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Cumulative Association of Twenty-Two Genetic Variants with Seropositive Rheumatoid Arthritis Risk

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Abstract

Background—Recent discoveries of risk alleles have made it possible to define genetic risk profiles for patients with rheumatoid arthritis (RA). We examined whether a cumulative score based on 22 validated genetic risk alleles for seropositive RA would identify high-risk, asymptomatic individuals who might benefit from preventive interventions.

Methods—We genotyped 14 single nucleotide polymorphisms (SNPs) at 13 validated RA risk loci and 8 HLA alleles among (1) 289 Caucasian seropositive cases and 481 controls from the US Nurses' Health Studies (NHS), and (2) 629 Caucasian CCP antibody positive cases and 623 controls from the Swedish Epidemiologic Investigation of RA (EIRA). We created a weighted genetic risk score (GRS), where the weight for each risk allele is the log of the published odds ratio. We used logistic regression to study associations with incident RA. We compared AUCs from a clinical-only model and clinical + genetic model in each cohort.

Results—Patients with GRS > 1.25 standard deviations of the mean had a significantly higher OR of seropositive RA in both NHS (OR=2.9, 95%CI 1.8–4.6) and EIRA (OR=3.4, 95% CI 2.3–5.0) referent to the population average. In NHS, the AUC for a clinical model was 0.57 and for a clinical + genetic model was 0.66, and in EIRA was 0.63 and 0.75, respectively.

Conclusion—The combination of 22 risk alleles into a weighted genetic risk score significantly stratifies individuals for RA risk beyond clinical risk factors alone. However, given the low incidence of RA, the clinical utility of a weighted genetic risk score is limited in the general population.

Keywords

rheumatoid arthritis; polymorphism; autoantibodies; anti-CCP; smoking

RA is a complex autoimmune disease thought to develop in genetically predisposed individuals when exposed to certain environmental factors. Early diagnosis and treatment strategies are

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critical to minimize disability from joint destruction[1]. Although epidemiologic research has produced convincing data linking cigarette smoking to RA risk[2–4], and identified genetic variants associated with RA risk in the major histocompatibility complex (MHC) region were discovered over 30 years ago[5], these risk factors are not used clinically for behavior modification, preventive therapy, or in establishing a diagnosis of RA. Similarly, the presence of RA-specific autoantibodies and inflammatory biomarkers appear years before disease onset and predict more severe disease, but are not used in clinical medicine prior to the onset of symptoms[6–9].

Advances in human genetics have led to a dramatic increase in the number of validated disease risk alleles in RA. There are now up to 22 risk alleles that explain approximately one-third of the genetic burden of seropositive RA risk[5, 10–20]. Much of the risk is derived from 8 alleles that reside within the MHC region[5], with up to 5% of risk explained by the 14 alleles outside of the MHC[20]. The discoveries of these alleles for RA, and similar discoveries for risk alleles in other diseases, has spurned much discussion about the clinical validity of using genetic results in personalized medicine[21–24].

Despite these advances, it is not clear how to utilize genetic information for prediction of RA risk in clinical practice. A critical first step is to understand the role of aggregate genetic risk factors, rather than associations of individual alleles with RA. Towards this end, we used 22 validated RA risk alleles to derive an aggregate genetic risk score (GRS) in seropositive RA patients derived from over 238,000 prospectively followed subjects from the United States (Nurses' Health Study) and seropositive RA patients derived from a large case control study of > 3600 subjects from Sweden (Epidemiologic Investigation of Rheumatoid Arthritis). We calculated odds ratios for seropositive RA relative to the median risk in these datasets and estimated genotype-specific incidence, which is a more useful measure of risk in a clinical setting. We compared predicted multi-locus odds ratios—formed by taking the product of individual-locus odds ratios estimated in a previous meta-analysis[20] — to multi-locus odds ratios estimated in this data set. We included the strongest epidemiologic risk factors for RA in the general population in the models (age, sex, and smoking) as “clinical” risk factors. Although the GRS is strongly associated with seropositive RA and adds significantly to the discrimination of a clinical model, the genotype-specific incidence remains low, suggesting that genetic information is not yet clinically useful in an asymptomatic individual patient.

Methods

STUDY SAMPLE

The Nurses' Health Study (NHSI) is a prospective cohort of 121,700 female nurses, aged 30–55 years in 1976 in which 32,826 (27%) NHSI participants aged 43 to 70 years provided blood samples for future studies and an additional 33,040 (27%) provided buccal cell samples, a total of 65,866 (54% of the cohort). The Nurses' Health Study II (NHSII) is a similar prospective cohort, established in 1989, with 116,609 female nurses aged 25–42 years in which 29,611 (25%) provided blood samples for future studies. In the current study, we combine both NHSI and NHSII, herein referred to simply as `NHS'. All women in both cohorts completed an initial questionnaire and have been followed biennially by questionnaire to update exposures and disease diagnoses. The specificity of CTD detection using a staged series design is very high, reducing misclassification of healthy subjects[25]. RA cases were validated, using previously described methods[4], in which two board-certified rheumatologists trained in chart abstraction independently conducted a medical record review blinded to the second reviewer's result, examining the charts for the American College of Rheumatology (ACR) classification criteria for RA[26], date of first RA symptom, evidence of RA-specific medication treatment, and the treating physician's diagnosis. Definite RA included subjects with four of the seven ACR criteria documented in the medical record or agreement by 2 rheumatologists on the diagnosis

of RA with 3 documented ACR criteria for RA and a diagnosis of RA by their physician. Seropositive status was determined by chart review, and in some cases by direct assay, as previously described[9]. Each NHS participant with confirmed incident or prevalent RA was matched by year of birth, race/ethnicity, menopausal status, and postmenopausal hormone use to a single healthy woman in the same cohort without RA.

This initial NHS nested case-control dataset consists of 585 RA cases and 585 matched controls. To minimize potential population stratification, we excluded non-Caucasian women (based on self-report), resulting in 564 total RA cases and 571 controls. We restricted our analysis to only seropositive RA, resulting in a sample of 327 seropositive RA cases and 571 controls. Covariate information was collected from the subjects in both cohorts via prospective biennial questionnaires regarding diseases, lifestyle, and health practices. All aspects of this study were approved by the Partners' HealthCare Institutional Review Board.

The Epidemiological Investigation of Rheumatoid Arthritis (EIRA) is a population based case-control study on incident RA in Sweden. Data on > 3,600 cases and controls was collected between May 1996 and December 2006. As described previously[3, 27], a case is defined as an individual who fulfills the American College of Rheumatology (ACR) 1987 criteria for the classification of RA, and had symptoms for less than 1 year. For each potential case, a control subject was randomly selected from the study base, taking into consideration the subject's age, sex and geographic location. In total, 659 confirmed CCP positive RA cases and 650 controls were included. All aspects of the EIRA study were approved by the Karolinska Institutet Institutional Review Board.

