP, Jara LJ, Samayoa EA, et al. Differences in idiopathic inflammatory myopathy phenotypes and genotypes between Mesoamerican Mestizos and North American Caucasians: ethnogeographic influences in the genetics and clinical expression of myositis. *Arthritis Rheum.* 2002; 46(7):1885–93. [PubMed: 12124873]
13. Miranda-Malpica E, Martinez-Rios MA, Fragoso JM, Delgadillo-Rodriguez H, Rodriguez-Perez JM, Gonzalez-Quesada C, et al. The interleukin 1B-511 polymorphism is associated with the risk of developing restenosis after coronary stenting in Mexican patients. *Hum Immunol.* 2008; 69(2): 116–21. [PubMed: 18361937]
14. Heinze G. A comparative investigation of methods for logistic regression with separated or nearly separated data. *Stat Med.* 2006; 25(24):4216–26. [PubMed: 16955543]
15. Maiti T, Pradhan V. A comparative study of the bias corrected estimates in logistic regression. *Stat Methods Med Res.* 2008; 15:15.
16. Varga J, Uitto J, Jimenez SA. The cause and pathogenesis of the eosinophilia-myalgia syndrome. *Ann Intern Med.* 1992; 116(2):140–7. [PubMed: 1727618]
17. Pincus T. Eosinophilia-myalgia syndrome: patient status 2-4 years after onset. *J Rheumatol Suppl.* 1996; 46:19–24. discussion 24-5. [PubMed: 8895178]
18. Ginn LR, Lin JP, Plotz PH, Bale SJ, Wilder RL, Mbauya A, et al. Familial autoimmunity in pedigrees of idiopathic inflammatory myopathy patients suggests common genetic risk factors for many autoimmune diseases. *Arthritis Rheum.* 1998; 41(3):400–5. [PubMed: 9506566]
19. Prelog M. Aging of the immune system: a risk factor for autoimmunity? *Autoimmun Rev.* 2006; 5(2):136–9. [PubMed: 16431345]
20. Candore G, Lio D, Colonna RG, Caruso C. Pathogenesis of autoimmune diseases associated with 8.1 ancestral haplotype: effect of multiple gene interactions. *Autoimmun Rev.* 2002; 1(1-2):29–35. [PubMed: 12849055]
21. Barth H, Klein R, Berg PA. L-tryptophan contaminant 'peak E' induces the release of IL-5 and IL-10 by peripheral blood mononuclear cells from patients with functional somatic syndromes. *Clin Exp Immunol.* 2001; 126(2):187–92. [PubMed: 11703359]
22. del P V, de AB, Gallardo S, Cardaba B, de Arruda-Chaves E, Cortegano MI, et al. Cytokine mRNA expression in lung tissue from toxic oil syndrome patients: a TH2 immunological mechanism. *Toxicology.* 1997; 118(1):61–70. [PubMed: 9074654]
23. Arnaiz-Villena A, Martinez-Laso J, Corell A, Allende L, Rosal M, Gomez-Reino JJ, et al. Frequencies of HLA-A24 and HLA-DR4-DQ8 are increased and that of HLA-B blank is decreased in chronic toxic oil syndrome. *Eur J Immunogenet.* 1996; 23(3):211–9. [PubMed: 8803534]

Table 1

Differences in the mean dose of L-tryptophan and age distributions in L-tryptophan users with different outcomes *

	LT-Affected Group (<i>LTA</i>)	LT-Unaffected Group (<i>LTU</i>)	OR (95% CI) <i>LTA</i> vs. <i>LTU</i>
	<i>EMS</i> (n=28)	<i>Unaffected</i> (n=37)	
Mean LT dose (mg/d)	4160.7	2898.7	1.35 (1.05-1.79)
Mean LT dose (mg/d)	4166.7	2777.8	1.43 (1.02-2.22)
> 45 years old (%)	70.3	44.1	3.01 (1.03-8.75)
> 45 years old (%)	69.2	44.4	2.81 (0.81-9.80)
	<i>EMSSD</i> (n=57)	<i>Unaffected</i> (n=37)	
Mean LT dose (mg/d)	3965.8	2898.7	1.39 (1.12-1.80)
Mean LT dose (mg/d)	4145.4	2777.8	1.58 (1.11-2.46)
> 45 years old (%)	66.1	44.1	2.47 (1.03-5.91)
> 45 years old (%)	69.0	44.4	2.79 (0.90-8.69)
	<i>Myalgia</i> (n=34)	<i>No Myalgia</i> (n=32)	
Mean LT dose (mg/d)	3648.5	3550.0	NS
Mean LT dose (mg/d)	3884.6	4350.0	NS
> 29 years old (%)	100	83.3	14.45 (1.53-1930)

