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Investigation of the *PRL*–1149 G/T Polymorphism and Rheumatoid Arthritis Susceptibility

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Abstract

Objectives—Prior evidence demonstrates that the *PRL* -1149 T (minor) allele decreases prolactin expression and may be associated with autoimmune diseases. Our goal 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 four separate collections consisting of 3,405 RA cases and 4,111 controls of self-described “white” European ancestry. Samples were genotyped using one of three genotyping platforms, and strict quality control metrics were applied. We tested for association with a two-tailed Cochran Mantel-Haenszel additive, fixed effects model.

Results—In the individual collections, odds ratios for an association between *PRL* -1149 T and RA risk ranged from 0.80 to 0.97. In a joint meta-analysis across all four collections, the odds ratio for an association between *PRL* -1149 T and RA risk was 0.90 (95% CI 0.84-0.96) with p = 0.001.

Conclusions—Our results suggest an association between the *PRL* -1149 T allele and decreased RA risk. The effect size is small but similar to odds ratios for other genetic polymorphisms associated with complex traits, including RA. Further studies will be necessary to confirm association unequivocally.

Prolactin, an important hormone in lactogenesis, has immunomodulatory properties and may play an important 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 IFN-γ production and enhances the effect of IL-2 in lymphocytes (2).

RA is a chronic inflammatory synovitis of autoimmune etiology that is two to four times more common in women than men. Clinical studies support a potential role for prolactin in RA pathogenesis. During the postpartum period, the incidence of RA increases. This time period coincides with elevated prolactin levels in women who choose to breastfeed. However, prospective cohort studies have suggested an inverse relationship between duration of past breastfeeding and future RA susceptibility (3–6). These observations suggest that breastfeeding, and possibly prolactin, may have differential effects on acute and

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long term risk for RA, but the exact mechanism by which prolactin affects RA risk is still unknown.

The prolactin gene (*PRL*) is controlled by two promoters, one that regulates production of prolactin from the pituitary and one that regulates production of extrapituitary prolactin. The *PRL* -1149 G/T polymorphism is located in the extrapituitary promoter region and influences mRNA 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 studies and reverse transcriptase-PCR studies of phytohemaglutinin-treated peripheral blood lymphocytesindicate that the G allele is associated with higher levels of promoter activity (7).

Previous studies have suggested an association between *PRL* -1149 G/T and autoimmunity, although the studies were small and inconclusive. The high-expressing G allele has been associated with increased risk of systemic lupus erythematosus (SLE) in one study of 143 SLE patients and 394 healthy controls (7), but not in two other studies (8,9). The only study published to date in RA, which included 173 RA patients and 123 healthy controls, found evidence that the heterozygous genotype of *PRL* -1149 G/T was associated with increased RA risk, but 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 Epidemiologic Investigation of Rheumatoid Arthritis (EIRA), and the North American Rheumatoid Arthritis Consortium (NARAC).

PATIENTS and METHODS

Patient Collections

We utilized data from four patient collections: NHS/NHSII (10,11), EIRA (12), and two collections that are part of NARAC (12) (Table 1). The NARAC collections are referred to in this manuscript as NARAC GWAS I and NARAC GWAS II (note that NARAC GWAS-II here is identified as NARAC-III in Raychaudhuri et al. (13)). All participants were of self-reported "white" European ancestry and either met 1987 American College of Rheumatology (ACR) criteria for RA or were diagnosed by board-certified rheumatologists. All EIRA, NARAC GWAS-I and NARAC GWAS-II cases were anti-cyclic citrullinated peptide (CCP) antibody positive. Sixty percent of NHS/NHSII cases were either anti-CCP or rheumatoid factor (RF) positive. The Institutional Review Board of each collecting site approved all study procedures, and informed consent was obtained from all participants. Detailed information on these collections can be found in Table 1 and in previously published studies (10,12,13).

Genotyping

For NHS/NHSII, DNA from blood samples was genotyped using Sequenom; buccal cell samples were genotyped with Taqman SNP allelic discrimination on the ABI 7900HT. EIRA samples were genotyped with the Illumina HumanHap300 Array (12) and Sequenom iPLEX platform. NARAC GWAS-I cases were genotyped with the Illumina HumanHap550 Array, while NARAC GWAS-I controls were genotyped with the Illumina HumanHap550 Array or the Illumina HumanHap300 + 240 Arrays (12). NARAC GWAS-II samples were genotyped using the Illumina HumanHap300 Array. Analyzed SNPs were required to pass quality control criteria, including: 1) minor allele frequency > 1% and 2) Hardy-Weinberg equilibrium ($p > 0.05$). Full genotyping details are described in previously published studies (10,12,13).

Statistical Analyses

χ^2 tests were used to assess the relationship between alleles and RA risk, using an allelic model with one degree of freedom. All analyses were first performed in each dataset separately. Data from the NHS/NHSII, EIRA, NARAC GWAS-I and NARAC GWAS-II collections were then combined in a meta-analysis, using an additive, two-tailed Cochran Mantel-Haenszel (CMH) 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 gender-specific subgroups. All data analysis was performed using MATLAB.

