Glutathione S-Transferase M Null Homozygosity and Risk of Systemic Lupus Erythematosus Associated with Sun Exposure: A Possible Gene-Environment Interaction for Autoimmunity

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ABSTRACT. ***Objective.*** Multiple genetic factors modulate predisposition to systemic lupus erythematosus (SLE). The glutathione S-transferase (GST) genes *GSTM1*, *GSTT1*, and *GSTP1* catalyze metabolic pathways for the excretion of reactive oxygen species that may be generated by cellular oxidative stress induced by ultraviolet radiation in sunlight. We hypothesized that risk of SLE associated with occupational sun exposure is modulated by *GSTM1*, *GSTT1*, and *GSTP1* genotypes.

Methods. DNA samples and occupational history were collected from 243 cases and 298 controls in the Carolina Lupus Study, a population based case-control study of patients with recently diagnosed SLE.

Results. There was no independent association between SLE and presence of the homozygous null *GSTM1* or *GSTT1* genotype, the homozygous *Val/Val* or heterozygous *Val/Ile GSTP1* genotype, or occupational sunlight exposure. The prevalence of Ro autoantibodies was significantly increased among Caucasians with the *GSTM1* null genotype (OR 2.6, 95% CI 1.0, 6.8), but was somewhat weaker among African-Americans (OR 1.5, 95% CI 0.7, 3.5). In the combined analysis of occupational sunlight exposure and *GSTM1* genotype, the effect of sun exposure among Caucasians varied depending on *GSTM1* genotype. There was a 3-fold increased risk (OR 3.1, 95% CI 0.9, 10.8) of SLE associated with 24 or more months' occupational sun exposure among Caucasians with the *GSTM1* null genotype, but sun exposure was not associated with risk among *GSTM1* positive Caucasians (OR 0.6, 95% CI 0.3, 1.5). The interaction was statistically significant (p = 0.028).

Conclusion. Our results suggest that *GSTM1* homozygous null genotype may modify the effect of occupational sun exposure on the risk of SLE in caucasians. (J Rheumatol 2003;30:276–82)

Key Indexing Terms:
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Systemic lupus erythematosus (SLE) is an autoimmune systemic rheumatic disease. Immunologic self-recognition of DNA and other nuclear macromolecules, a common feature in humans and animal models of SLE[1-3], is reflected in the extensive array of autoantibodies that are often seen in this disease. The mechanisms leading to immune recognition of these autoantigens in human SLE are not well defined. Events that lead to DNA damage may expose or alter the conformation of cryptic epitopes that are then recognized by autoreactive T cells. Environmental exposures that trigger these cellular mechanisms may predispose some individuals to SLE.

The metabolism and detoxification of many reactive oxygen intermediate (ROI) compounds can involve members of the glutathione S-transferase (GST) family of enzymes, *GSTM1*, *GSTT1*, and *GSTP1*[4]. Individuals who

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are homozygous for *GSTM1* or *GSTT1* null genes theoretically clear ROI more slowly, and may be at greater risk of damage to intracellular macromolecules. Reduced catalytic activity has also been reported with the *GSTP1* variant involving *Val* amino acid substitution at codon 105[5-9].

Recent research has focused on the interaction between GST enzymes and cancer risk in connection with environmental or occupational exposures (e.g., smoking, solvents)[10-12]. There are few reports that have addressed these metabolizing enzymes or gene-environment interactions in SLE, and the available data are somewhat inconsistent[13,14]. Ollier, *et al* reported an increased prevalence of *GSTM1* null genotype among Caucasian patients with SLE in the United Kingdom with anti-Ro, but not anti-La antibodies[13]. No association between *GSTM1* or *GSTT1* null genotype and risk of SLE was seen in the LUMINA (Lupus in Minority Populations, Nature versus Nurture) study in Alabama and Texas[14].

Ultraviolet radiation in sunlight can damage keratinocytes and other mammalian cells in a variety of ways, including induction of reactive oxygen species[15-18], which may damage DNA. We hypothesize that this latter molecular mechanism may be the basis for photosensitivity in SLE. Although sun exposure may exacerbate preexisting lupus[19,20], it is not known whether sun exposure is a risk factor for development of SLE.