SELECTION OF GENETIC RISK FACTORS AND GENOTYPING

We selected all validated seropositive RA susceptibility SNPs established prior to September 2008. We define validated as those alleles demonstrating $p < 5\times10^{-7}$ with evidence of replication at $p < 0.05$ in at least one independent study[10–17, 20]. One locus, *CDK6*, has a strong but not unequivocal evidence of association based on these criteria. In NHS, low resolution *HLA-DRB1* genotyping was performed using polymerase chain reaction with sequence specific primers (PCR-SSP) using OLERUP SSP kits (QIAGEN, West Chester, PA), as previously described.[28] For samples with positive 2-digit *HLA* signals, sequence specific primers were used for high-resolution 4-digit allele detection of *DRB1*0401*, **0404*, **0405*, **0408*, **0101*, **0102*, **09* and **1001*. In EIRA, low-resolution HLA typing was performed using Olerup PCR-SSP (DR low resolution and DR4 kits, Olerup SSP AB, Saltsjöbaden, Sweden). High resolution typing was performed for positive *04 samples. Thus 4 digit HLA subtypes were available from EIRA for **0401*, **0404*, **0405*, **0408* and 2 digit subtypes were available for other alleles. All non-MHC risk alleles for both NHS and EIRA were genotyped using iPlex (Sequenom) at the Broad Institute, as previously described[20]. All SNPs had call rates >95% and Hardy-Weinberg equilibrium p-values > 0.01.

We filtered our data to account for missing genotype information, dropping individuals with >10% missing SNP data and dropping individuals missing any HLA data. In NHS, among 327 seropositive RA cases, 6 (2%) were missing HLA data and 32 (10%) were missing >10% SNP information, leaving 289 seropositive RA cases in the analysis. Among 571 controls, 20 (4%) were missing HLA and 70 (12%) were missing >10% SNP information data, leaving us with 481controls in the analysis. In EIRA, among 659 cases, 3 (0.5%) were missing HLA data and 27 (4%) were missing > 10% SNP information leaving 629 cases in the analysis. Among 650 controls 1 (0.1%) was missing HLA results and 25 (4%) were missing > 10% SNP information, leaving 623 controls in the analysis. in a sample of 656 cases and 648 controls. The higher rates of genotyping failure in NHS were due primarily to poor quality cheek cell DNA samples. We are confident that this missingness is completely at random, and therefore does not bias our results, since the case and control samples were randomly interspersed on the genotyping

plate and our resulting odds ratios are consistent with previously published results (see Table 2).

STATISTICAL METHODS

Characteristics of RA cases and controls were summarized by means and standard deviations for continuous variables and frequency and percent for categorical variables. Data for NHS was presented separately from data from EIRA. All analyses were performed using SAS version 9.1 or version 9.2 (SAS Institute, Cary, NC).

SELECTION OF EPIDEMIOLOGIC COVARIATES

In NHS and EIRA, lifetime history of smoking was collected at baseline. In the NHS cohorts, data concerning current smoking, and number of cigarettes smoked per day were updated in two year questionnaire cycles and data on pack years of smoking (number of packs per day × number of years smoking) was selected from the questionnaire cycle prior to the date of RA diagnosis (or index date in controls). In EIRA, pack-years of smoking was calculated prior to RA onset for cases or index date for controls. We included age, sex, and pack-years of smoking as "clinical" risk factors in the models.

ASSOCIATION BETWEEN GENETIC RISK ALLELES AND RA

We used logistic regression to study the association of each allele with risk of seropositive RA according to an additive log-odds model in NHS and in EIRA (Table 2).

WEIGHTED GENETIC RISK SCORE

We developed a "weighted-GRS" (wGRS) that utilized the allelic odds ratios (OR) from published studies to account for the strength of the genetic association within each allele. We calculated a wGRS22 that included 8 *HLA-DRB1* "shared epitope" (*HLA-SE*) alleles and 14 non-MHC risk alleles, and a wGRS14 (no HLA) that included only the 14 non-MHC risk alleles. This is preferred over a simple count GRS, equal to the sum the number of risk alleles carried, since *PTPN22* and *HLA-SE* have substantially higher odds ratios for RA than do the more recently discovered SNPs. The weights used in the wGRS were calculated as the natural log of the published OR with respect to the risk allele as presented in Table 2. The odds ratios for *HLA-SE* alleles were derived from a recent meta-analysis of all published studies[29]. The ORs for the 14 non-MHC alleles were derived from published studies in which results have been extensively replicated, including the following alleles: *PTPN22* (rs2476601)[10], *TRAF1-C5* (rs3761847)[13], *STAT4* (rs7574865)[12], *TNFAIP3* (rs17066662, in LD with 10499194, R^2 = 1.0)[14]), *TNFAIP3* (rs6920220)[14]. We also included 9 alleles from a meta-analysis of GWAS data for 3,393 cases and 12,462 controls with replication in 3,929 seropositive RA cases and 5,807 matched controls by Raychaudhuri et al.[20]: *CD40* (rs4810485), *CCL21* (rs2812378), *CTLA4* (rs3087243), *PADI4* (rs2240340), *CDK6* (rs42041), *TNFRSF14* (rs3890745), *PRKCQ* (rs4750316), *KIF5A* (rs1678542), and *4q27* (rs6822844). For each non-MHC allele, we chose the OR in replication samples to avoid over-estimation of the true effect size[30]. In EIRA, we used a proxy SNP for STAT4 (rs11889341, r^2=1.0 with rs7574865) and a proxy SNP for KIF5A rs775322, r^2=1.0 with rs 1678542). For any individual with missing genotype data for a particular SNP, we assigned the expected allele count (twice the risk allele frequency) to that individual. We tested for epistasis and did not find any significant gene-gene interaction, in agreement with our previous studies[13, 14, 20]. Our results are consistent with a multiplicative genetic model. We did not consider more complex HLA associations, including analysis of compound heterozygotes that have substantially higher risk such as HLA 0401/0404 (9 cases and 3 controls (n=12 total) in NHS and 52 cases and 4 controls (n=56 total) in EIRA).

To determine the cumulative effect of the 14 or 22 alleles on risk of RA we first divided wGRS scores into 7 categories based on the mean and standard deviation (SD) of the wGRS distribution in the controls. Dividing our score into 7 categories provided the most robust distribution, allowing us to parse out the highest and lowest risk groups while assuring that there were sufficient numbers of cases and controls in these extreme categories of interest. Additional details on determination of the groupings are available in the Supplementary Methods. We used logistic regression models adjusting for year of birth, sex and total pack-years of smoking to study the association of wGRS22 with seropositive RA and wGRS14 (no HLA) with seropositive RA (Table 3), comparing each group to a referent median group. An ordinal wGRS variable based on our groupings was used to calculate a p-value for trend. Finally, we calculated the odds of RA for the top group (group 7) as compared to the bottom group (group 1) in two ways. First, by using group 1 as the referent group, the method used in other GRS analyses of complex diseases (eg. macular degeneration[31], prostate cancer[32, 33], lipid levels and heart disease[34–37], and diabetes[38–40]). Second, because group 1 has few cases and the first method only considers subjects in group 7 and 1, we also compared the median wGRS score in group 7 to the median wGRS score in group 1 using a model derived from an ordinal wGRS variable in which each group was given its median wGRS value as a score.

ADDITIONAL STATISTICAL ANALYSIS

To determine how well our wGRS predictors discriminate between cases and controls, we generated ROC curves by plotting sensitivity of the wGRS22 score (continuous) against 1-specificity and calculated the area-under-the-curve (AUC) for both NHS and EIRA. Because there are few established epidemiologic predictors other than age, sex and smoking in the asymptomatic general population, any improvement in the ROC curve contributed by the wGRS may have value in a clinical setting. ROC curves were plotted for a “clinical” model that included year of birth and pack-years of smoking in NHS and age, sex, pack-years of smoking, and geographic region in EIRA, for a “clinical + genetic” model based on adding wGRS14 (no HLA) and a full “clinical + genetic” model that included age, smoking, (residential area in EIRA only) and wGRS22. The AUCs were compared using a non-parametric approach with each “clinical + genetic” model compared to the “clinical” model as described by DeLong et.al.[41]

To judge how well previously-reported association results could be used to distinguish cases and controls in this data set, using a likelihood ratio test we studied the calibration of a model for the multilocus odds ratio, formed by multiplying the individual-locus odds ratios, from the published odds ratios in Table 2 (i.e. exponentiating the continuous wGRS) (see Supplementary Methods).