* Abbreviations: EMS, eosinophilia myalgia syndrome; EMSSD, EMS spectrum disorder (EMS or characteristic features of EMS); LTA, L-tryptophan affected group; LTU, L-tryptophan unaffected group; OR, odds ratio; CI, confidence interval; NS, not significant. Bolded variables were significantly different in those who took only implicated L-tryptophan (total N=60, for EMS N=27, for EMSSD N=43, for myalgia N=26, for unaffected N=18).

Table 2

Differences in HLA types in L-tryptophan users with different outcomes and healthy controls *

HLA Allele	LT-Affected Group (LTA)	LT-Unaffected Group (LTU)	Healthy controls	OR (95% CI)** LTA vs LTU	Power*** LTA vs LTU	OR (95% CI) LTA vs Healthy controls	Power*** LTA vs Healthy controls
	EMS (% n=28)	Unaffected (% n=37)	Healthy (% n=872)		(%)		(%)
HLA-DRB1							
*03	39.3	24.3	21.9	3.89 (1.15-15.20)	25.3	2.31 (1.06-5.02)	37.8
*03	22.2	22.2	21.9	NS	4.0	NS	1.2
*04	32.1	16.2	28.1	3.93 (1.08-16.37)	33.6	NS	2.7
*04	33.3	5.6	28.1	7.52 (1.30- 86.05)	60.0	NS	3.2
*07	14.3	40.5	25.0	0.12 (0.02-0.48)	64.7	NS	5.9
*07	14.3	50.0	20	0.12 (0.02-0.48)	73.7	NS	4.1
HLA-DQA1							
*0201	17.4	23.1	24.0	NS	3.1	NS	1.3
*0201	17.4	57.1	24.0	0.08 (0.003-0.64)	56.9	NS	0.9
*0501	26.1	50.0	40.2	0.23 (0.05-0.85)	49.0	NS	7.6
*0501	26.1	71.4	40.2	0.008 (0.01- 0.36)	59.4	NS	7.6
*0601	21.7	0	0.7	13.74 (1.32-1874)	76.8*****	38.61 (9.56-155.96)	97.4
*0601	21.7	0	0.7	NS	1.6*****	38.61 (9.56-155.96)	97.4
	EMSSD (% n=57)	Unaffected (% n=37)	Healthy (% n=872)				
HLA-DRB1							
*03	38.6	24.3	21.9	NS	28.8	2.24 (1.29-3.92)	64.3
*03	39.5	22.2	21.9	NS	24.7	2.33 (1.24-4.39)	61.2
*04	26.3	2.7	28.1	NS	92.4	NS	1.3
*04	30.2	5.6	28.1	6.30 (1.20- 67.09)	58.8	NS	2.8
*07	26.3	43.2	25.0	NS	38.8	NS	1.8
*07	16.7	50.0	25.0	0.13 (0.03-0.50)	76.1	NS	19.9
HLA-DQA1							

HLA Allele	L/T-Affected Group (<i>LTA</i>)	L/T-Unaffected Group (<i>LTU</i>)	Healthy controls	OR (95% CI) <i>LTA</i> vs <i>LTU</i>	Power <i>LTA</i> vs <i>LTU</i>	OR (95% CI) <i>LTA</i> vs <i>Healthy controls</i>	Power <i>LTA</i> vs <i>Healthy controls</i>
*0201	26.0	23.0	24.0	NS	5.2	NS	4.4
*0201	13.5	57.1	24.0	0.07 (0.004-0.56)	27.2	NS	17.7
*0601	10.0	0	0.7	NS	23.0	15.44 (4.01-59.54)	93.7
*0601	13.5	0	0.7	NS	0	21.72 (5.56-84.81)	94.1

* Abbreviations per Table 1; *alleles in italics are protective factors*. Bolded alleles were those that were significantly different in those who took only implicated L-tryptophan (total N=60, for EMS N=27, for EMSSD N=43, for unaffected N=18).