We hypothesized that chronic exposure to sunlight may predispose to SLE and that the risk associated with exposure to sunlight may be altered by homozygosity for *GSTM1*, *GSTT1* null genes, or by the presence of the lower activity variant of *GSTP1*. To test these hypotheses, we analyzed the relationship between occupational sun exposure, *GSTM1* and *GSTT1* null homozygosity, *GSTP1* genotype, and SLE in the Carolina Lupus Study, a population based case-control study designed to assess the role of hormonal, environmental, and genetic risk factors for SLE.

MATERIALS AND METHODS

Subjects. The Carolina Lupus Study is based in 60 contiguous counties in eastern and central North Carolina and South Carolina. Eligibility was based on fulfillment of the American College of Rheumatology classification criteria for SLE[21,22], diagnosis between January 1, 1995, and July 31, 1999, age 18 years or older at study enrollment, residence within the study area during at least 6 months of the year prior to diagnosis, and ability to speak and understand English. Cases were referred from community based and university based rheumatology practices. We received 285 referrals of patients who were eligible for the study based on medical record data pertaining to the diagnostic criteria. Six patients refused screening and 14 declined to participate, for a total of 265 case participants (93% of the referrals). Details of the subject recruitment procedures have been described[23]. About 50% of the patients were referred from university based rheumatology practices and the median time from diagnosis to study interview was 13 months. The study protocol was approved by the review boards of all participating institutions.

Population based controls were identified through drivers license records corresponding to the counties in the study area and were frequency matched to the cases by age (5 year age groups), sex, and state. Eligibility criteria were the same as the nonmedical criteria used for cases with the additional criterion of never having been diagnosed with any kind of lupus. We enrolled 355 controls (75% of those who were screened and eligible). Controls were randomly assigned a reference month and year to correspond with the frequency distribution of the diagnosis month and year of cases.

As described, 90% of the SLE cases in the Carolina Lupus Study are female and 60% are African-American, 34% are white, and 6% are Native American, Asian or members of other racial or ethnic groups. The mean age at diagnosis was 39 years. Because of the age and sex matching procedure we used, these characteristics are similar in the controls. Our sample of controls is representative of the population in the study area: 28% African-American, 65% white, and 7% other racial and ethnic groups[23].

Occupational sun exposure and clinical features. Data collection included a structured 60 minute in-person interview with an extensive occupational history. For each job held for 12 or more months we asked if the participant worked outside in the sun for at least 10 hours per week for at least 3 months of the year. Information about ages worked in a particular job was used to eliminate jobs held at or after the diagnosis age (cases) or reference age (controls). For the exposure variable, we calculated total number of months in a sun-exposed job (defined as above, that is, work outside in the sun for 10 or more hours a week for 3 or more months of the year). This variable was dichotomized into 2 groups (< 24 mo, ≥ 24 mo) for analysis. We repeated the analyses using groups defined as 0 and 1 or more months and found similar results indicating that the specific cut-point used for categorization did not affect the results.

We abstracted data pertaining to the photosensitivity and other clinical features of SLE from patients' medical records. The abstraction was done by a combination of the investigators (one rheumatologist at each institution and an epidemiologist trained for this purpose by 2 of the rheumatologists). Details of the procedures have been described[24].

Blood samples, GST genotyping, and analysis of nuclear antibodies. We obtained one blood sample from 244 (92%) cases and 303 (85%) controls at the time of the interview. One case and 2 controls did not give permission to use their samples for genetic research. DNA was not extracted from 3 other control samples, leaving 243 cases and 298 controls in the analysis.

DNA was obtained from previously frozen blood samples after removal of red blood cells. Samples were extracted with phenol, phenol:chloroform:isoamyl alcohol (25:24:1), aliquoted, and stored at –70°C until further use.

GST genotyping was accomplished by multiplex polymerase chain reactions (PCR) to determine the presence or absence of *GSTM1* and *GSTT1* null homozygosity status, according the methods of Chen, *et al*[25]. This molecular typing strategy distinguishes between individuals who are homozygous for the *GSTM1* and *GSTT1* null alleles and those who are not. *GSTP* genotypes were determined by PCR-restriction fragment length polymorphism, according to a modification of the methods of Nedelcheva, *et al*[26].

To identify precipitating antibodies against Sm, RNP, and La/SSB, undiluted sera were tested by double immunodiffusion using a saline nuclear extract from rabbit thymus acetone powder (Pel-Freez, Rogers, AR, USA). Precipitin lines were observed at 48 h and identified by comparison with standard sera. For antibody to Ro/SSA, sera were similarly tested using a human spleen cell nuclear extract as antigen.