To determine whether wGRS22 is clinically useful on an individual patient basis, we estimated risk-score specific incidence among US women. We used the average annual incidence estimated from the full NHS cohort: λ=33/100,000; the risk-score specific odds ratios OR_G; and one minus the population attributable risk 1-PAR = $1/(\Sigma_G\, OR_G\, \pi_G)$, where π_G is the prevalence of genotype G in the controls. The risk score specific incidence is then: λ (1-PAR) $OR_G\pi_G$[42]. To estimate risk-score specific absolute risks among Swedish men and women we used data on RA incidence rates in Northern Europe from Alamanos et al, and estimated Swedish annual incidence rates λ=40/100,000 for women, and λ=20/100,000 for men[43].

RESULTS

PATIENTS

Characteristics of RA cases and controls for NHS and EIRA are presented in Table 1. The demographics of both groups are similar although (a) seropositive status in NHS was defined

as either rheumatoid factor (RF) or CCP positive and in EIRA as those who were CCP positive, (b) NHS includes patients with new-onset and long-standing disease, whereas EIRA patients are of new-onset only, and (c) NHS is all female, whereas EIRA is both female and male (at the expected ratio of approximately 3:1).

ASSOCIATION BETWEEN GENETIC RISK ALLELES AND RA

The results for each of the 22 risk alleles with risk of RA are presented in Table 2. The majority of the odds ratios are in the same direction for the risk allele and of the same magnitude as from published discovery studies. Not surprisingly, many of the 95% confidence intervals cross 1.0, as might be expected given the modest odds ratios of the non-MHC alleles and the sample size of the two cohorts.

OBSERVED RELATIVE RISK WITH GENETIC RISK SCORE

The results for wGRS22 as a predictor of seropositive RA are presented in Table 3 and Figure 1. For wGRS22, the median level of risk (group 4, containing 20% of controls) was used as the referent group. Those with the highest risk (group 7) had a significantly higher odds of RA as compared to group 4 in both NHS (OR = 2.85, 95% CI 1.75 – 4.64) and in EIRA (OR = 3.36, 95% CI 2.27 – 4.97) (Table 3, Figure 1a and 1b). Using group 1 (lowest level of risk) as a reference group, group 7 had a higher odds of RA, 5.61 (95% CI 2.41 – 13.07) in NHS and 8.83 (95% CI 4.77 – 16.32) in EIRA. In the ordinal model that takes into account all data in the model, group 7 had even higher odds of RA, 6.30 (95% CI 3.78 – 10.48) for NHS and 12.31 (95% CI 8.12 – 18.67) for EIRA. The trends across all 7 categories of risk were highly significant, with $p < 0.0001$ for both NHS and EIRA.

A similar analysis was performed using only the 14 non-HLA risk alleles (Table 3, Figure 1c and 1d). For wGRS14 (no HLA), those in group 7 (highest risk) relative to group 4 (median) had an elevated OR of 2.52 (95% CI 1.49 – 4.28) and 2.43 (95% CI 1.62 – 3.63) in both NHS and EIRA, respectively. Using group 1 as the reference, group 7 had a higher odds of RA 3.43 (95% CI 1.74 – 6.74) and 2.81 (95% CI 1.66 – 4.73) in NHS and EIRA respectively. The OR from an ordinal model for group 7 was 2.39 (95% CI 1.44 – 3.98) in NHS and 3.22 (95% CI 2.14 – 4.86) in EIRA. The trends across all 7 categories were highly significant ($p = 0.002$ for NHS, $p < 0.0001$ for EIRA).

DISCRIMINATION OF CASES AND CONTROLS BY GRS SCORES

The statistics used during the discovery phase of research (such as odds ratios or P-values for association) are not the most appropriate measures for evaluating the predictive value of genetic profiles in clinical practice. Other measures - sensitivity, specificity, and risk classification - are more useful when proposing a genetic profile for risk prediction[23, 24, 44]. ROC curves that plot sensitivity of the GRS score (continuous) against 1-specificity, and calculated the area-under-the-curve (AUC), also known as the c-statistic, for both NHS and EIRA are shown in Figure 2. In the NHS, the AUC for the clinical model including age and pack-years of smoking was 0.566. Adding wGRS14 (no HLA) to this model did not significantly improve discrimination (AUC = 0.589; p=0.31). Adding HLA subtypes to the clinical + genetic model significantly improved discrimination relative to both the clinical model and the clinical + wGRS14 model (AUC = 0.660; $p < 0.001$ for both comparisons). In EIRA, ROC curves for the clinical model adjusted for age, sex, geographic region, and pack-years of smoking demonstrate significant improvements in discrimination with the addition of wGRS14 (no HLA) or wGRS18 scores, with AUCs of 0.627 0.662, and 0.752 (clinical + wGRS22 vs. wGRS14 (no HLA), $p< 0.0001$; clinical + wGRS22 vs. clinical, $p< 0.0001$; clinical + wGRS14 vs. clinical p=0.002).

GENOTYPE-SPECIFIC RISK AND COMPARISON BETWEEN PREDICTED AND OBSERVED ODDS RATIOS

Figure 3 plots the distribution of genotype (or genotype-category) annual incidence for predicted models based on previous locus-specific odds ratio estimates and the observed categorized wGRS models fit to these data sets. For NHS and women in EIRA, the observed risks from our groupings approximate the predicted risk from a continuous wGRS, except for the lowest risk group (group 1) where observed risk exceeds predicted risk. For men in EIRA the observed risks from our groupings approximate the predicted risk from a continuous wGRS except for the highest risk group (group 7) where predicted risk exceeds observed risk, suggesting that in the highest risk group the risk based on grouping the wGRS is biased toward the null or the predicted risk is an overestimate. Figure 3 also shows that despite the statistically significant improvement in the AUC after incorporating the wGRS22, the predicted risks of RA were still small (<1% annual risk) for all of the observed genotypes.

Discussion

Until 2004, only two genetic loci had been unequivocally associated with risk of RA susceptibility: *HLA-DRB1* and *PTPN22*[5, 10]. Recent large studies using genome-wide scans or related methodologies have discovered and replicated 12 additional non-MHC risk loci[12–15, 20]. In the current study, we develop a weighted genetic risk score including established 14 risk alleles from 13 non-MHC RA loci and 8 HLA subtypes based on high resolution genotyping. We demonstrate that a composite genetic risk score improves significantly the discrimination ability of the model for seropositive RA compared to no RA when compared to a risk model with epidemiologic variables alone when applied in the general population.

We found that in our top wGRS group with 22 alleles there was a 2.9 fold increase in the odds of seropositive RA compared to the most common wGRS group, and a 5.6 fold increase in odds of RA compared to the wGRS group with the lowest score in the NHS. In EIRA, the top wGRS group with 22 alleles had a higher increase in the odds of RA than in the US cohort, with a 3.4 fold compared to the most common wGRS group and 8.8 fold compared to the lowest wGRS group. However, comparing results from the cumulative score with 14 alleles, without the *HLA-SE* alleles, there were similar increased odds ratios for RA in both cohorts (2.5 fold in NHS and 2.4 fold in EIRA). This suggests that the increased risk in the Swedish cohort is primarily due to the higher frequency of *HLA-SE* alleles in that population, which may reflect the higher percentage of patients seropositive for CCP autoantibodies (Table 1).