** OR, odds ratio obtained by multivariate analysis;

*** Power to detect a significant difference (p<0.05, two-tailed) between the groups being compared.

**** These power calculations use the proportion of DQA1*0601 in the LTU group as 0.000001.

Table 3
Differences in HLA types in L-tryptophan users who developed myalgia or muscle weakness*

HLA Allele	LT-Affected Group	LT-Unaffected Group	Healthy controls	OR** (95% CI) LTA vs LTU	Power*** LTA vs LTU	OR (95% CI) LTA vs. Healthy controls	Power*** LTA vs. Healthy controls
	Myalgia (%; n=34)	No Myalgia (%; n=32)	Healthy (%; n=872)		(%)		(%)
HLA-DRB1							
*07	25.9	46.7	25.0	0.19 (0.04–0.89)	41.9	NS	0.8
*07	11.5	40.0	25.0	NS	41.7	NS	5.4
HLA-DQA1							
*0501	32.3	53.3	40.2	NS	36.7	NS	2.3
*0501	26.9	80.0	40.2	0.09 (0.02–0.54)	92.9	NS	7.4
*0601	8.8	6.7	0.7	NS	3.8	13.5 (2.89–62.86)	73.4
*0601	11.5	20.2	0.7	NS	11.3	16.59 (3.53–77.97)	82.0
	Muscle Weakness (%; n=22)	No Muscle Weakness (%; n=41)	Healthy (%; n=872)				
HLA-DQA1							
*0101	31.8	7.7	27.5	7.22 (1.26–76.66)	69.1	NS	2.6
*0101	28.6	5.6	27.5	NS	44.3	NS	1.1
*0501	27.2	53.8	40.2	NS	52.9	NS	4.5
*0501	14.3	72.2	40.2	0.06 (0.01–0.40)	93.9	0.21 (0.05–0.94)	12.5
*0601	9.1	2.4	0.7	NS	17.8	9.55 (1.81–50.29)	64.5
*0601	14.3	5.6	0.7	NS	13.0	20.53 (3.47–121.56)	73.1

* Abbreviations per Table 1; *alleles in italics are protective factors*; bolded alleles were significantly different in those who took only implicated L-tryptophan (for myalgia N=26, for no myalgia N=10, for muscle weakness N=14, for no muscle weakness N=18).

** OR, odds ratio obtained by multivariate analysis.

*** Power to detect a significant difference ($p < 0.05$, two-tailed) between the groups being compared.

Table 4

Summary of risk and protective factors for different syndromes following L-tryptophan ingestion in this study*

Syndrome	Risk factors	Protective factors
EMS developing after ingestion of LT from multiple manufacturers	LT dose Age > 45 years DRB1*03 DRB1*04 DQA1*0601	<i>DRB1*07</i> <i>DQA1*0501</i>
EMS developing after ingestion of LT from a single implicated manufacturer	LT dose Age > 45 years DRB1*04	<i>DRB1*07</i> <i>DQA1*0201</i> <i>DQA1*0501</i>
EMS spectrum disorder developing after ingestion of LT from multiple manufacturers	LT dose Age > 45 years	<i>None</i>
EMS spectrum disorder developing after ingestion of LT from a single implicated manufacturer	LT dose Age > 45 years DRB1*04	<i>DRB1*07</i> <i>DQA1*0201</i>
Myalgia developing after ingestion of LT from multiple manufacturers	Age > 29 years No HLA alleles	<i>DRB1*07</i>
Myalgia developing after ingestion of LT from a single implicated manufacturer	<i>None</i>	<i>DQA1*0501</i>
Muscle weakness developing after ingestion of LT from multiple manufacturers	DQA1*0101	<i>None</i>
Muscle weakness developing after ingestion of LT from a single implicated manufacturer	<i>None</i>	<i>DQA1*0501</i>

* Abbreviations and conventions per Table 1.