Sera were tested for antibody to native DNA by fixed *Crithidia luciliae* immunofluorescence. Sera diluted 1:10 were added to prepared slides (Sanofi), incubated 1 h, washed, and binding was detected using a FITC anti-IgG reagent as described above. Positive sera were titered until reaching the endpoint of immunofluorescence.

Statistical analysis. We examined the frequency of GST genotypes (*GSTM1*, *GSTT1*, *GSTP1*) among cases and controls using contingency tables. Because of the known racial difference in risk of SLE[27] and because GST genotype distributions vary by race[11,12], we conducted separate analyses among African-Americans and among Caucasians to determine if associations were similar in these 2 groups. The association between genotype and risk of developing SLE was estimated by the odds ratio (OR) and

95% confidence interval (CI) using logistic regression and adjusting for age, sex, and state. Analyses of the full sample also included a 2 level variable for race (Caucasians, African-Americans and other non-Caucasians). The adjusted OR were very similar to the crude (unadjusted) OR. We also adjusted for education (4 groups: did not complete high school, completed high school or equivalency, some college, completed college) in analyses of occupational sunlight exposure in relation to disease risk, since education could be related to work history. We used contingency tables to compare the frequency of specific autoantibodies by genotype, and estimated the association between genotype and autoantibodies adjusting for age and race (for the analysis of the full sample).

To evaluate gene-environment interactions, we included the main effects for the genotype variable (e.g., *GSTM1* null vs positive) and the occupational sun exposure variable (≥ 24 mo vs < 24 mo) and a variable representing their combined effects in a logistic regression model. We compared the likelihood ratio statistic of this model to the model without the interaction term. A statistically significant p value implies that the model with the interaction term is a better fit with the data, and thus that the interaction should be considered when interpreting the effects of either genotype or sunlight. In the full interaction model, the effects within 3 strata (null genotype with low exposure, positive genotype with high exposure, and null genotype with high exposure) were compared to the referent group of positive (non-null) genotype and low exposure. We also ran separate models to assess the effect of sunlight exposure by genotype (i.e., effect of sunlight exposure among null genotype and effect of sunlight exposure among positive genotype).

RESULTS

There was no association between sex or age and frequency of *GSTM1* null, *GSTT1* null, or *GSTP1* genotypes among controls (data not shown), but racial differences in the genotype distributions were seen (Table 1). *GSTM1* null genotype was less common among African-American controls compared with white controls, and the *GSTT1* null genotype and *GSTP1 Val/Val* genotype were more common among African-Americans. The frequencies among controls follow the distributions seen among controls in other population based studies in North Carolina[10,11]. Allele frequencies among cases and among controls are in Hardy-Weinberg equilibrium.

There was no evidence of an increased risk of SLE due to the *GSTM1* null genotype or *GSTP1 Val/Val* or *Val/Ile* genotypes. The *GSTT1* null genotype was associated with a small increased risk (OR 1.4) of SLE in the full sample, but this association was not statistically significant (Table 1). Similar results were seen in race-stratified analyses. The combination of *GSTM1* and *GSTT1* null genotypes was suggested as a risk factor for SLE in African-Americans (OR 3.9), but not in Caucasians (OR 0.8), but these are imprecise estimates because they are based on small numbers.

We saw little evidence of an association between occupational sunlight exposure and risk of SLE: 67% of cases and 67% of controls reported 0 months, 11% of cases and 10% of controls reported 1–24 months, 5% of cases and 6% of controls reported 24–45 months, 9% of cases and 8% of controls reported 46–110 months, and 8% of cases and 8% of controls reported > 110 months in a sunlight-exposed job. The association comparing 24 or more months to less than 24 months was OR 0.8, 95% CI 0.5, 1.3. Similar associations were seen in analyses stratified by race (among whites, OR 0.9, 95% CI 0.5, 1.7 and among African-Americans, OR 0.9, 95% CI 0.4, 2.0).

Photosensitivity was not associated with presence of anti-Ro antibodies: 40% of 86 cases with anti-Ro antibodies

Table 1. GSTM1 null, *GSTT1* null, and *GSTP1* genotypes and risk of developing SLE*.