Publications on genetic risks for other complex human diseases and quantitative traits such as macular degeneration[31], prostate cancer[32, 33], lipid levels and heart disease[34–37], height[45, 46] and diabetes[38–40]. These studies have combined risk alleles into a single risk score simply by summing the number of risk alleles carried. Our study extends the methodology by weighting the risk score by the published allelic odds ratios, thus accounting for the different strengths of association for genes such as the *HLA-SE* and *PTPN22*. Although models have been developed to identify which patients presenting with early inflammatory arthritis will progress to RA[47], this is the first demonstration of risk models that include all known genetic risk factors and the two strongest epidemiologic factors, age and smoking, in prediction of incident RA among healthy subjects without symptoms.

Our wGRS is a first step towards development of RA risk prediction models that incorporate aggregate genetic factors. In contrast to other complex diseases such as diabetes[38, 39] and heart disease[34–37], where adding genetic markers to clinical risk factors does not add to discrimination, the addition of genetic factors to a clinical model that includes epidemiologic risk factors improves discrimination significantly for RA, which supports the clinical validity of this approach. The AUCs of 0.566 and 0.627 in NHS and EIRA, respectively, suggest that

clinical risk factors alone – in subjects without symptoms – do not provide much discrimination between RA cases and controls. Adding genetic alleles to the aggregate score significantly improves the model AUCs to 0.660 in NHS and 0.752 in EIRA. However, there is variance in risk that remains unexplained, suggesting that further work is needed to incorporate environmental exposure data and gene-environment interactions into risk models and to discover additional genetic variants. We note that in patients with early symptoms consistent with an inflammatory arthritis, clinical prediction models that include sex, age, localization of symptoms, morning stiffness, the tender joint count, the swollen joint count, the C-reactive protein level, rheumatoid factor positivity, and the presence of anti-cyclic citrullinated peptide antibodies accurately predict who will go on to develop RA[47, 48]. Under this clinical scenario, it will be important to test whether genetic factors helps discriminate which patients will develop RA.

Odds ratios alone are difficult to interpret for patients and physicians in a clinical setting[24]. However, as suggested by Kraft et. al.[24], measures of absolute risk (i.e. risk that a disease-free subject will develop disease) such as the results shown in Figure 3, provide a more intuitive context of RA risk at the individual level. A strength of our study is that we have data on the entire prospective NHS cohort from which our nested samples were taken and thus we have an accurate estimate of the population annual incidence. Using data from the full NHS cohort, we see an absolute risk of RA among US women aged 25–50 of 0.3%, thus a wGRS22 in group 7 increases the absolute risk to 0.7%. In EIRA women, the wGRS22 score in group 7 increases the absolute risk from 0.4% to 1.3%. In EIRA men, the wGRS22 score in group 7 increases the absolute risk from 0.2% to 0.7%. These predictive models demonstrate that there is a small portion of the general population at very high risk

Although the hope is that we will soon be able to apply genetic information to individual patients, the wGRS for RA is unlikely to be useful in routine clinical practice for assessing risk among the healthy asymptomatic patients. Even the highest risk category - group 7 - has a modest absolute risk of RA. It is possible genetic results might eventually help us to identify subsets of patients who are at substantially elevated absolute risk, and would be willing to undergo potentially toxic therapies, to prevent RA. It will be important to perform studies in among subsets of patients at higher risk for RA, eg. patients with early undifferentiated arthritis, patients with anti-CCP + arthralgia, and first degree relatives of RA patients[49]. We propose that wGRS22 may be clinically useful as part of an overall risk assessment tool among high risk groups.

We recognize that the ideal setting to perform prognostic modelling analyses is a prospective cohort study, such as the Framingham Heart Study or the full Nurses' Health Study cohorts. However, no such large study has blood samples available on the full dataset and validated RA cases. Instead, we approximated risk by use of the odds, which in a population based case-control study with a proper sampling of controls approximates relative risk well. We calculated risk-score specific absolute risks using these odds ratios and the average population risk estimated from the full NHS cohort, and from the literature for Northern Europe. The estimated incidence in NHS is consistent with RA incidence rates observed in other studies in women of Northern European ancestry[43], except for a single study from North America[50]. The NHS dataset is limited by the absence of CCP antibody information on cases that were diagnosed prior to the widespread use of the test. Thus the phenotype used in NHS analyses is seropositive RA, while the phenotype used in EIRA analyses is CCP positive RA, which is more strongly associated with genetic factors such as the HLA-SE. Although stronger associations are demonstrated in EIRA, the results from NHS are very consistent, suggesting that the general category of seropositive RA is associated with these genetic factors.

Despite the rapid advances in our understanding of the genetic basis of complex human diseases such as RA, it is not clear how to utilize this information for clinical care, prediction, or prevention. Although a combination of known genetic factors for RA aggregated into a weighted score has a 3-fold increased odds for the development of RA, the absolute risk of this disease remains low, suggesting that genetic risk scores, calculated as in this paper, have little clinical utility in predicting RA risk in asymptomatic individuals. More research to identify genetic and environmental risk factors, as well as gene-environment interactions, is critical to understanding the determinants of RA risk before this information can be used in patient counseling or preventive trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Finckh A, Liang MH, van Herckenrode CM, de Pablo P. Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: A meta-analysis. Arthritis Rheum 2006;55(6):864–72. [PubMed: 17139662]
2. Criswell LA, Merlino LA, Cerhan JR, et al. Cigarette smoking and the risk of rheumatoid arthritis among postmenopausal women: results from the Iowa Women's Health Study. Am J Med 2002;112 (6):465–71. [PubMed: 11959057]
3. Stolt P, Bengtsson C, Nordmark B, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. Ann Rheum Dis 2003;62(9):835–41. [PubMed: 12922955]
4. Costenbader KH, Feskanich D, Mandl LA, Karlson EW. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. Am J Med 2006;119(6):503–11. [PubMed: 16750964]
5. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30(11):1205–13. [PubMed: 2446635]
6. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48(10): 2741–9. [PubMed: 14558078]
7. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004;50(2): 380–6. [PubMed: 14872479]
8. Chibnik LB, Mandl LA, Costenbader KH, Schur PH, Karlson EW. Comparison of threshold cutpoints and continuous measures of anti-cyclic citrullinated peptide antibodies in predicting future rheumatoid arthritis. J Rheumatol 2009;36(4):706–11. [PubMed: 19228654]

