



OPEN ACCESS

TRANSLATIONAL SCIENCE

Polygenic Risk Scores have high diagnostic capacity in ankylosing spondylitis

Zhixiu Li ,¹ Xin Wu,² Paul J Leo,¹ Erika De Guzman,³ Nurullah Akkoc ,⁴ Maxime Breban ,^{5,6,7} Gary J Macfarlane ,^{8,9} Mahdi Mahmoudi ,¹⁰ Helena Marzo-Ortega ,^{11,12} Lisa K Anderson,³ Lawrie Wheeler,³ Chung-Tei Chou,^{13,14} Andrew A Harrison ,¹⁵ Simon Stebbings ,¹⁶ Gareth T Jones ,^{8,9} So-Young Bang,¹⁷ Geng Wang,¹⁸ Ahmadreza Jamshidi,¹⁰ Elham Farhadi,¹⁰ Jing Song,² Li Lin,² Mengmeng Li,² James Cheng-Chung Wei ,^{19,20,21} Nicholas G Martin,²² Margaret J Wright,²³ MinJae Lee ,²⁴ Yuqin Wang,²⁵ Jian Zhan,²⁶ Jin-San Zhang,^{27,28} Xiaobing Wang ,²⁹ Zi-Bing Jin ,³⁰ Michael H Weisman,³¹ Lianne S Gensler,³² Michael M Ward ,³³ Mohammad Hossein Rahbar,³⁴ Laura Diekman,³⁵ Tae-Hwan Kim ,¹⁷ John D Reveille ,³⁵ Bryan Paul Wordsworth ,³⁶ Huji Xu ,^{2,37,38} Matthew A Brown ,^{27,39} TCRI AS Group

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/annrheumdis-2020-219446>).

For numbered affiliations see end of article.

Correspondence to

Professor Matthew A Brown, NIHR Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London, London, SE1 9RT, United Kingdom; matt.brown@kcl.ac.uk and Professor Huji Xu, Department of Rheumatology and Immunology, Changzheng Hospital, The Second Military Medical University, Shanghai, China; xuhuji@smmu.edu.cn

ZL and XW contributed equally. HX and MAB contributed equally.

Received 4 November 2020
Revised 23 March 2021
Accepted 29 March 2021
Published Online First
20 April 2021



© Author(s) (or their employer(s)) 2021. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Li Z, Wu X, Leo PJ, et al. *Ann Rheum Dis* 2021;**80**:1168–1174.

ABSTRACT

Objective We sought to test the hypothesis that Polygenic Risk Scores (PRSs) have strong capacity to discriminate cases of ankylosing spondylitis (AS) from healthy controls and individuals in the community with chronic back pain.

Methods PRSs were developed and validated in individuals of European and East Asian ethnicity, using data from genome-wide association studies in 15 585 AS cases and 20 452 controls. The discriminatory values of PRSs in these populations were compared with other widely used diagnostic tests, including C-reactive protein (CRP), *HLA-B27* and sacroiliac MRI.

Results In people of European descent, PRS had high discriminatory capacity with area under the curve (AUC) in receiver operator characteristic analysis of 0.924. This was significantly better than for *HLA-B27* testing alone (AUC=0.869), MRI (AUC=0.885) or C-reactive protein (AUC=0.700). PRS developed and validated in individuals of East Asian descent performed similarly (AUC=0.948). Assuming a prior probability of AS of 10% such as in patients with chronic back pain under 45 years of age, compared with *HLA-B27* testing alone, PRS provides higher positive values for 35% of patients and negative predictive values for 67.5% of patients. For PRS, in people of European descent, the maximum positive predictive value was 78.2% and negative predictive value was 100%, whereas for *HLA-B27*, these values were 51.9% and 97.9%, respectively.

Conclusions PRS have higher discriminatory capacity for AS than CRP, sacroiliac MRI or *HLA-B27* status alone. For optimal performance, PRS should be developed for use in the specific ethnic groups to which they are to be applied.

INTRODUCTION

Ankylosing spondylitis (AS) affects approximately 0.2%–0.6% of individuals of European descent and Chinese.^{1,2} Early treatment with biologic therapies

Key messages

What is already known about this subject?

► *HLA-B27* testing is widely used in the diagnostic pathway in ankylosing spondylitis (AS), but only captures a moderate proportion (~20%) of the overall genetic risk for the disease.

What does this study add?

► Polygenic Risk Scores (PRSs) for AS perform better than *HLA-B27* testing and other standard diagnostic tests employed in AS including C-reactive protein measurement and MRI scanning.

How might this impact on clinical practice or future developments?

► PRS for AS should be used to assist diagnosing AS among patients with chronic back pain.

in those with more severe forms of the disease achieves more effective clinical responses³ and probably reduces the rate joint fusion in the long term.⁴ However, other causes of chronic back pain are common in the community, and AS is responsible for only a minority of these cases. It can be difficult to distinguish AS from other causes of back pain, particularly early in the disease with the consequence that the diagnosis of AS is often significantly delayed; many surveys undertaken in a variety of different health systems suggest an average delay of 6–10 years.^{5–7} A recent North American survey reported that fewer than half (37.1%) of patients with AS reported that they were correctly diagnosed within 1 year of seeking medical attention, and 32.8% waited more than a decade to receive the diagnosis.⁷ Population surveys suggest that as many as 80% of cases in the community remain

undiagnosed⁸ and therefore may not receive appropriate effective treatment. There is thus a great need for improved testing to improve early accurate diagnosis.