	Total Sample (243 cases, 298 controls)			Caucasians (85 cases, 202 controls)			African-Americans (144 cases, 73 controls)		
	Cases, N(%)	Controls, N(%)	OR (95%CI)	Cases, N(%)	Controls, N(%)	OR (95%CI)	Cases, N(%)	Controls, N(%)	OR (95%CI)
GSTM1									
Positive	168 (69)	179 (60)	1.0 (referent)	48 (57)	110 (55)	1.0 (referent)	111 (77)	54 (75)	1.0 (referent)
Null	75 (31)	119 (40)	0.9 (0.6, 1.3)	37 (44)	92 (46)	0.9 (0.5, 1.5)	33 (23)	18 (25)	0.9 (0.5, 1.8)
GSTT1									
Positive	199 (82)	262 (88)	1.0 (referent)	73 (86)	181 (90)	1.0 (referent)	114 (79)	58 (82)	1.0 (referent)
Null	44 (18)	35 (12)	1.5 (0.9, 2.5)	12 (14)	21 (10)	1.5 (0.7, 3.2)	30 (21)	13 (18)	1.2 (0.6, 2.4)
GSTT1 and *GSTM1*									
Both positive	135 (56)	154 (52)	1.0 (referent)	39 (46)	98 (49)	1.0 (referent)	89 (62)	43 (60)	1.0 (referent)
GSTT null only	33 (14)	25 (8)	1.4 (0.7, 2.5)	9 (11)	12 (6)	2.0 (0.8, 5.1)	22 (15)	12 (17)	0.9 (0.4, 2.0)
GSTM null only	64 (26)	108 (36)	0.9 (0.6, 1.3)	34 (40)	83 (41)	1.0 (0.6, 1.8)	25 (17)	16 (22)	0.8 (0.4, 1.6)
Both null	11 (5)	10 (3)	1.5 (0.6, 3.9)	3 (4)	9 (5)	0.9 (0.2, 3.4)	8 (6)	1 (1)	3.8 (0.5, 31.5)
GSTP1									
Val/Val	41 (17)	43 (14)	0.9 (0.5, 1.6)	11 (13)	24 (12)	1.1 (0.5, 2.5)	28 (19)	16 (23)	0.6 (0.3, 1.4)
Val/Ile	110 (45)	140 (47)	0.9 (0.6, 1.3)	39 (46)	91 (45)	1.1 (0.6, 1.9)	68 (47)	38 (54)	0.6 (0.3, 1.3)
Ile/Ile	92 (38)	114 (38)	1.0 (referent)	35 (41)	87 (43)	1.0 (referent)	48 (33)	17 (24)	1.0 (referent)

*Odds ratio (OR) and 95% confidence interval (CI) estimated by logistic regression adjusting for age, sex, and state. Analyses for the full sample also adjusted for race as a 2 level variable (Caucasians, African-Americans and other non-Caucasians).
Total sample includes other minorities (14 cases, 23 controls) in addition to Caucasians and African-Americans.
One African-American control was missing *GSTT1* analyses.

had a history of photosensitivity compared with 40% of 150 cases without anti-Ro antibodies (race adjusted OR 1.0, 95% CI 0.6, 1.7). There was no association between photosensitivity and *GSTM1*, *GSTT1*, or *GSTP1* genotype (data not shown).

The prevalence of anti-Ro autoantibodies was significantly increased among Caucasians with the *GSTM1* null genotype compared with whites who were *GSTM1* positive (i.e., not null) (OR 2.6, p = 0.045) (Table 2). The association among African-Americans was not as strong and was not statistically significant (OR 1.5, p = 0.30). A similar pattern was also seen when limited to those who were positive for anti-Ro antibodies but negative for anti-La autoantibodies. There were no associations between specific autoantibodies and *GSTT1* or *GSTP1* genotypes (data not shown).

Occupational exposure to sunlight was somewhat associated with the presence of anti-Ro antibodies among cases, but the difference was not statistically significant (age, sex, and race adjusted OR 1.9, 95% CI 0.9, 4.0 for ≥ 24 mo compared with < 24 mo occupational sunlight exposure). This association was similar in Caucasians (OR 2.2, 95% CI 0.6,7.6) and African-Americans (OR 1.6, 95% CI 0.6, 4.3).