9. Karlson EW, Chibnik LB, Tworoger SS, et al. Biomarkers of inflammation and development of rheumatoid arthritis in women from two prospective cohort studies. Arthritis Rheum 2009;60(3):641–52. [PubMed: 19248103]
10. Begovich AB, Carlton VE, Honigberg LA, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75(2):330–7. [PubMed: 15208781]
11. Plenge RM, Padyukov L, Remmers EF, et al. Replication of Putative Candidate-Gene Associations with Rheumatoid Arthritis in >4,000 Samples from North America and Sweden: Association of Susceptibility with PTPN22, CTLA4, and PADI4. Am J Hum Genet 2005;77(6):1044–60. [PubMed: 16380915]
12. Remmers EF, Plenge RM, Lee AT, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med 2007;357(10):977–86. [PubMed: 17804842]
13. Plenge RM, Seielstad M, Padyukov L, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. N Engl J Med 2007;357(12):1199–209. [PubMed: 17804836]
14. Plenge RM, Cotsapas C, Davies L, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet 2007;39(12):1477–82. [PubMed: 17982456]
15. Consortium WTCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447(7145):661–78. [PubMed: 17554300]
16. Thomson W, Barton A, Ke X, et al. Rheumatoid arthritis association at 6q23. Nat Genet 2007;39(12): 1431–3. [PubMed: 17982455]
17. Kurreeman FA, Padyukov L, Marques RB, et al. A candidate gene approach identifies the TRAF1/ C5 region as a risk factor for rheumatoid arthritis. PLoS medicine 2007;4(9):e278. [PubMed: 17880261]
18. Barton A, Thomson W, Ke X, et al. Rheumatoid arthritis susceptibility loci at chromosomes 10p15, 12q13 and 22q13. Nat Genet 2008;40(10):1156–9. [PubMed: 18794857]
19. Suzuki A, Yamada R, Kochi Y, et al. Functional SNPs in CD244 increase the risk of rheumatoid arthritis in a Japanese population. Nat Genet 2008;40(10):1224–9. [PubMed: 18794858]
20. Raychaudhuri S, Remmers EF, Lee AT, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat Genet 2008;40(10):1216–23. [PubMed: 18794853]
21. Burke W, Psaty BM. Personalized medicine in the era of genomics. Jama 2007;298(14):1682–4. [PubMed: 17925520]
22. Ioannidis JP. Personalized genetic prediction: too limited, too expensive, or too soon? Annals of internal medicine 2009;150(2):139–41. [PubMed: 19153414]
23. Jakobsdottir J, Gorin MB, Conley YP, Ferrell RE, Weeks DE. Interpretation of genetic association studies: markers with replicated highly significant odds ratios may be poor classifiers. PLoS genetics 2009;5(2):e1000337. [PubMed: 19197355]
24. Kraft P, Wacholder S, Cornelis MC, et al. Beyond odds ratios-communicating disease risk based on genetic profiles. Nat Rev Genet [epub]. 2009
25. Liang MH, Meenan RF, Cathcart ES, Schur PH. A screening strategy for population studies in systemic lupus erythematosus. Series design. Arthritis Rheum 1980;23(2):153–7. [PubMed: 6965858]
26. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31(3):315–24. [PubMed: 3358796]
27. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum 2004;50(10):3085–92. [PubMed: 15476204]
28. Costenbader KH, Chang SC, De Vivo I, Plenge R, Karlson EW. Genetic polymorphisms in PTPN22, PADI-4, and CTLA-4 and risk for rheumatoid arthritis in two longitudinal cohort studies: evidence of gene-environment interactions with heavy cigarette smoking. Arthritis Res Ther 2008;10(3):R52. [PubMed: 18462498]
29. Fernando MM, Stevens CR, Walsh EC, et al. Defining the role of the MHC in autoimmunity: a review and pooled analysis. PLoS genetics 2008;4(4):e1000024. [PubMed: 18437207]

30. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33(2):177–82. [PubMed: 12524541]

31. Maller J, George S, Purcell S, et al. Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. Nat Genet 2006;38(9):1055–9. [PubMed: 16936732]

32. Zheng SL, Sun J, Wiklund F, et al. Cumulative association of five genetic variants with prostate cancer. N Engl J Med 2008;358(9):910–9. [PubMed: 18199855]

33. Thomas G, Jacobs KB, Yeager M, et al. Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 2008;40(3):310–5. [PubMed: 18264096]

34. Kathiresan S, Melander O, Anevski D, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008;358(12):1240–9. [PubMed: 18354102]

35. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 2009;41(1):56–65. [PubMed: 19060906]

36. Aulchenko YS, Ripatti S, Lindqvist I, et al. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat Genet 2009;41(1):47–55. [PubMed: 19060911]

37. Kathiresan S, Voight BF, Purcell S, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009;41(3):334–41. [PubMed: 19198609]

38. Lyssenko V, Jonsson A, Almgren P, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2008;359(21):2220–32. [PubMed: 19020324]

39. Meigs JB, Shrader P, Sullivan LM, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359(21):2208–19. [PubMed: 19020323]

40. Cornelis MC, Qi L, Zhang C, et al. Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry. Annals of internal medicine 2009;150(8):541–50. [PubMed: 19380854]

41. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44(3):837–45. [PubMed: 3203132]

42. Rebbeck, TR.; Ambrosone, CB.; Shields, PG., editors. Molecular epidemiology: applications in cancer and other human diseases. Informa Healthcare; New York: 2008.

43. Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. Semin Arthritis Rheum 2006;36(3):182–8. [PubMed: 17045630]

44. Kraft P, Hunter DJ. Genetic risk prediction--are we there yet? N Engl J Med 2009;360(17):1701–3. [PubMed: 19369656]

45. Weedon MN, Lango H, Lindgren CM, et al. Genome-wide association analysis identifies 20 loci that influence adult height. Nat Genet 2008;40(5):575–83. [PubMed: 18391952]

46. Lettre G, Jackson AU, Gieger C, et al. Identification of ten loci associated with height highlights new biological pathways in human growth. Nat Genet 2008;40(5):584–91. [PubMed: 18391950]

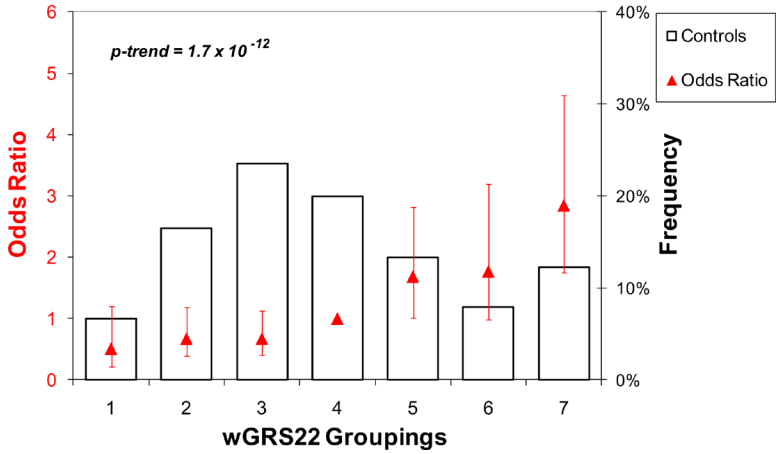
47. van der Helm-van Mil AH, le Cessie S, van Dongen H, Breedveld FC, Toes RE, Huizinga TW. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. Arthritis Rheum 2007;56(2):433–40. [PubMed: 17265478]

48. van der Helm-van Mil AH, Detert J, le Cessie S, et al. Validation of a prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: moving toward individualized treatment decision-making. Arthritis Rheum 2008;58(8):2241–7. [PubMed: 18668546]