RESULTS

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

DISCUSSION

We performed a comprehensive analysis of the association between *PRL* -1149 G/T and RA susceptibility in four separate patient collections. In the individual collections, an association between the *PRL* -1149 T allele and lower RA risk was suggested, with odds ratios ranging from 0.80 to 0.97. In a joint analysis of all 3,405 RA cases and 4,111 controls, the combined odds ratio was 0.90 [0.84–0.96] with 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.

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). 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, but of somewhat higher magnitude, compared to the association we observed with RA. However, two other studies of SLE patients were unable to replicate the association between *PRL* -1149 G/T and SLE (8,9). These studies were relatively small, involving approximately 150 SLE cases each, and may have been underpowered to detect a small to moderate effect size.

Only one study has examined the association between *PRL* -1149 G/T and RA risk. In this 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). A separate study involving 463 patients with juvenile inflammatory arthritis (JIA) and 263 healthy controls of European ancestry was unable to find any association between *PRL* -1149 G/T and JIA (14). 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), but 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 four large, independent collections. By combining data from these collections, 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 odds ratio 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 (12,13,15).

False-positive results may occur as a result of population stratification, but we do not believe that population stratification played a significant role in this study. The control minor allele frequencies were similar across all four collections, consistent with the assumption that all samples were drawn from similar populations, and the Breslow-Day test of heterogeneity of odds ratios across all four collections was not significant.

Although these analyses indicate that the collections are sufficiently similar to combine in a meta-analysis, notable differences did exist between the NHS/NHSII cohorts and the EIRA, NARAC GWAS-I and NARAC GWAS-II collections. 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 individuals, whereas the other cohorts only included anti-CCP positive individuals. To determine whether the association between *PRL* -1149 G/T and RA risk was different between the men and women, we performed gender specific analyses. However, there was no evidence for a gender effect, as 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 compared to women, but this difference was likely due to the small number of men in these cohorts. We 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 small sample size. Future studies involving large collections of anti-CCP negative RA patients will be necessary to clarify the effect of *PRL* -1149 G/T on RA risk.

A recent meta-analysis by Raychaudhuri et al. 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). This study revealed an odds ratio of 0.85 for the association between a *CD40* SNP and RA risk and odds ratios of ~ 1.15 for associations between five other loci and RA risk (13). The size of these odds ratios 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 in RA.

In summary, our results add substantial information to 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 p-value of 0.001 for the association between *PRL* -1149 T and decreased RA susceptibility is modest, but the odds ratio of 0.90 is comparable to reported odds ratios 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 lower risk for RA. Although studies have shown that *PRL* -1149 G/T is a functional polymorphism in vitro, future studies are necessary to clarify the functional role of *PRL* -1149 G/T in vivo.

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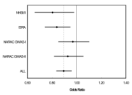

Figure 1. Association of *PRL* -1149 T across four collections

For each collection, we plot the odds ratio (diamond) and the 95% confidence interval. A dashed line indicates the odds ratio across all four collections.

Table 1

Collections used in meta-analysis

The four collections were genotyped as part of other studies. For each collection, we list the number of cases and controls and the source of controls. We also provide information regarding ethnicity and auto-antibody status.

Study	Collection	Cases		Controls	
		Description	N	Description	N
Nurses Health Study (NHS) and NHSII	NHS/NHSII	Caucasian female nurses from North America with incident RA. 62% with seropositive RA. All met ACR criteria for RA (10)	437	Caucasian female nurses from North America, matched to cases by age, menopausal status and hormone use (10)	437
Epidemiologic Investigation of Rheumatoid Arthritis (EIRA)	EIRA	Swedish residents (97% white) with anti-CCP positive RA, fulfilling ACR criteria for RA (12)	1191	Healthy Swedish residents (97% white), matched to cases by age, sex and geographic location (12)	1111
North American Rheumatoid Arthritis Consortium (NARAC)	NARAC GWAS-I	Caucasian North American residents with anti-CCP positive RA, fulfilling ACR criteria for RA (12)	908	Healthy Caucasian New York residents, participating in the New York Cancer Project (12)	1260
	NARAC GWAS-II	Caucasian North American residents with anti-CCP positive RA, fulfilling ACR criteria for RA (13)	869	Healthy Caucasian, New York residents, participating in the New York Cancer Project (13)	1303
Total			**3405**		**4111**

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Table 2

Genotype counts, minor allele frequencies, odds ratios and p-values for the association between *PRL* -1149 G/T and RA.

Collection	Genotype Counts*						Case MAF	Control MAF	OR [95% CI]	p-value
	Cases			Control						
	GG	GT	TT	GG	GT	TT				
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	509	583	207	0.37	0.38	0.93[0.82, 1.05]	0.26
Meta-analysis	1378	1483	446	1560	1779	653	0.36	0.39	0.90[0.84, 0.96]	0.001

*Genotype counts may not sum exactly to the number of cases and controls described in Table 1. The discrepancy includes missing data due to samples that were not adequately genotyped for *PRL* -1149 G/T.