In the combined analysis of occupational sunlight exposure and *GSTM1* genotype, there was evidence of a gene-environment interaction among Caucasians. That is, the effect of sun exposure varied depending on *GSTM1* genotype (Table 3), and the interaction was statistically significant (p = 0.028). Among *GSTM1* positive Caucasians sun exposure was not associated with risk (OR 0.6, 95% CI 0.3, 1.2), but among Caucasians with the *GSTM1* null genotype there was a 3-fold increased risk (OR 3.1, 95% CI 0.9, 10.8) of SLE associated with 24 or more months occupational sun exposure (Table 4). An alternative way of expressing the interaction is that the effect of genotype varied depending on sun exposure. Among African-Americans, however, there was no evidence of a gene-environment interaction. No association with sun exposure was seen in either *GSTM1* null or *GSTM1* positive genotype groups, and the p value for the interaction was 0.91. There was no evidence of an interaction, in either race, with the *GSTT1* null genotype or with *GSTP1* genotype (data not shown).

DISCUSSION

We did not observe an association between *GSTM1* or *GSTT1* null homozygosity and risk of developing SLE in either African-Americans or Caucasians. We also did not observe an association between occupational sun exposure and risk of SLE overall or by racial group. There was evidence, however, of an association between *GSTM1* null genotype and presence of anti-Ro autoantibodies and between occupational sunlight exposure and anti-Ro autoantibodies. These associations were stronger among Caucasians than among African-Americans (Table 4).

Our data also suggest a gene-environment interaction between sun exposure and *GSTM1* null homozygosity and risk of SLE. To our knowledge, this is the first observation of this kind in SLE. Interestingly, this gene-environment interaction was limited to Caucasians. This finding may relate to differences in the skin reflectance between African-Americans and Caucasians due to the melanin content of the skin[28]. Although there is a wide variation in skin pigmentation in populations of African ancestry, it is likely that on average, the African-American subjects in this study had greater melanin content than their Caucasian counterparts. Melanin protects against ultraviolet induced genotoxicity by absorbing ultraviolet energy[29,30].

In vitro experiments using human keratinocytes have

Table 2. Number (%) of SLE cases with specific autoantibodies by race and *GSTM1* genotype*.

	Total Sample (n = 75 null, 168 positive)			Caucasians (n = 37 null, 48 positive)			African–Americans (n = 33 null, 111 positive)		
	GSTM null, N (%)	*GSTM* positive, N (%)	OR (95% CI) (p value)	*GSTM* null, N (%)	*GSTM* positive, N (%)	OR (95% CI) (p value)	*GSTM* null, N (%)	*GSTM* positive, N (%)	OR (95% CI) (p value)
Anti-Ro	33 (44)	57 (34)	1.7 (1.0, 3.0) (0.061)	16 (43)	11 (23)	2.6 (1.0, 6.8) (0.045)	15 (45)	41 (37)	1.5 (0.7, 3.5) (0.30)
Anti-La	10 (13)	13 (8)	1.8 (0.7, 4.6) (0.19)	5 (14)	3 (6)	2.2 (0.5, 9.9) (0.32)	5 (15)	8 (7)	2.5 (0.7, 8.4) (0.15)
Anti-Ro, not anti-La	24 (32)	44 (26)	1.6 (0.9, 3.0) (0.14)	11 (30)	8 (17)	2.4 (0.8, 7.0) (0.11)	11 (33)	33 (30)	1.3 (0.5. 3.1) (0.58)
Anti-dsDNA	21 (28)	45 (27)	1.3 (0.7, 2.5) (0.42)	6 (16)	9 (19)	0.8 (0.3, 2.7) (0.77)	12 (36)	36 (32)	1.1 (0.5, 2.7) 1.2 (0.75)
Anti-RNP	16 (21)	52 (31)	0.8 (0.4, 1.7) (0.63)	1 (3)	3 (6)	0.4 (0.0, 4.2) (0.44)	14 (42)	48 (43)	0.9 (0.4, 2.1) (0.88)
Anti-Sm	10 (13)	22 (13)	1.4 (0.6, 3.2) (0.45)	1 (3)	2 (4)	0.6 (0.0, 7.1) (0.67)	9 (27)	19 (17)	1.9 (0.8, 5.0) (0.35)

*Logistic regression, adjusting for age and sex, estimating odds ratio (OR) and 95% confidence interval (CI) for the association between genotype and presence of specific autoantibody, by race.

Table3. Interaction between *GSTM1* null genotypes, occupational sunlight exposure, and risk of developing SLE, by race*.