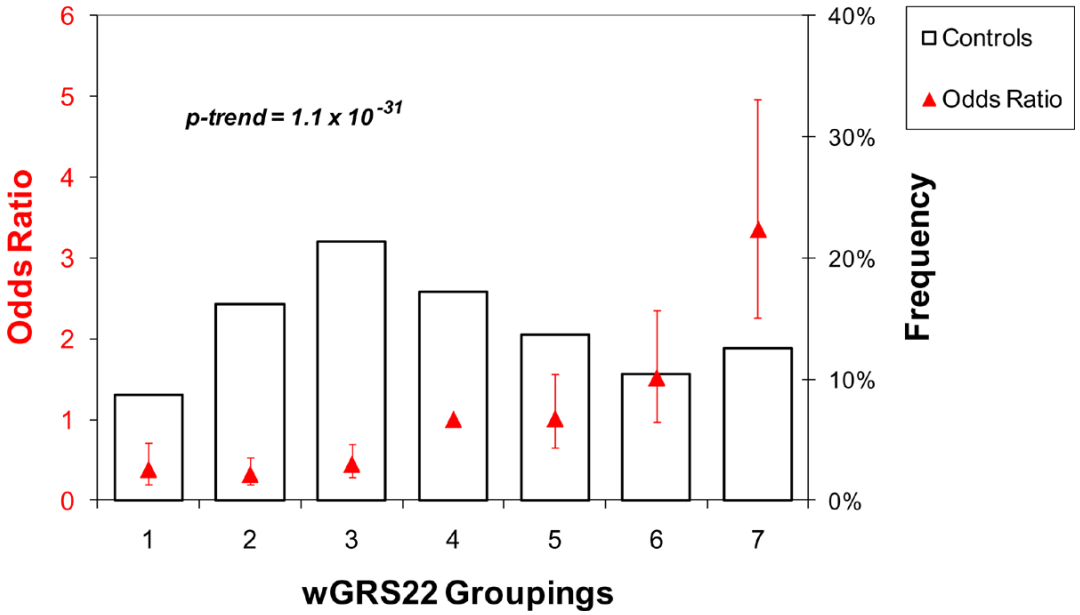
49. Hemminki K, Li X, Sundquist J, Sundquist K. Familial associations of rheumatoid arthritis with autoimmune diseases and related conditions. Arthritis Rheum 2009;60(3):661–8. [PubMed: 19248111]

50. Gabriel SE, Crowson CS, O'Fallon WM. The epidemiology of rheumatoid arthritis in Rochester, Minnesota, 1955–1985. Arthritis Rheum 1999;42(3):415–20. [PubMed: 10088762]

(a) wGRS22 ORs and Distribution for Seropositive RA in NHS



(b) wGRS22 ORs and Distribution in CCP+ RA in EIRA



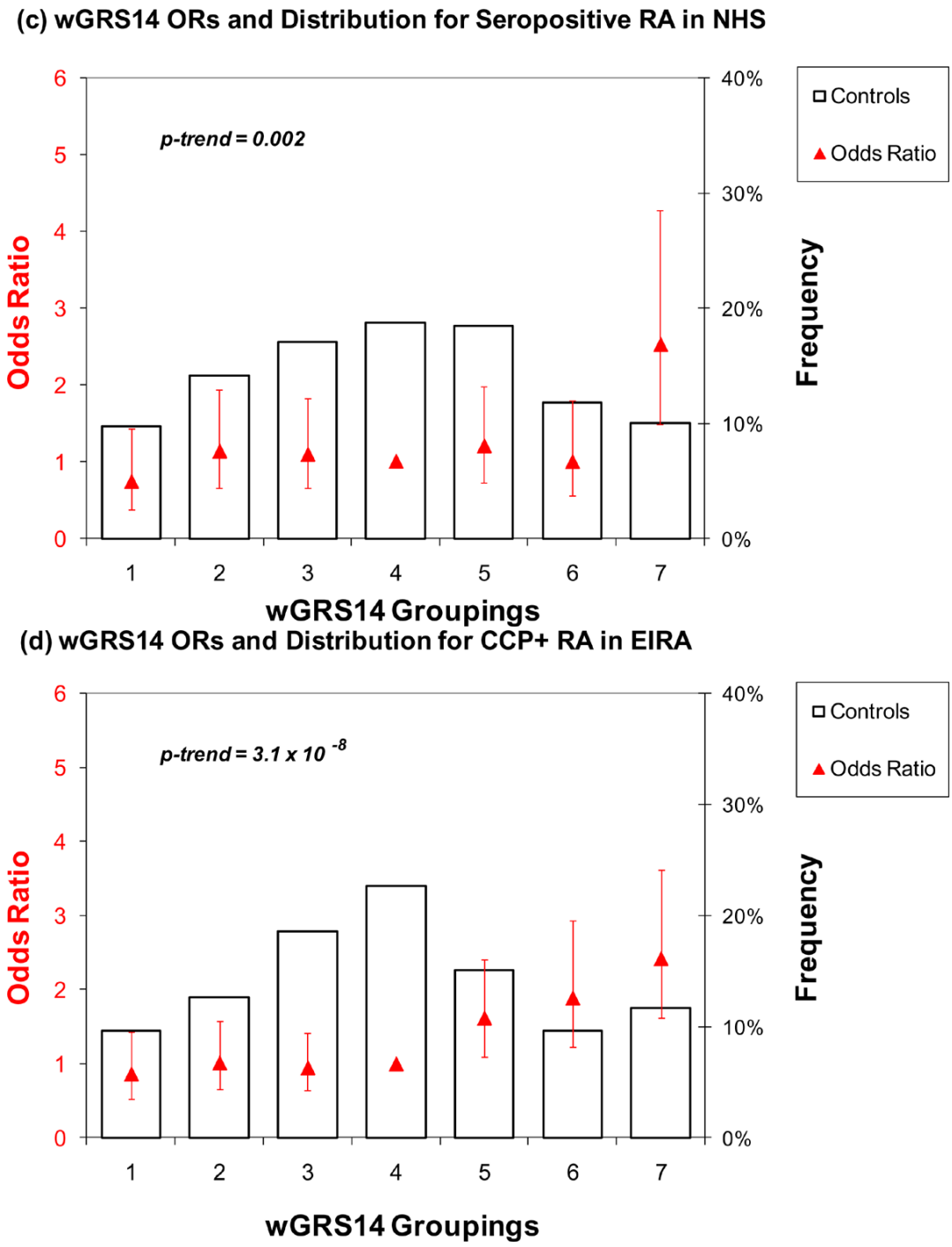


Figure 1.

Odds ratios for wGRS22 and wGRS14 (no HLA) in NHS and EIRA. Weighted GRS distribution among controls shown in bars, odds ratios shown in red triangles. (a) Odds ratios for wGRS22 and seropositive RA in NHS; b) Odds ratios for wGRS22 and CCP+ RA in EIRA; (c) Odds ratios for wGRS14 (no HLA) and Sero+ RA in NHS; (d) Odds ratios for wGRS14 (no HLA) and CCP+ RA in EIRA. NHS: Nurses' Health Studies, EIRA: Epidemiologic Investigation of Rheumatoid Arthritis, CCP: cyclic-citrullinated peptide antibody, wGRS22:

weighted genetic risk score with 22 alleles; wGRS14 (no HLA): weighted genetic risk score with 14 alleles, without HLA alleles.