Currently, the most widely used tests for AS in those with chronic back pain are measurements of acute phase reactants, such as erythrocyte sedimentation rate and C-reactive protein (CRP), genetic testing for *HLA-B27* and imaging—either plain radiographs or MRI of the sacroiliac joints.⁹ However, each of these tests has limitations. In brief, acute phase reactants and MRI are only positive after disease develops and are therefore not useful for predicting disease risk. Acute phase reactants have only moderate sensitivity and specificity, particularly in early disease. MRI is expensive and is not universally available. Genetic factors are the major determinants of the risk of developing AS, with heritability assessed in twins of >90%.^{10 11} Although *HLA-B27* alone contributes 20% of the variation in disease risk,¹² the remainder of the genetic risk is determined by thousands of common genetic variants, each of which has only a very small effect. Polygenic Risk Scores (PRS) use combinations of hundreds to thousands of genetic variants to quantify an individual's genetic risk of disease. Unlike *HLA-B27* testing which is categorical or dichotomous in outcome, PRS are continuous measures. They are of particularly strong predictive value for low-frequency diseases with high heritability,¹³ such as AS. Here, we describe the development and validation of PRS for AS in two different ethnic groups and compare its performance to standard screening or diagnostic tests.

METHODS

Study population

AS was defined according to the modified New York criteria.¹⁴ Following genotyping quality control, there were 8244 cases and 14274 controls of western European descent; 6001 cases and 4493 controls of East Asian (Chinese) descent; and 1340 cases and 1685 controls of Turkish and Iranian origin, respectively. Written informed consent was obtained from all cases, with approval from the relevant research ethics authorities at each participating centre. Cohort details are provided in online supplemental table S1.

Genetic data

Samples were genotyped using the Illumina Core-Exome SNP genotyping microarray, according to the manufacturer's recommendations (chip versions used per cohort are provided in online supplemental table S1). Bead intensity data were processed and normalised for each sample, and genotypes called, using Genome Studio V.2.0 software (GenomeStudio Software Downloads (illumina.com)). Standard quality control measures as outlined in the Supplementary Methods were applied including identification and exclusion of cryptic-related samples, exclusion of samples with an outlying heterozygosity rate (3 SD from the mean in each cohort) or excess missingness (>5%). Single nucleotide polymorphisms (SNPs) with genotyping missing rate >2%, p value of Hardy-Weinberg equilibrium test <1×10⁻⁶, or with allele frequency <1% were removed. Population stratification was assessed using Shellfish (<http://www.stats.ox.ac.uk/~davison/software/shellfish/shellfish.php>). PRS analyses were performed with and without inclusion of principal components and gender as covariates. Results including principal components and gender as covariates are reported in online supplemental table S2 and are very similar to the results not including these covariates.

HLA-B27 imputation was performed using SNP2HLA, using a deep sequencing Chinese reference panel (n=10689)¹⁵ for East Asian samples and Type 1 Diabetes Genetics Consortium (n=5225) panel of combined HLA types and MHC SNP genotypes for all other subjects.¹⁶

PRS were calculated for each individual using the adaptive MultiBLUP algorithm (implemented in the software LDAK V.5.0).¹⁷ LDAK first divides the genetic data into chunks of size 75 000 bp and then performs association test for all the chunks and thinned out SNPs in strong linkage disequilibrium. The significant chunks with p value <1×10⁻⁵ and all adjacent chunks with p value <0.01 are merged into regions. Then the variance components and effect size of SNPs are estimated, and the effect size of the SNPs used to calculate the PRS. A 10-fold cross-validation analysis was performed as internal validation; a separate external validation was performed in the British and North American subjects, as well as through comparison of performance of PRS trained in either European descent or East Asian subjects, then validated in a separate ethnic group. In regard to cross-validation studies, the case-control cohort being studied is divided into 10 equal folds randomly with same case-control ratio. Nine folds of samples were used as a training set and the remaining fold of samples was retained as the validation data for testing the model generated by the training set. The process was repeated 10 times, with each of the 10-folds used only once as the validation data. The out-of-fold predictions based on the effect sizes of the selected SNPs were obtained for the test fold. All the predictions of 10 test folds were merged, after which statistical analysis was performed using all out-of-fold test set predictions to maximise sample size for internal testing. The resulting weighted predictors were then applied to the test cohort to obtain per sample scores from which the area under the curve (AUC) was obtained using receiver operator characteristic (ROC) analysis. R package pROC was used to calculate the 95% CI of the AUC and also compare AUCs from two models.¹⁸ Positive (PPV) and negative predictive values (NPV) were then calculated for PRS centiles, assuming different prior probabilities of AS. The continuous net reclassification improvement (NRI),¹⁹ a statistic that aims to quantify differences in classification performance of different models, was calculated using the R package PredictABEL²⁰ and used to compare accuracy of diagnostic assignment by *HLA-B27* testing and PRS.