		GSTM1 Positive			*GSTM1* Null			
	Sun Exposure, mo**	Cases, n (%)	Controls, n (%)	OR (95% CI)	Cases, n (%)	Controls, n (%)	OR (95% CI)	p Value for *GSTM1*-Sun Interaction†
Caucasians††	< 24	39 (46)	83 (41)	1.0 (referent)	23 (27)	78 (39)	0.6 (0.3, 1.5)	
	≥ 24	9 (11)	27 (13)	0.6 (0.3, 1.2)	14 (16)	14 (7)	1.6 (0.5, 1.5)	
								0.028
African-Americans†††	< 24	89 (62)	45 (62)	1.0 (referent)	22 (15)	10 (14)	1.0 (0.4, 2.6)	
	≥ 24	26 (18)	14 (19)	0.9 (0.4, 2.0)	7 (5)	4 (5)	1.0 (0.2, 3.8)	
								0.91

*Logistic regression adjusting for age, sex, state, and education, estimating odds ratio (OR) and 95% confidence interval (CI).
**Occupational sunlight exposure based on job history obtained for each job lasting 12 or more months, and based on a question asking if the job involved work in the sun for 10 or more hours per week for at least 3 months of the year; months in these jobs was summed up to the diagnosis age for cases or corresponding reference age for controls.
† Interaction p value based on comparison of the likelihood ratio statistic for the model with only the variables representing the main effects of *GSTM* null genotype and of sunlight with the model that also included a variable representing their combined effects. A statistically significant p value implies that the model with the interaction term is a better fit with the data, and thus that the interaction should be considered when interpreting the effects of either genotype or sunlight.
†† 85 Caucasian cases, 202 Caucasians controls, †††144 African-American cases, 73 African-American controls.

Table 4. Summary of associations between *GSTM1*, occupational sunlight exposure, risk of SLE, and prevalence of anti-Ro antibodies.

	Total Sample OR (95% CI)	Caucasians OR (95% CI)	African-Americans OR (95% CI)
GSTM1 null and risk of SLE	0.8 (0.6, 1.3)	0.9 (0.5, 1.5)	0.9 (0.5, 1.9)
Occupational sunlight and risk of SLE	0.8 (0.5, 1.3)	0.9 (0.5, 1.7)	0.9 (0.4, 2.0)
GSTM1 null and anti–Ro antibodies	1.7 (1.0, 3.0)	2.6 (1.0, 6.8)	1.5 (0.7, 3.5)
Occupational sunlight and anti–Ro antibodies	0.9 (0.9, 4.0)	2.2 (0.6, 7.6)	1.6 (0.6, 4.3)
High sun exposure among *GSTM1* positive	—*	0.6 (0.3, 1.2)	1.0 (0.4, 2.6)
High sun exposure among *GSTM1* null	—*	3.1 (0.9, 10.8)	0.9 (0.1, 5.1)

*Not estimated because associations differed by race.

shown that exposure to ultraviolet-B radiation results in a redistribution of nuclear autoantigens (Ro, La, and snRNP) from the nucleus to the cell surface[31]. Some of this redistribution may occur in response to ultraviolet induced apoptosis, and apoptotic cells may serve as reservoirs for autoantigens, which could drive autoimmune responses in susceptible hosts[32]. Golan, *et al*[33] demonstrated that ultraviolet-B exposed keratinocytes from patients with lupus exhibit enhanced binding of IgG autoantibodies and suggest that the increased expression of autoantigens at the cell surface could provide the initial antigenic stimulus for the development of autoantibodies.

Ollier, *et al* reported an increased prevalence of *GSTM1* null genotype among Caucasian patients with SLE in the United Kingdom with anti-Ro, but not anti-La antibodies[13]. In contrast, no association between *GSTM1* null genotype and anti-Ro positive- anti-La negative status was seen in the analysis of African-American, caucasian, and Hispanic patients with SLE in the LUMINA study[14], but race-specific or race adjusted data were not presented. Ollier, *et al* postulated that *GSTM1* null genotype may increase susceptibility to oxidative stresses such as ultraviolet light, resulting in increased ultraviolet associated damage and expression of nuclear autoantigens on the cell surface. This idea is supported by the association between *GSTM1* null homozygosity, sun exposure, and anti-Ro antibody status and SLE in Caucasians observed in our study. Chronic sun exposure may lead to sufficient genotoxicity, DNA damage, in keratinocytes to alter cell surface expression of Ro and other nuclear proteins. In genetically susceptible individuals, these altered proteins become immunogenic and may lead to the development of SLE. Since we did not observe any association between sun exposure and anti-Ro antibody status in African-Americans, we hypothesize that different molecular mechanisms lead anti-Ro antibody production in this group.