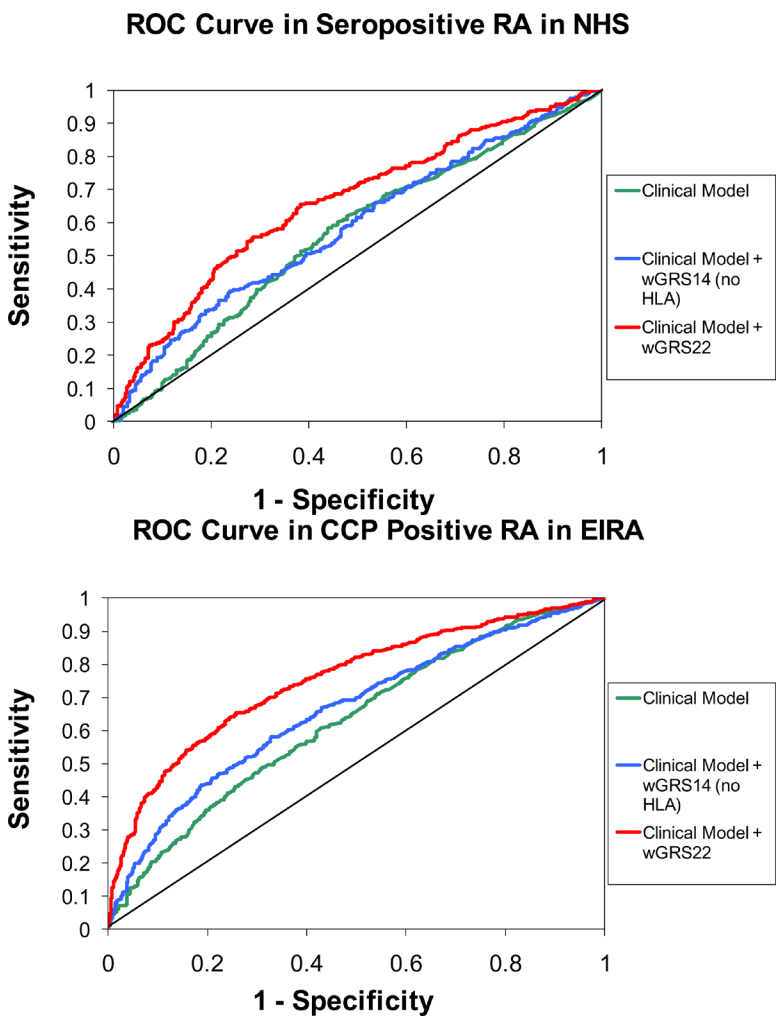


Figure 2.

Receiver Operator-Characteristic (ROC) curves for predicting seropositive RA in NHS and CCP+ RA in EIRA. NHS clinical model is adjusted for year of birth and pack-years of smoking. EIRA clinical model is adjusted for age, sex, geographic region and pack-years of smoking. NHS AUCs: clinical model: AUC=0.566; clinical + wGRS14 (no HLA): AUC=0.589; clinical + wGRS22: AUC=0.660. NHS AUC comparisons: clinical + wGRS22 vs. clinical + wGRS14 (no HLA), $p < 0.001$; clinical + wGRS22 vs. clinical, $p < 0.001$; clinical + wGRS14 vs. clinical $p = 0.31$. EIRA AUCs: clinical model: AUC=0.626; clinical + wGRS14 (no HLA): AUC=0.662; clinical + wGRS22: AUC=0.752. EIRA AUC comparisons: clinical + wGRS22 vs. clinical + wGRS14 (no HLA), $p < 0.0001$; clinical + wGRS22 vs. clinical, $p < 0.0001$; clinical + wGRS14 vs. clinical p=0.002. NHS: Nurses' Health Studies, EIRA: Epidemiologic Investigation of Rheumatoid Arthritis, CCP: cyclic-citrullinated peptide antibody, wGRS22: weighted genetic risk score with 22 alleles; wGRS14 (no HLA): weighted genetic risk score with 14 alleles, without HLA alleles

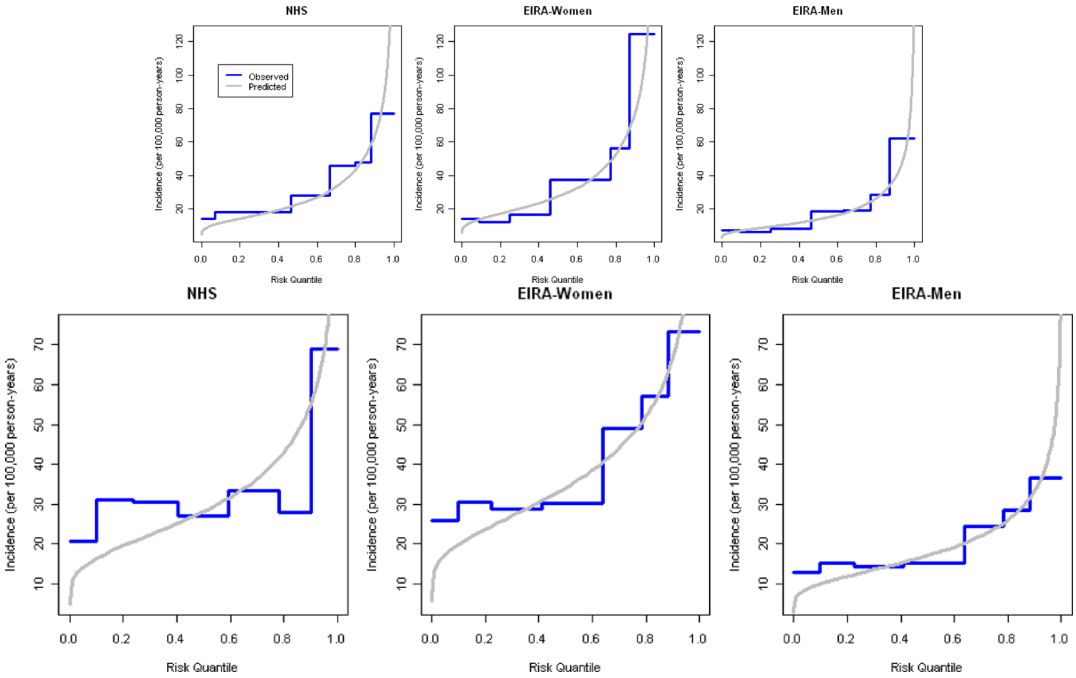


Figure 3.

a) predicted vs. observed incidence rates for wGRS22 in NHS women, EIRA women and EIRA men; b) predicted vs. observed incidence rates for wGRS14 in NHS women, EIRA women and EIRA men; NHS: Nurses' Health Studies, EIRA: Epidemiologic Investigation of Rheumatoid Arthritis, CCP: cyclic-citrullinated peptide antibody, wGRS22: weighted genetic risk score with 22 alleles; wGRS14 (no HLA): weighted genetic risk score with 14 alleles, without HLA alleles

Table 1

Characteristics of Seropositive RA cases and matched controls in the Nurses' Health Studies and CCP Positive RA in Epidemiologic Investigation of RA (NHS)

	NHS		EIRA	
	RA cases (n=289)	**Controls (n=481)**	**RA cases[1] (n=629)**	**Controls[2] (n=623)**
Age, mean (SD)[3]	55.3 (±8.1)	55.7 (±7.9)	51.2 (±11.7)[5]	52.1 (±11.8)[6]
Pack-years among smokers, mean (SD)	25.2 (±17.5)	23.0 (±21.4)	18.3 (±15.3)	14.7 (±13.8)
RA features				
Mean age at diagnosis, (SD)	56.2 (±9.9)	-	51.2 (±11.7)[5]	-
Rheumatoid factor, positive (%)	270 (93.4%)	-	523 (87.3%)[5]	-
Anti-CCP[4] positive (%)	106 (55.5%)	-	629 (100%)	-
Seropositive (%)	289 (100.0%)	-	629 (100%)	-
Rheumatoid nodules, (%)	45 (15.6%)	-		
Radiographic changes, (%)	95 (32.9%)	-		

[1] EIRA: 184 male cases; 446 female cases

[2] EIRA: 163 male controls, 460 female controls

[3] Age at blood draw

[4] Citric citrullinated protein antibodies assayed in subset of NHS cases (n=191) with stored blood samples at collected at different points with respect to RA onset, up to 12 years prior to onset or after diagnosis

