ARTHRITIS & RHEUMATISM
Vol. 60, No. 5, May 2009, pp 1250–1254
DOI 10.1002/art.24468

The *PRL* –1149 G/T Polymorphism and Rheumatoid Arthritis Susceptibility

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Objective. **Previous studies have demonstrated that the *PRL* −1149 T (minor) allele decreases prolactin expression and may be associated with autoimmune disease. The aim of this study was to determine the role of the *PRL* −1149 G/T polymorphism (rs1341239) in rheumatoid arthritis (RA) susceptibility.**

Methods. **We examined the association between *PRL* −1149 G/T and RA risk in 4 separate study populations, consisting of a total of 3,405 RA cases and 4,111 controls of self-reported white European ancestry. Samples were genotyped using 1 of 3 genotyping platforms, and strict quality control metrics were applied. We tested for association using a 2-tailed Cochran-Mantel-Haenszel additive, fixed-effects model.**

Results. **In the individual populations, odds ratios (ORs) for an association between *PRL* −1149 T and RA risk ranged from 0.80 to 0.97. In a joint meta-analysis across all 4 populations, the OR for an association between *PRL* −1149 T and RA risk was 0.90 (95% confidence interval 0.84–0.96, $P = 0.001$).**

Conclusion. **Our findings indicate a possible association between the *PRL* −1149 T allele and decreased RA risk. The effect size is small but similar to ORs for other genetic polymorphisms associated with complex traits, including RA.**

Prolactin, an important hormone in lactogenesis, has immunomodulatory properties and may play a vital role in the development of rheumatoid arthritis (RA). Prolactin binds to the prolactin receptor, a member of the class I cytokine superfamily, to induce lymphocyte proliferation, inhibit apoptosis, and stimulate antibody formation (1). Extrapituitary prolactin, which is produced by lymphocytes and endometrial cells, increases interferon-γ production and enhances the effect of interleukin-2 in lymphocytes (2).

RA is a chronic inflammatory synovitis of autoimmune etiology that is 2–4 times more common in women than in men. Findings of clinical studies have supported the idea that prolactin has a potential role in RA pathogenesis. For example, the incidence of RA increases during the postpartum period, which coincides

Dr. Lee's work was supported by the Scholars in Clinical Science Program, which is funded by NIH grant K30-RR-022292-07. Dr. Raychaudhuri's work was supported by an NIH/National Institute of Arthritis and Musculoskeletal and Skin Diseases Career Development award (K08-AR-055688A). Dr. Padyukov's work was supported by the Swedish National Research Council. Dr. Costenbader is recipient of an Arthritis Foundation/American College of Rheumatology Arthritis Investigator award and an NIH Building Interdisciplinary Research Careers in Women's Health award (K12-HD-051959). Dr. Klareskog's work was supported by the Swedish National Research Council and the Swedish Rheumatism Association. Dr. Plenge's work was supported by NIH grant K08-AI-55314-3, the William Randolph Hearst Fund of Harvard University, and a Career Award for Medical Scientists from the Burroughs Wellcome Fund. Dr. Karlson's work was supported by NIH grants R01-AR-49880, P60-AR-047782, and K24-AR-0524-01. The Broad Institute Center for Genotyping and Analysis is supported by grant U54-RR020278 from the National Center for Research Resources.

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Dr. Gregersen has received consulting fees, speaking fees, and/or honoraria from Roche (less than $10,000) and owns stock or stock options in Amgen, Illumina, and Genentech.

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Submitted for publication September 5, 2008; accepted in revised form January 31, 2009.

Table 1. Description of the 4 study populations used in the meta-analysis*

	Cases (n = 3,405)		Controls (n = 4,111)	
Study population (ref.)	No.	Description	No.	Description
NHS/NHSII (10)	437	Caucasian female nurses from North America with incident RA (62% seropositive), fulfilling ACR criteria for RA	437	Caucasian female nurses from North America, matched to cases by age, menopause status, and hormone use
EIRA (12)	1,191	Swedish residents (97% white) with anti-CCP–positive RA, fulfilling ACR criteria for RA	1,111	Healthy Swedish residents (97% white), matched to cases by age, sex, and geographic location
NARAC GWAS-I (12)	908	Caucasian North American residents with anti-CCP–positive RA, fulfilling ACR criteria for RA	1,260	Healthy Caucasian New York residents participating in the New York Cancer Project
NARAC GWAS-II (13)	869	Caucasian North American residents with anti-CCP–positive RA, fulfilling ACR criteria for RA	1,303	Healthy Caucasian New York residents participating in the New York Cancer Project

* NHS = Nurses' Health Study; RA = rheumatoid arthritis; ACR = American College of Rheumatology; EIRA = Epidemiological Investigation of Rheumatoid Arthritis; anti-CCP = anti–cyclic citrullinated peptide; NARAC = North American Rheumatoid Arthritis Consortium; GWAS = genome-wide association study.

with elevated levels of prolactin in women who choose to breastfeed. However, prospective cohort studies have suggested an inverse relationship between the duration of past breastfeeding and future RA susceptibility (3–6). These observations suggest that breastfeeding, and possibly prolactin, may have differential effects on short- and long-term risk for RA, though the exact mechanism by which prolactin affects RA risk is still unknown.