RESULTS

ROC analyses of test discriminatory capacity are summarised in table 1. In 10-fold cross-validation in this case-control cohort, the PRS had AUC of 0.924 (95% CI 0.920 to 0.928) (figure 1). The AUC of *HLA-B27* testing alone was 0.869 (95% CI 0.865 to 0.874), which was statistically significantly less discriminatory than the PRS (p<2.2×10⁻¹⁶). Additionally, the NRI was positive (0.717, 95% CI 0.692 to 0.743), confirming that the PRS is an improvement on *HLA-B27* alone. A PRS including only non-MHC SNPs performed less well (AUC 0.782), as did a PRS including only 103 (genotyped or imputed) loci previously reported to have achieved genome-wide significance in AS (AUC=0.659).²¹ MRI has a reported sensitivity of 85% and specificity of 92% in AS,²² which correlates with an AUC of 0.885. CRP has a reported sensitivity of 50% and specificity of 80% for the disease (AUC=0.7).²³

To test the performance of the PRS using external validation, the European descent cases were divided into British and North American cohorts, and controls divided in the same proportion as the two case cohorts. PRS was then

Table 1 ROC analysis findings (AUC) of genetic risk scores in different populations

Predictors	Population tested in			
	European	East Asian	Iranian	Turkish
HLA-B27 alone	0.869 (0.865–0.874)	0.901 (0.895–0.906)	0.831 (0.807–0.854)	0.821 (0.804–0.838)
European non-MHC PRS	0.782 (0.776–0.788)*	0.594 (0.539–0.560)	0.534 (0.500–0.569)	0.568 (0.542–0.595)
European overall PRS	0.924 (0.920–0.928)*	0.788 (0.779–0.796)	0.852 (0.826–0.879)	0.854 (0.836–0.872)
East Asian non-MHC PRS	0.555 (0.547–0.563)	0.731 (0.722–0.741)*	0.565 (0.531–0.598)	0.554 (0.528–0.581)
East Asian overall PRS	0.880 (0.875–0.887)	0.948 (0.943–0.952)*	0.872 (0.848–0.895)	0.840 (0.821–0.860)
MRI EUR	0.885			
MRI CH ⁴¹	0.62			
CRP	0.7			

*10-fold cross-validation. All other PRS AUC values are external validation statistics.

AUC, area under the curve; CRP, C-reactive protein; PRS, Polygenic Risk Score; ROC, receiver operator characteristic .

developed in the British training set (n=6499 cases, 12 163 controls) and externally validated in the North American case-control cohort (n=1128 cases, 2111 controls). The PRS in the North American cohort had AUC of 0.928 (95% CI 0.918 to 0.939), significantly higher than *HLA-B27* alone (0.895, 95% CI 0.883 to 0.906, $p=1.73 \times 10^{-5}$) (online supplemental figure S1). These findings are very similar to the cross-validation analysis of the overall dataset reported above.

The PRS developed in all the European descent subjects, with 3994 SNPs (including 2244 major histocompatibility complex (MHC) SNPs), had moderate discriminatory capacity in East Asian, Iranian and Turkish cases and controls (AUC=0.788, 0.852 and 0.854, respectively), better than the performance of *HLA-B27* alone in the Iranian and Turkish cohorts, but not in East Asians. In contrast, the PRS developed in East Asian subjects, then tested by cross-validation (i.e. also in East Asian subjects), had much better discriminatory capacity (AUC=0.948, 95% CI 0.943 to 0.952)

than did the PRS developed in European descent subjects when tested in East Asian subjects. The PRS involving 8659 SNPs (including 2417 MHC SNPs) developed with all the East Asian subjects also performed well in European descent subjects (AUC=0.880, online supplemental figure S2), better than the discriminatory performance of *HLA-B27* in each of the other three populations tested.

In clinical practice, the utility of all such tests depends on the prior probability of the disease concerned. The PPV and NPV of the PRS and *HLA-B27* in European subjects are presented in figure 2 in the setting of a patient under 45 years of age, attending a physician with a history of back pain for 3 months or more. Published studies report that in this setting the prior probability of AS is ~30%,^{24–26} but as this may vary according to referral patterns, we have additionally provided findings for prior probabilities of 10% and 20% (online supplemental figures S5 and S6; East Asian specific findings are presented in online supplemental figures S7–S9). Assuming a prior probability for AS of 30%,