A major strength of this report is that it is a large population based study of recently diagnosed patients with SLE. We used a systematic, active surveillance system (medical record review of all potentially eligible patients) at the universities, larger community based practices, and prac-

tices known for SLE care. Data collection involved a standardized in-person interview with an extensive occupational history. However, the occupational sunlight exposure measure we used was based on recall of jobs held at least 12 months, so shorter-term occupational exposures and recreational sunlight exposure are not included. We did not include recreational sources of sun exposure because of poorer reproducibility in self-reported history of these measures[34]. Both nondifferential and differential misclassification of sunlight exposure are possible because of general difficulties in recall accuracy, and because of possible over- or under-reporting of sun exposure by lupus patients. Another limitation that should be noted is that even with the large total sample size, the lower frequency of *GSTM1* null genotype among African-Americans resulted in small sample sizes (and relatively imprecise estimates) in some of the race-specific analyses. The frequency distribution of the sunlight data did not permit us to examine with any kind of precision a dose-response relationship between sun exposure and SLE risk.

In the Carolina Lupus Study, self-reported occupational sunlight exposure in Caucasians with the *GSTM1* null genotype was associated with an increased risk of developing SLE. If feasible, additional prospective studies that combine personal dosimetry of sun exposure[35], serial testing of anti-Ro antibodies, and biomarkers of genotoxicity[36,37] may elucidate the relationships between sunlight, *GSTM1* null genotype, anti-Ro antibodies, and SLE.

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REFERENCES

1. Lu L, Kaliyaperumal A, Boumpas DT, Datta SK. Major peptide autoepitopes for nucleosome-specific T cells of human lupus. J Clin Invest 1999;104:345-55.
2. Mohan C, Adams S, Stanik V, Datta SK. Nucleosome: a major immunogen for pathogenic autoantibody-inducing T cells of lupus. J Exp Med 1993;177:1367-81.
3. Burlingame RW, Boey ML, Starkebaum G, Rubin RL. The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. J Clin Invest 1994;94:184-92.
4. Hayes JD, Strange RC. Glutathione-S-transferase polymorphisms and their biological consequences. Pharmacology 2000;61:154-66.
5. Watson M, Steward R, Smith G, Massey T, Bell D. Glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 1998;19:275-80.
6. Landi S, Norppa H, Frenzilli G, et al. Individual sensitivity to cytogenetic effect of 1,2:3,4—diepoxybutane in cultured human lymphocytes: influence of glutathione S-transferase M1, P1 and T1 genotypes. Pharmacogenetics 1998;8:461-71.
7. Ollikainen T, Hirvonen A, Norppa H. Influence of GSTT1 genotype on sister chromatid exchange induction by styrene-7,8-oxide in cultured human lymphocytes. Environ Mol Mutagen 1998; 31:311-5.
8. Xu X, Wiencke JK, Niu T, et al. Benzene exposure, glutathione S-transferase theta homozygous deletion, and sister chromatid exchanges. Am J Ind Med 1998;33:157-63.
9. Park JY, Schantz SP, Stern JC, Kaur T, Lazarus P. Association between glutathione S-transferase p polymorphisms and oral cancer risk. Pharmacogenetics 1999;9:497-504.
10. Shinka T, Ogura H, Morita T, Nishikawa T, Fuginaga T, Ohkawa T. Relationship between glutathione S-transferase M1 deficiency and urothelial cancer in dye workers exposed to aromatic amines. J Urol 1998;159:380-3.
11. Olshan AF, Weissler MC, Watson MA, Bell DA. GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 polymorphisms, tobacco use, and the risk of head and neck cancer. Cancer Epidemiol Biomarkers Prev 2000;9:185-91.
12. Millikan R, Pittman G, Tse C-K, Savitz DA, Newman B, Bell D. Gluathione S-transferases M1, T1, and P1 and breast cancer. Cancer Epidemiol Biomarkers Prev 2000;9:567-73.
13. Ollier W, Davies E, Snowden N, et al. Association of homozygosity for glutathione-S-transferase GSTM1 null alleles with the Ro+/La- autoantibody profile in patients with systemic lupus erythematosus. Arthritis Rheum 1996;39:1763-4.
14. Tew MB, Ahn CW, Friedman AW, et al. Systemic lupus erythematosus in three ethnic groups. VIII. Lack of association of glutathione S-transferase null alleles with disease manifestations. Arthritis Rheum 2001;44:981-3.
15. Griffiths HR, Mistry P, Herbert KE, Lunec J. Molecular and cellular effects of ultraviolet light-induced genotoxicity. Crit Rev Clin Lab Sci 1998;35:189-237.
16. Muller L, Kasper P, Kersten B, Zhang J. Photochemical genotoxicity and photochemical carcinogenesis — two sides of a coin? Toxicol Lett 1998;102-3:383-7.
17. Record IR, Jannes M, Dreosti IE. Protection by zinc against UVA- and UVB-induced cellular and genomic damage in vivo and in vitro. Biol Trace Elem Res 1996;53:19-25.
18. Runger TM, Epe B, Moller K. Processing of directly and indirectly ultraviolet-induced DNA damage in human cells. Recent Results Cancer Res 1995;139:31-42.
19. Nived O, Johansen PB, Sturfelt G. Standardized ultraviolet-A exposure provokes skin reaction in systemic lupus erythematosus. Lupus 1993;2:247-50.
20. Nived O, Sturfelt G, Ryberg B. Two UV-induced episodes of myelitis in a patient with systemic lupus erythematosus. J Intern Med 1992;232:461-3.
21. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
22. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
23. Cooper GS, Dooley MA, Treadwell EL, St. Clair EW, Gilkeson GS. Hormonal and reproductive risk factors for development of systemic lupus erythematosus. Arthritis Rheum 2002;46:1830-9.