[5] 31 EIRA cases missing covariate data

[6] 5 EIRA controls missing covariate data. NHS: Nurses' Health Studies, EIRA: Epidemiologic Investigation of Rheumatoid Arthritis, anti-CCP: cyclic-citrullinated peptide antibody

Table 2

Allele frequencies and association with seropositive RA in NHS and CCP positive RA in EIRA for 22 alleles

				NHS			EIRA		
Loci	**SNP**	**Risk Allele**	**Published OR[1]**	**Cases RAF**	**Controls RAF**	**OR (95% CI)**	**Cases RAF**	**Controls RAF**	**OR (95% CI)**
DRB*0401	SE	SE	3.30	0.15	0.09	1.70 (1.25–2.29)	0.30	0.13	3.04 (2.45–3.77)
DRB*0404	SE	SE	1.85	0.07	0.04	1.81 (1.13–2.90)	0.10	0.04	2.94 (2.07–4.18)
DRB*0405	SE	SE	3.84	0.02	0.003	6.31 (1.75–22.81)	0.008	0.004	1.98 (0.67–5.83)
DRB*0408	SE	SE	1.04	0.003	0.006	0.55 (0.11–2.76)	0.02	0.004	5.07 (1.923–13.34)
DRB*0101	SE	SE	1.60	0.11	0.06	1.83 (1.27–2.63)	0.14[2]	0.10	1.47 (1.15–1.89)
DRB*0102	SE	SE	1.10	0.01	0.01	1.69 (0.63–4.54)	---	---	---
DRB*1001	SE	SE	2.35	0.01	0.01	2.26 (0.78–6.58)	0.02	0.01	1.53 (0.79–2.97)
DRB*09	SE	SE	1.48	0.01	0.01	1.34 (0.36–5.03)	0.02	0.02	0.94 (0.53–1.67)
PTPN22	rs2476601	T	1.75	0.14	0.09	1.75 (1.27–2.41)	0.17	0.12	1.49 (1.19–1.86)
TRAF1-C5	rs3761847	G	1.32	0.44	0.40	1.18 (0.95–1.45)	0.51	0.45	1.29 (1.1–1.51)
STAT4	rs7574865	T	1.27	0.24	0.21	1.18 (0.93–1.51)	0.24	0.22	1.07 (0.89–1.29)
TNFAIP3	rs17066662	C*	1.33	0.73	0.73	1.00 (0.80–1.25)	0.80	0.79	1.04 (0.86–1.26)
TNFAIP3	rs6920220	A	1.22	0.22	0.21	1.05 (0.82–1.35)	0.26	0.22	1.23 (1.02–1.48)
CD40	rs4810485	G*	1.15	0.76	0.75	1.05 (0.83–1.35)	0.78	0.72	1.36 (1.13–1.64)
CCL21	rs2812378	C	1.12	0.35	0.34	1.07 (0.87–1.33)	0.36	0.34	1.11 (0.94–1.31)
CTLA4	rs3087243	G*	1.11	0.54	0.52	1.06 (0.87–1.30)	0.63	0.59	1.20 (1.02–1.4)
PADI4	rs2240340	A	1.02	0.43	0.40	1.10 (0.89–1.35)	0.42	0.39	1.14 (0.97–1.33)
CDK6	rs42041	G	1.11	0.27	0.24	1.21 (0.96–1.53)	0.25	0.24	1.02 (0.85–1.23)
TNFRSF14	rs3890745	T*	1.12	0.67	0.68	0.93 (0.75–1.16)	0.70	0.70	1.02 (0.86–1.21)
PRKCQ	rs4750316	C*	1.14	0.85	0.82	1.25 (0.95–1.65)	0.82	0.80	1.09 (0.89–1.34)
KIF5A	rs1678542	G*	1.12	0.70	0.64	1.28 (1.02–1.60)	0.59	0.58	1.06 (0.91–1.24)
IL2/IL21	rs6822844	G*	1.09	0.84	0.84	0.99 (0.74–1.31)	0.85	0.82	1.29 (1.04–1.62)

*Major Allele

[1]Published OR with respect to the risk allele

[2]Only 2 digit DR1 data available. NHS: Nurses' Health Studies, EIRA: Epidemiologic Investigation of Rheumatoid Arthritis, RAF: risk allele frequency, OR: odds ratio, SE: *HLA-DRB1* shared epitope.

Table 3

Weighted GRS scores and odd ratios of seropositive RA in NHS and CCP positive RA in EIRA

	NHS			EIRA		
	Sero+ Cases	**Controls**	**OR (95%CI)[1]**	**CCP+ Cases**	**Controls**	**OR (95% CI)[2]**
wGRS22						
Groups						
1	8	32 (7%)	0.51 (0.22–1.19)	20	54 (9%)	0.38 (0.20–0.71)
2	27	79 (16%)	0.67 (0.38–1.19)	32	101 (16%)	0.32 (0.19–0.53)
3	37	113 (23%)	0.67 (0.40–1.13)	54	133 (21%)	0.44 (0.28–0.70)
4	49	96 (20%)	1.00 (ref)	96	107 (17%)	1.00 (ref)
5	51	64 (13%)	1.69 (1.01–2.82)	78	85 (14%)	1.01 (0.65–1.57)
6	32	38 (8%)	1.77 (0.98–3.20)	96	65 (10%)	1.52 (0.98–2.36)
7	85	59 (12%)	2.85 (1.75–4.64)	253	78 (13%)	3.36 (2.27–4.97)
7 vs. 1[3]			5.61 (2.41–13.07)			8.83 (4.77–6.32)
wGRS22						
Ordinal Model						
7 vs. 1[4]			6.30 (3.78–10.48)			12.31 (8.12–18.67)
wGRS14(noHLA)						
Groups						
1	17	47 (10%)	0.74 (0.38–1.43)	42	60 (10%)	0.86 (0.53–1.42)
2	38	68 (14%)	1.13 (0.66–1.94)	61	79 (13%)	1.01 (0.65–1.58)
3	46	82 (17%)	1.09 (0.65–1.83)	88	116 (19%)	0.95 (0.64–1.41)
4	45	90 (19%)	1.00 (ref)	114	141 (23%)	1.00 (ref)
5	55	89 (19%)	1.20 (0.73–1.98)	107	94 (15%)	1.62 (1.09–2.41)
6	28	57 (12%)	1.00 (0.55–1.80)	85	60 (10%)	1.89 (1.22–2.94)
7	60	48 (10%)	2.52 (1.49–4.28)	132	73 (12%)	2.43 (1.62–3.63)
7 vs. 1[3]			3.43 (1.74–6.74)			2.81 (1.66–4.73)
wGRS14 (no HLA)						
Ordinal Model						
7 vs. 1[4]			2.39 (1.44–3.98)			3.22 (2.14–4.86)

[1]Odds ratio adjusted for age and pack-years smoking

[2]Odds ratio adjusted for age, sex, geographic region and pack-years smoking

[3]Odds ratio adjusted for age, sex, geographic region and pack-years smoking using group 1 as referent group

[4]Model based estimate of group 7 vs. group 1 using an ordinal model and takes into account all data in the model. NHS: Nurses' Health Studies, EIRA: Epidemiologic Investigation of Rheumatoid Arthritis, sero+: seropositive, CCP: cyclic-citrullinated peptide antibody, OR: odds ratio, CI: confidence interval; wGRS22: weighted genetic risk score with 22 alleles; wGRS14 (no HLA): weighted genetic risk score with 14 alleles, without HLA alleles