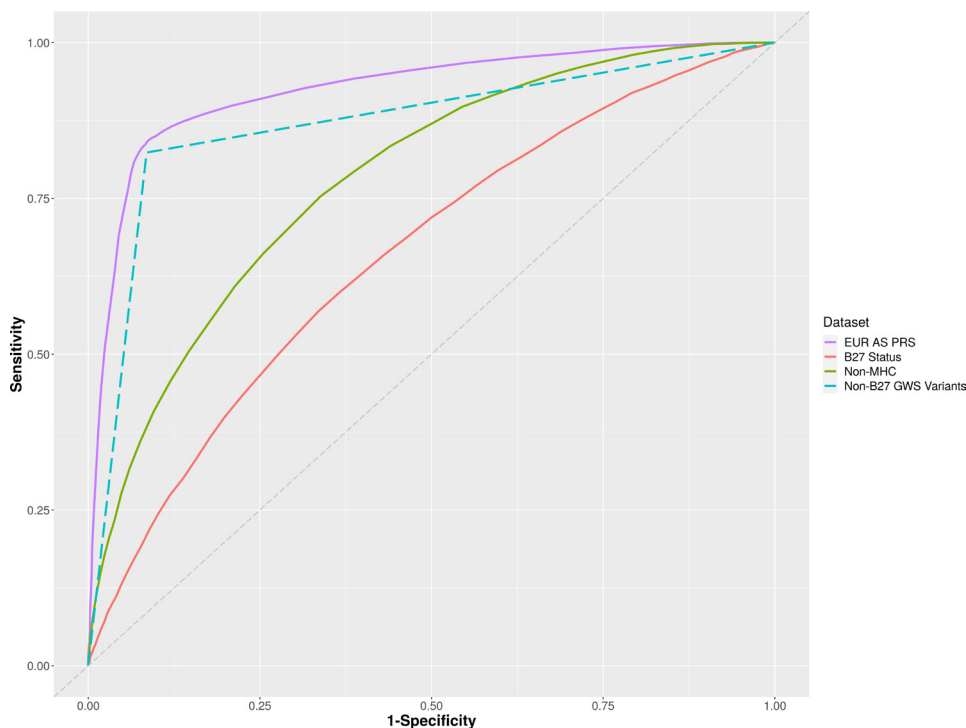


Figure 1 Receiver operating characteristic curve plot of performance of Polygenic Risk Scores (PRS) (purple dashes, area under the curve (AUC)=0.924), *HLA-B27* (aqua dashes, AUC=0.869), PRS less major histocompatibility complex (MHC) (green line, AUC=0.782) and genome-wide significant loci only (red line, AUC=0.659).

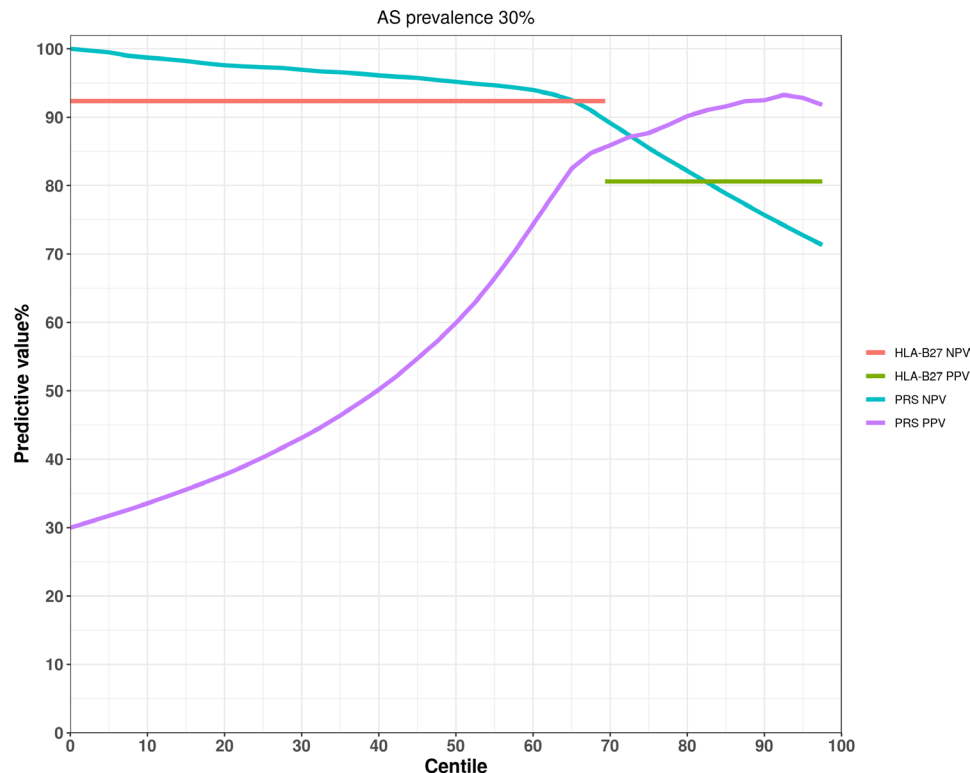


Figure 2 Positive (PPV) and negative predictive values (NPV) of Polygenic Risk Scores (PRS) and HLA-B27 for ankylosing spondylitis (AS), assuming prior probability of AS of 30%, among Europeans. Centiles refer to the population distribution of the PRS.

an *HLA-B27* test will be positive in 31% of those tested with a PPV of 80.6%, and in the 69% of those with a negative test, the NPV is 92.4%. Using the PRS, the PPV is >80.6% for top 35% of those screened, and achieves a higher maximum value (93.3%) than does *HLA-B27* (80.6%) (figure 2). The PRS NPV will be >92.4% for 65% of those screened, and also achieves a higher maximum value (99.6%) than does *HLA-B27* (92.4%). Considering the situation where only 10% of screened patients have AS, then *HLA-B27* will be positive in 16% of those tested. In this group, *HLA-B27* positivity has a PPV of 51.9%, and a negative result (seen in 84% of screened patients) has an NPV of 97.9%. Using the PRS, the PPV is >51.9% for 35% of patients and has a much higher maximum value (78.2% vs 51.9%). The NPV for the PRS is >97.9% for 65% of patients and achieves a slightly higher maximum value than *HLA-B27* testing (100% vs 97.9%).