The prolactin gene (*PRL*) is controlled by 2 promoters. One of these promoters regulates production of prolactin from the pituitary gland, and the other regulates production of extrapituitary prolactin. The *PRL* −1149 G/T polymorphism is located in the extrapituitary promoter region and influences messenger RNA expression levels. Electrophoretic mobility shift assays have shown that the *PRL* −1149 G/T polymorphism alters binding of a GATA-related transcription factor (7). Transient transfection and reverse transcriptase–polymerase chain reaction assays of phytohemagglutinin-treated peripheral blood lymphocytes indicate that the G allele is associated with higher levels of promoter activity (7).

Results of previous studies have suggested an association between *PRL* −1149 G/T and autoimmunity, although the studies were small and the findings inconclusive. The high-expressing G allele has been associated with increased risk of systemic lupus erythematosus (SLE) in 1 study of 143 SLE patients and 394 healthy controls (7), but not in 2 other studies (8,9). To date, the only study demonstrating an association with RA, which included 173 RA patients and 123 healthy controls, showed evidence that the heterozygous genotype of *PRL* −1149 G/T was associated with increased RA risk, but that neither homozygous genotype was associated with RA risk (8).

In this study, we examined the association between the *PRL* −1149 G/T polymorphism and RA risk in case–control samples collected as part of the Nurses' Health Study/Nurses' Health Study II (NHS/NHSII), the Epidemiological Investigation of Rheumatoid Arthritis (EIRA), and the North American Rheumatoid Arthritis Consortium (NARAC).

PATIENTS AND METHODS

Study populations. We used data from 4 study populations: the NHS/NHSII (10,11), the EIRA (12), and 2 populations that were part of the NARAC (12) (Table 1). (The NARAC populations are referred to herein as NARAC genome-wide association study I [GWAS-I] and NARAC GWAS-II [note that NARAC GWAS-II is identified as NARAC-III in ref. 13].) All participants were of self-reported white European ancestry, and either met the 1987 American College of Rheumatology (formerly, the American Rheumatism Association) criteria for RA (14) or were diagnosed as having RA by a board-certified rheumatologist. All cases in the EIRA, NARAC GWAS-I, and NARAC GWAS-II populations were positive for anti–cyclic citrullinated peptide (anti-CCP) antibodies. Sixty percent of the NHS/NHSII cases were either anti-CCP positive or rheumatoid factor positive. The Institutional Review Board at each collection site approved all study procedures, and informed consent was obtained from all participants. Detailed information on these populations can be found in Table 1 and in previously published reports (10,12,13).

Genotyping. For NHS/NHSII cases, DNA from blood samples was genotyped using Sequenom genotyping technology (Sequenom, San Diego, CA); buccal cell samples were genotyped using the TaqMan single-nucleotide polymorphism (SNP) allelic discrimination method with an ABI 7900HT

Table 2. Genotypes, minor allele frequencies, ORs, and *P* values for the association between *PRL* −1149 G/T and rheumatoid arthritis in 4 study populations and the joint meta-analysis*

Study population	Genotype: Cases G/G	Cases G/T	Cases T/T	Controls G/G	Controls G/T	Controls T/T	Minor allele frequency: Cases	Controls	OR (95% CI)	*P*
NHS/NHSII	180	202	49	158	196	72	0.35	0.40	0.80 (0.66–0.98)	0.03
EIRA	514	511	144	425	493	169	0.34	0.38	0.84 (0.74–0.95)	0.005
NARAC GWAS-I	334	397	129	469	507	205	0.38	0.39	0.97 (0.85–1.10)	0.63
NARAC GWAS-II	350	373	124	508	583	207	0.37	0.38	0.93 (0.82–1.05)	0.26
Meta-analysis	1,378	1,483	446	1,560	1,779	653	0.36	0.39	0.90 (0.84–0.96)	0.001

* Genotype counts may not equal the exact number of cases and controls described in Table 1; the discrepancy is a result of missing data from samples that were not adequately genotyped for *PRL* −1149 G/T. OR = odds ratio; 95% CI = 95% confidence interval (see Table 1 for other definitions).

system (Applied Biosystems, Foster City, CA). EIRA samples were genotyped with the HumanHap300 Array (Illumina, San Diego, CA) (12) and the Sequenom iPlex platform. GWAS-I cases were genotyped with the Illumina HumanHap550 Array, while NARAC GWAS-I controls were genotyped with either the Illumina HumanHap550 Array or the Illumina HumanHap300 + 240 Arrays (12). All NARAC GWAS-II samples were genotyped using the Illumina HumanHap300 Array. We removed from analysis data on individuals for whom >5% of the genotype data were missing. All SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$). Full genotyping details have been described previously (10,12,13).