24. Cooper GS, Parks CG, Treadwell EL, et al. Differences by race, sex, and age in the clinical and immunologic features of recently-diagnosed systemic lupus erythematosus patients in the Southeastern United States. Lupus 2002;11:161-7.
25. Chen CL, Liu Q, Relling MV. Simultaneous characterization of glutathione S-transferase M1 and T1 polymorphisms by polymerase chain reaction in American whites and blacks. Pharmacogenetics 1996;6:187-91.
26. Nedelcheva KV, Andersen TI, Erikstein B, et al. Single tube multiplex polymerase chain reaction genotype analysis of GSTM1, GSTT1 and GSTP1: relation of genotypes to TP53 tumor status and clinicopathological variables in breast cancer patients. Pharmacogenetics 1998;8:441-7.
27. Cooper GS, Dooley MA, Treadwell EL, St. Clair EW, Parks CG, Gilkeson GS. Hormonal, environmental, and infectious disease risk factors for the development of systemic lupus erythematosus. Arthritis Rheum 1998;41:1714-24.
28. Berardesca E, Maibach H. Racial differences in skin pathophysiology. J Am Acad Dermatol 1996;34:667-72.
29. Gilchrest BA, Eller MS. DNA photodamage stimulates melanogenesis and other photoprotective responses. J Invest Dermatol Symp Proc 1999;4:35-40.
30. Shono S, Imura M, Ota M, Ono S, Toda K. The relationship of skin color, UVB-induced erythema, and melanogenesis. J Invest Dermatol 1985;84:265-7.
31. Casciola-Rosen L, Rosen A. Ultraviolet light-induced keratinocyte apoptosis: a potential mechanism for the induction of skin lesions and autoantibody production in LE. Lupus 1997;6:175-80.
32. Rosen A, Casciola-Rosen L. Clearing the way to mechanisms of autoimmunity. Nature Med 2001;7:664-5.
33. Golan T, Elkon K, Ghatavi A, Krueger J. Enhanced membrane binding of autoantibodies to cultured keratinocytes of systemic lupus erythematosus. J Clin Invest 1992;90:1067-76.
34. English DR, Armstrong BK, Kricker A. Reproducibility of reported measurements of sun exposure in a case-control study. Cancer Epidemiol Biomarkers Prev 1998;7:857-63.
35. Moehrle M, Garbe C. Personal UV dosimetry by Bacillus subtilis spore films. Dermatology 2000;200:1-5.
36. Heenen M, Giacomoni PU, Golstein P. Individual variations in the correlation between erythemal threshold, UV-induced DNA damage and sun-burn cell formation. J Photochem Photobiol B 2001; 63:84-7.
37. Marrot L, Belaidi JP, Chaubo C, Meunier JR, Perez P, Agapakis-Causse C. An in vitro strategy to evaluate the phototoxicity of solar UV at the molecular and cellular level: application to photoprotection assessment. Eur J Dermatol 1998;8:403-12.