Considering general population screening, at least 8% of the European population carry *HLA-B27*,²⁷ yet only 5% of carriers of this allele will develop AS²⁸; as such, no higher PPV can be achieved using *HLA-B27* testing alone. In contrast, for the PRS, the PPV for the top 8% of the population is three times higher (15.1%), and it is higher than 5% for the top 35% of the population. The NPV for *HLA-B27*-negative status is 99.9%, which is exceeded by the PRS for 62.5% of the population.

DISCUSSION

Distinguishing AS from other causes of chronic back pain remains an important issue in rheumatology. *HLA-B27* testing can have a valuable PPV for AS, particularly in clinical settings where the pretest probability of the disease is relatively high compared with the general population. It is therefore included in the Assessment of Spondyloarthritis

International Study Group (ASAS) axial spondyloarthritis (axSpA) classification criteria and is an essential criterion for those with no available imaging evidence of disease. *HLA-B27* testing has also been recommended for screening patients with chronic back pain to identify those at higher risk of AS or the related group of diseases axSpA, for referral to specialist services.^{23 25} However, *HLA-B27* only contributes ~20% of the overall heritability of AS, which is estimated to be ≥90% overall, indicating a substantial non-MHC component.²⁹ This suggests that PRS, which capture the common-variant component of heritability, are likely to be much more informative than *HLA-B27* tests alone. Our study confirms this, with the PRS performing better than *HLA-B27* testing in both AUC and continuous NRI analyses, irrespective of the prevalence of AS among those being tested. We confirm these findings both by internal cross-validation and by external validation. For 35% of the population, the PPV is higher for the PRS than for *HLA-B27* testing, and the NPV is higher for >65%. In particular, the peak PPV is substantially higher for the PRS than for *HLA-B27* and is informative for a far higher proportion of patients, as it is a continuous variable whereas *HLA-B27* is dichotomous. PRS testing also has higher discriminatory capacity for AS than MRI, and far higher than CRP. Accurate interpretation of MRI scans is known to be dependent on training and experience, and particularly in inexperienced, untrained hands may perform worse than the average reported performance, in which setting PRS may be particularly valuable.

Chronic back pain of >3 months' duration has previously been shown to have very low heritability attributable to common genetic variants (minor allele frequency >0.01) such as those included in our AS PRS (common variant heritability=6.43%³⁰–7.6%³¹) and not to be genetically correlated with AS. Therefore, it is unlikely that the AS

PRS will prove less discriminatory in practice in the clinical setting of patients presenting with chronic back pain than the estimates presented here. A limitation of this study is that the performance of the PRS has not been formally tested in this setting, where it will require further evaluation.

axSpA refers to a spectrum of diseases. Patients with radiographic sacroiliitis are classified as having AS, whereas those without X-ray changes are classified as having non-radiographic (nr)-axSpA. The current PRS may have prognostic value in distinguishing the 16%–24% of nr-axSpA cases that are likely to go on to develop AS.^{32–33} Whether the PRS we report here will prove more informative than *HLA-B27* testing alone in patients with nr-axSpA itself is unknown. The ASAS have previously demonstrated that patients meeting the ASAS classification criteria for axSpA who do not yet have AS have a much lower average genetic risk score than patients with AS, using only genome-wide significant AS loci.³⁴ Whether this is because nr-axSpA is actually genetically distinct from AS, or reflects the greater clinical and likely aetiopathogenic heterogeneity of nr-axSpA,³⁵ will require further study.

As with the use of PRS in the screening of individuals with chronic back pain, its performance in nr-axSpA will also require further study. Similarly, the performance of the PRS in males compared with females, in subjects with environmental risk factors for the disease such as cigarette smoking,³⁶ and in subsets of patients such as those with extraskeletal manifestations of AS requires further study. In that regard, the excellent performance of a PRS in patients with acute anterior uveitis complicating AS (AUC=0.96; 95% CI 0.955 to 0.966) suggests that at least in some AS subsets the performance of the PRS will be even better than reported here.³⁷

PRS testing can be performed using data from any dense SNP microarray. Indeed, the performance of the PRS reported here was high despite our use of a relatively low density SNP microarray—the Illumina Core-Exome chip (>520 000 variants, including many rare and non-polymorphic variants that do not contribute to the PRS). The performance of PRS testing would be likely to improve further with use of microarrays with better SNP coverage, or with whole genome sequencing. It has been estimated that up to 12 million Americans have had SNP microarray testing performed by commercial services such as 23andMe and Ancestry.³⁸ At little additional cost, these data would probably prove suitable for the calculation of the AS PRS we report, as well as enabling PRS for many other diseases in which they have been shown to be informative. The cost-effectiveness of the PRS we report here needs to be confirmed in further studies. As the genetic profile of AS becomes better understood, the discriminatory capacity of these tests is also likely to increase. For example, it is likely that many of the SNPs included in the PRS at present are not truly associated with AS, but just add noise to the test.