Statistical analysis. Chi-square tests were used to assess the relationship between alleles and RA risk, using an allelic model with 1 df. All analyses were first performed in each data set separately. Data from the individual NHS/NHSII, EIRA, NARAC GWAS-I, and NARAC GWAS-II study populations were then combined in a meta-analysis, using an additive, 2-tailed Cochran-Mantel-Haenszel fixed-effects model. Using the same techniques, post hoc analyses were performed to examine the association between *PRL* −1149 G/T and RA risk in sex-specific subgroups. All data analysis was performed using MatLab software (MathWorks, Natick, MA).

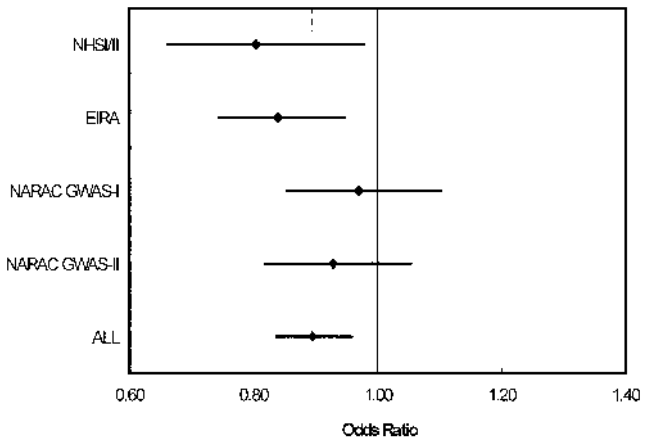

Figure 1. Association of *PRL* −1149 T with rheumatoid arthritis in the 4 study populations and the joint meta-analysis. Diamonds represent odds ratios (ORs); solid lines show 95% confidence intervals. Broken line represents the OR from the meta-analysis. NHS = Nurses' Health Study; EIRA = Epidemiological Investigation of Rheumatoid Arthritis; NARAC = North American Rheumatoid Arthritis Consortium; GWAS = genome-wide association study.

RESULTS

The clinical features of subjects in the NHS/NHSII, the EIRA, and the NARAC GWAS-I and NARAC GWAS-II populations are described in Table 2. The minor allele frequencies were similar (ranging from 0.38 to 0.40) in the controls in all 4 populations. The odds ratios (ORs) for the association of the *PRL* −1149 T allele with RA risk ranged between 0.80 and 0.97: for the NHS/NHSII, the OR was 0.80 (95% confidence interval [95% CI] 0.66–0.98, $P = 0.03$); for the EIRA, the OR was 0.84 (95% CI 0.74–0.95, $P = 0.005$); for the NARAC GWAS-I, the OR was 0.97 (95% CI 0.85–1.10, $P = 0.63$); and for the NARAC GWAS-II, the OR was 0.93 (95% CI 0.82–1.05, $P = 0.26$). The Breslow-Day *P* value was 0.40, indicating no evidence of heterogeneity; thus, these populations were combined via a Cochran-Mantel-Haenszel fixed-effects model. A joint meta-analysis of all 3,405 RA cases and 4,111 controls resulted in a combined OR of 0.90 (95% CI 0.84–0.96, $P = 0.001$ by 2-tailed test) (Table 2 and Figure 1). Sex-specific analyses revealed similar effect sizes among men and women (OR 0.91 [95% CI 0.80–1.02]; $P = 0.11$ in men and OR 0.89 [95% CI 0.82–0.97]; $P = 0.005$ in women).

DISCUSSION

We performed a comprehensive analysis of the association between *PRL* −1149 G/T and RA susceptibility in 4 separate study populations. In the individual populations, an association between the *PRL* −1149 T allele and decreased risk of RA was suggested, with ORs ranging from 0.80 to 0.97. In a joint meta-analysis of all 3,405 RA cases and 4,111 controls, the combined OR was 0.90 (95% CI 0.84–0.96, $P = 0.001$). Though these results are not definitive, they are intriguing, suggesting a modest protective effect of the *PRL* −1149 T allele on RA risk.

Results of previous studies have suggested an association between *PRL* −1149 G/T and autoimmunity. In a study of 143 SLE patients and 394 healthy controls of European ancestry, Stevens et al reported that the *PRL* −1149 G allele was associated with SLE risk (OR 2.51, 95% CI 1.14–6.28) (7). This OR is equivalent to an OR of 0.4 for the association between the *PRL* −1149 T allele and SLE susceptibility (7). This association is in the same direction as, but of somewhat higher magnitude than, the association we observed with RA. However, in 2 other studies of SLE patients, the association between *PRL* −1149 G/T and SLE could not be replicated (8,9). Those studies were relatively small, involving ~150 SLE cases each, and may have been underpowered to detect a small-to-moderate effect.

Only one study has examined the association between *PRL* −1149 G/T and RA risk. In that study of 173 Czech RA patients and 123 healthy controls, the heterozygous genotype of −1149 G/T was associated with RA risk, but neither homozygous genotype was associated with RA risk (8). In a separate study, involving 463 patients with juvenile inflammatory arthritis (JIA) and 263 healthy controls of European ancestry, an association between *PRL* −1149 G/T and JIA could not be demonstrated (15). Similarly, a study of 83 Czech psoriatic arthritis patients and 123 healthy controls did not demonstrate an association between *PRL* −1149 G/T and psoriatic arthritis risk (11). However, these studies may have been underpowered to detect a small-to-modest effect.

Our study is unique because it is the first to examine data on *PRL* −1149 G/T and RA risk in 4 large, independent populations. By combining data from these populations, we were able to assemble a cohort of 3,405 cases and 4,111 controls. The large sample size provided adequate power to detect modest associations. The combined OR of 0.90 is consistent with effect sizes seen in recently published studies of RA susceptibility genes and in other studies of genetic associations in complex diseases (12,13,16).

False-positive results may occur due to population stratification, but we do not believe that population stratification played a significant role in this study. The minor allele frequencies of the control groups were similar across all 4 populations, consistent with the assumption that all samples were drawn from similar populations. The *P* value derived from the Breslow-Day test of heterogeneity of ORs across all 4 populations was not significant.

Although these analyses indicate that the study populations were sufficiently similar to combine in a meta-analysis, notable differences did exist between the NHS/NHSII, the EIRA, and the NARAC GWAS-I and NARAC GWAS-II populations. First, the NHS/NHSII cohort included only women, while the other cohorts also included men. Second, the NHS/NHSII cohort included both seropositive and seronegative RA patients, whereas the other cohorts only included anti-CCP–positive patients. To determine whether the association between *PRL* −1149 G/T and RA risk was different between men and women, we performed sex-specific analyses. However, there was no evidence of a sex effect; the effect size for the association between *PRL* −1149 G/T and RA risk was similar among men and women. The *P* value for association was higher among men than women, but this difference was likely due to the small number of men in these cohorts. Our study did not have sufficient statistical power to examine the association between *PRL* −1149 G/T and RA risk in anti-CCP–negative patients because of the small sample size. Future studies involving large populations of anti-CCP–negative RA patients will be necessary to clarify the effect of *PRL* −1149 G/T on RA risk.

The findings of a previous meta-analysis by our group suggested that the majority of genetic variation in RA can be explained by polymorphisms with modest effect sizes that are difficult to detect in current genome-wide association studies due to insufficient sample sizes (13). The findings of our previous study revealed an OR of 0.85 for the association between a *CD40* SNP and RA risk, and ORs of ~1.15 for associations between 5 other loci and RA risk (13). The size of these ORs is similar to our results involving *PRL* −1149 G/T. These recent findings suggest the need for future studies with larger sample sizes and combined studies using meta-analysis techniques to analyze the risk of RA.

In summary, our results add substantial information to the data from previous studies suggesting an association between the *PRL* −1149 G/T polymorphism

and autoimmunity. This association has not been detected in previous genome-wide association scans, possibly because these scans were underpowered to detect small-to-modest associations. The association between *PRL* −1149 T and decreased RA susceptibility is modest ($P = 0.001$), but the OR (0.90) is comparable to the reported ORs for genetic associations of complex traits. The *PRL* −1149 T allele is associated with lower levels of *PRL* promoter activity, possibly corresponding to lower levels of prolactin, which may be associated with a decreased risk for RA. Although studies have shown that *PRL* −1149 G/T is a functional polymorphism in vitro, future studies are necessary to clarify its functional role in vivo.

AUTHOR CONTRIBUTIONS

Dr. Lee had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Lee, Raychaudhuri, Alfredsson, Costenbader, Seielstad, Graham, Klareskog, Plenge, Karlson.

Acquisition of data. De Vivo, Alfredsson, Padyukov, Costenbader, Seielstad, Gregersen, Karlson.

Analysis and interpretation of data. Lee, Raychaudhuri, Cui, Ding, Alfredsson, Costenbader, Plenge, Karlson.

Manuscript preparation. Lee, Raychaudhuri, Cui, De Vivo, Alfredsson, Padyukov, Costenbader, Klareskog, Plenge, Karlson.

Statistical analysis. Raychaudhuri, Cui, Ding, Plenge.

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