As there is no preventive therapy yet for AS, general population screening to identify patients at high risk of the disease is not recommended except, perhaps, for those at increased risk, such as the relatives of those with AS (given the high sibling recurrence risk of 8.2%).³⁹ PRS performs significantly better than *HLA-B27* testing alone in the general population, with the PPV of the ~8% of the general population who carry *HLA-B27* being 5%, compared with the peak PPV of the PRS of 15.1%. Similarly, the NPV for the PRS exceeds that of *HLA-B27* testing for most of the population. Although the PPV for PRS testing for general population screening is modest, the test performs well compared with other widely used screening tests. For example, the PPVs for 10-year risk of coronary heart disease of a high total cholesterol (≥ 240 mg/dL)—a threshold above which many

patients will be prescribed cholesterol-lowering therapy—are 10.3% in women and 18.6% in men,⁴⁰ similar to the top 20% of PPVs of PRS for AS in general population screening. Among those who have already had SNP microarray testing performed, knowledge of a high AS-PRS even in the absence of symptoms may heighten clinician awareness of the possible diagnosis, reduce delay and assist with earlier appropriate and effective treatment, given the current long diagnostic delays.

Our study shows that the performance of the PRS varies between ethnic groups, although it remains moderately high even when a PRS developed in subjects of (western) European descent is tested in eastern European/west Asian subjects such as Turks and Iranians. The PRS developed specifically for East Asians performed far better in that population than did the European PRS, indicating that at least for populations that are remotely related, ethnic-specific PRSs are preferable.

We conclude that PRS testing for AS has greater discriminatory capacity than *HLA-B27* testing, MRI scanning or CRP testing, either alone or in combination. PRS could be used to screen patients with chronic back pain to identify subjects at increased risk of the disease for referral to secondary care and to assist in diagnosing the condition.

Author affiliations

¹Queensland University of Technology, Centre for Genomics and Personalised Health, School of Biomedical Sciences, Faculty of Health, Translational Research Institute, Woolloongabba, Queensland, Australia

²Department of Rheumatology and Immunology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, Shanghai, China

³Australian Translational Genomics Centre, Queensland University of Technology (QUT), Translational Research Institute, Woolloongabba, Queensland, Australia

⁴Department of Internal Medicine, Division of Rheumatology, School of Medicine, Manisa Celal Bayar University, Manisa, Turkey

⁵UMR 1173, Inserm, University of Versailles Saint-Quentin, Montigny-le-Bretonneux, France

⁶Service de Rhumatologie, Hôpital Ambroise Paré, Assistance Publique-Hôpitaux de Paris, Boulogne-Billancourt, France

⁷Laboratoire d'Excellence Inflammex, Université Paris Diderot, Sorbonne Paris Cité, Paris, France

⁸Epidemiology Group, Institute of Applied Health Sciences, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Foresterhill, Aberdeen, UK

⁹Aberdeen Centre for Arthritis and Musculoskeletal Health, University of Aberdeen, Foresterhill, Aberdeen, UK

¹⁰Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Tehran, Iran (the Islamic Republic of)

¹¹NIHR Leeds Biomedical Research Centre, Leeds Teaching Hospitals NHS Trust, Leeds, UK

¹²Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK

¹³Division of Allergy, Immunology, Rheumatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

¹⁴School of Medicine, National Yang-Ming University, Taipei, Taiwan

¹⁵Department of Medicine, University of Otago Wellington, Wellington, New Zealand

¹⁶Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand

¹⁷Hanyang University Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea (the Republic of)

¹⁸University of Queensland Diamantina Institute, University of Queensland, Brisbane, Queensland, Australia

¹⁹Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan

²⁰Department of Medicine, Chung Shan Medical University, Taichung, Taiwan

²¹Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan

²²QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia

²³Queensland Brain Institute, University of Queensland, Brisbane, Queensland, Australia

²⁴Population & Data Sciences, University of Texas Southwestern Medical Center, Dallas, Texas, USA

²⁵State Key Laboratory of Optometry, Ophthalmology, and Vision Science, Affiliated Eye Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, China

²⁶Institute for Glycomics, Griffith University, Nathan, Queensland, Australia

²⁷Center for Precision Medicine, First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

²⁸Institute of Life Sciences, Wenzhou University, Wenzhou, Zhejiang, China
²⁹Rheumatology Department, First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China
³⁰Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology & Visual Sciences Key Lab, Beijing, Beijing, China
³¹Department of Medicine/Rheumatology, Cedars-Sinai Medical Center, Los Angeles, California, USA
³²Division of Medicine/Rheumatology, University of California San Francisco, San Francisco, California, USA
³³Intramural Research Program, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland, USA
³⁴Internal Medicine, The University of Texas Health Science Center at Houston John P and Katherine G McGovern Medical School, Houston, Texas, USA
³⁵Department of Internal Medicine, Division of Rheumatology, McGovern Medical School at The University of Texas Health Science Center, Houston, Texas, USA
³⁶NIHR Oxford Musculoskeletal Biomedical Research Unit, Botnar Research Centre, University of Oxford, Oxford, UK
³⁷School of Clinical Medicine, Tsinghua University, Beijing, Beijing, China
³⁸Peking-Tsinghua Center for Life Sciences, Tsinghua University, Beijing, China
³⁹NIHR Biomedical Research Centre at Guy's and Saint Thomas' NHS Foundation Trust and King's College London, London, UK

Twitter Nurullah Akkoc @nurullahakkoc, Gary J Macfarlane @UAbderdeenEpi and Gareth T Jones @hteraG_seno

Acknowledgements We would like to thank all participating subjects with ankylosing spondylitis and healthy individuals who provided the DNA and clinical information necessary for this study. The TASC study was funded by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) grants P01-052915 and R01-AR046208. Funding was also received from the University of Texas Health Science Center at Houston CTSA grant UL1R02418, Cedars-Sinai GCRC grant MO1-RR00425, Intramural Research Program, NIAMS/NIH and Rebecca Cooper Foundation (Australia).

Collaborators TCRI AS Group Jian Yin¹, Lei Jiang¹, Lin Zhou¹, Ting Li¹, Qingwen Wang², Tianwang Li², Guanmin Gao⁴, Shengqian Xu², Weiguo Xiao⁶, Hui Shen⁶, Jingguo Zhou⁷, Yuquan You⁸, Dongbao Zhao⁹, Qing Cai⁹, Shengming Dai¹⁰, Lan He¹¹, Ping Zhu¹², Zhenyu Jiang¹³, Jian Xu¹⁴, Huaxiang Wu¹⁵, Lie Dai¹⁶, Yang Li¹⁷, Feng Ding¹⁸, Xiaochun Zhu¹⁹, Chongyang Liu²⁰, Dongyi He²¹, Liyun Zhang²², Zhijun Li²³, Futao Zhao²⁴, Hanshi Xu²⁵, Niansong Wang¹⁰, Youlian Wang²⁶, Lindi Jiang²⁷, Yu Zhang²⁸, Jinwei Chen²⁹, Fang Cheng²⁴, Zhiyi Zhang³⁰, Yifang Mei³⁰, Liangjing Lv³¹, Lingli Dong³², Jing Yang³³, Yinong Li³⁴, Xiaodong Wang³⁵, Xiaofeng Li³⁶, Hongsheng Sun³⁷, Xianming Long³⁸, Xiao Zhang³⁹, Qinghong Yu⁴⁰, Xiaodan Kong⁴¹, Yi Zheng⁴², Miaojia Zhang⁴³, Yi Tao⁴⁴, Yisha Li⁴⁵, Xinwang Duan⁴⁶, Qianghua Wei⁴⁷, Xiaofei Wang⁴⁸, Jie Han⁴⁹, Rong Mu⁵⁰, Yiping Lin⁵¹, Jian Zhu⁵², Xiaoyuan Chen⁵³.
 1. Department of Rheumatology and Immunology, Shanghai Changzheng Hospital, The Second Military Medical University; 2. Peking University Shenzhen Hospital; 3. Guangdong Second Provincial General Hospital; 4. The First Affiliated Hospital of Zhengzhou University; 5. The First Affiliated Hospital of Anhui Medical University; 6. The First Affiliated Hospital of China Medical University; 7. The Affiliated Hospital of North Sichuan Medical College; 8. Quanzhou Orthopedic-Traumatological Hospital of Fujian Traditional Chinese Medical University; 9. Shanghai Changhai hospital; 10. Shanghai Sixth People's Hospital; 11. The First Affiliated Hospital of Xi'an Jiaotong University; 12. Xijing Hospital; 13. Bethune First Hospital of Jilin University; 14. The First Affiliated Hospital of Kunming Medical University; 15. The Second Affiliated Hospital of Zhejiang University School of Medicine; 16. The Second Affiliated Hospital of Sun Yat-sen University; 17. The Second Affiliated Hospital of Harbin Medical University; 18. Qilu Hospital of Shandong University; 19. The First Affiliated Hospital of Wenzhou Medical University; 20. The Third Affiliated Hospital of Chongqing Medical University; 21. Shanghai GuangHua Hospital of Integrated Traditional Chinese and Western Medicine; 22. Shanxi Dayi Hospital; 23. The First Affiliated Hospital of Bengbu Medical College; 24. Shanghai Ninth People's Hospital; 25. The First Affiliated Hospital of Sun Yat-sen University; 26. Jiangxi Provincial People's Hospital; 27. Shanghai Zhongshan Hospital; 28. Northern Jiangsu People's Hospital; 29. The Second Xiangya Hospital of Central South University; 30. The First Affiliated Hospital of Harbin Medical University; 31. Shanghai Renji Hospital; 32. Tongji Hospital of Huazhong University of Science & Technology; 33. Mianyang Hospital of Traditional Chinese Medicine; 34. Fujian armed police hospital; 35. Affiliated Hospital of Weifang Medical University; 36. The Second Affiliated Hospital of Shanxi Medical University; 37. Shandong Provincial Hospital; 38. The First Affiliated Hospital to Soochow University; 39. Guangdong General Hospital; 40. Zhujiang Hospital of Southern Medical University; 41. The Second Affiliated Hospital of Dalian Medical University; 42. Beijing Chao-Yang Hospital; 43. Jiangsu Province Hospital; 44. The Second Affiliated Hospital of Guangzhou Medical University; 45. The First Xiangya Hospital of Central South University; 46. The Second Affiliated Hospital of Nanchang University; 47. Shanghai General Hospital; 48. Shengjing Hospital of China Medical University; 49. Shanghai Dongfang Hospital; 50. Peking University People's Hospital; 51. The People's Liberation Army's 202 Hospital; 52. Beijing Tsinghua Changgung

Hospital, School of Clinical Medicine, Tsinghua University; 53. Tsinghua Clinical Research Center, School of Medicine, Tsinghua University. Ann Morgan, NIHR Leeds Biomedical Research Centre, Leeds Teaching Hospitals NHS Trust, Leeds, UK, and Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK.

Contributors Study design was performed by ZL, XW, PL, MHW, LG, MMW, MHR, JDR, BPW, HX and MAB. Case recruitment was performed by XW, NA, MA, GM, MM, HMO, CTC, AAH, SS, GTJ, SYB, GW, AJ, EF, JS, LL, ML, JCCW, NM, MJW, MJL, YW, JZ, JSZ, XW, JZB, MHW, LG, MMW, MHR, LD, THK, JDR, BPW, HX and MAB. Genotyping and/or analysis were performed by ZL, XW, ML, PL, GW, EDG, LA, LW, XH and MAB. The manuscript was prepared by ZL, JDR, BPW, XH and MAB. All authors were involved in completion of, and approved, the final manuscript.

Funding This study was funded, in part, by Arthritis Research UK (Grants 19536 and 18797), by the Wellcome Trust (grant number 076113) and by the Oxford Comprehensive Biomedical Research Centre ankylosing spondylitis chronic disease cohort (Theme Code: A91202). XH is supported by the National Natural Science Foundation of China (Grant No. 31821003), National Key Research and Development Project (Grant No. 2018AAA0100302), Shanghai Municipal Key Clinical Specialty (shslczdzk02602), and Shanghai Science and Technology Development Funds (2020-SH-XY-2). ZBJ was funded by a grant from the Zhejiang Provincial Natural Science Foundation of China (LD18H120001LD). The New Zealand data were derived from participants in the Spondyloarthritis Genetics and the Environment Study (SAGE) and was funded by The Health Research Council, New Zealand. We acknowledge the Understanding Society: The UK Household Longitudinal Study. This is led by the Institute for Social and Economic Research at the University of Essex and funded by the Economic and Social Research Council. The survey was conducted by NatCen and the genome-wide scan data were analysed and deposited by the Wellcome Trust Sanger Institute. Information on how to access the data can be found on the Understanding Society website <https://www.understandingsociety.ac.uk/>. HMO is supported by the National Institute for Health Research (NIHR) Leeds Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. French sample collection was performed by the Groupe Française d'Etude Génétique des Spondylarthrites, coordinated by Professor Maxime Breban and funded by the Agence Nationale de Recherche GEMISA grant reference ANR-10-MIDI-0002. We acknowledge and thank the TCRI AS Group for their support in recruiting patients for the study (see below). The authors acknowledge the sharing of data and samples by the BSRBR-AS Register in Aberdeen. Chief Investigator, Professor Gary Macfarlane and Dr Gareth Jones, Deputy Chief Investigator created the BSRBR-AS study which was commissioned by the British Society for Rheumatology, funded in part by Abbvie, Pfizer and UCB. We are grateful to every patient, past and present staff of the BSRBR-AS register team and to all clinical staff who recruited patients, followed them up and entered data—details here: <https://www.abdn.ac.uk/iahs/research/epidemiology/spondyloarthritis.php#panel1011>. The QIMR control samples were from parents of adolescent twins collected in the context of the Brisbane Longitudinal Twin Study 1992–2016, support by grants from NHMRC (NGM) and ARC (MJW). We thank Anjali Henders, Lisa Bowdler, Tabatha Goncales for biobank collection and Kerrie McAloney and Scott Gordon for curating samples for this study. MAB is funded by a National Health and Medical Research Council (Australia) Senior Principal Research Fellowship (1024879), and support for this study was received from a National Health and Medical Research Council (Australia) program grant (566938) and project grant (569829), and from the Australian Cancer Research Foundation and Rebecca Cooper Medical Research Foundation. We are also very grateful for the invaluable support received from the National Ankylosing Spondylitis Society (UK) and Spondyloarthritis Association of America in case recruitment. Additional financial and technical support for patient recruitment was provided by the National Institute for Health Research Oxford Musculoskeletal Biomedical Research Unit and NIHR Thames Valley Comprehensive Local Research and an unrestricted educational grant from Abbott Laboratories. This research was funded/supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London and/or the NIHR Clinical Research Facility. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Written informed consent was obtained from all cases, with approval from the relevant research ethics authorities at each participating centre. The overall programme was reviewed and approved by Metro South Hospital Research Ethics Committee (approval reference HREC/05/QPAH/221).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. Details of the polygenic risk scores will be made available depending on completion of data transfer agreements with King's College London.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Zhixiu Li <http://orcid.org/0000-0002-2924-9120>
 Nurullah Akkoc <http://orcid.org/0000-0002-3718-171X>
 Maxime Breban <http://orcid.org/0000-0002-6932-9395>
 Gary J Macfarlane <http://orcid.org/0000-0003-2322-3314>
 Mahdi Mahmoudi <http://orcid.org/0000-0002-8164-8831>
 Helena Marzo-Ortega <http://orcid.org/0000-0002-9683-3407>
 Andrew A Harrison <http://orcid.org/0000-0002-4372-3252>
 Simon Stebbings <http://orcid.org/0000-0002-2824-4440>
 Gareth T Jones <http://orcid.org/0000-0003-0016-7591>
 James Cheng-Chung Wei <http://orcid.org/0000-0003-0310-2769>
 MinJae Lee <http://orcid.org/0000-0002-4329-506X>
 Xiaobing Wang <http://orcid.org/0000-0002-4302-2213>
 Zi-Bing Jin <http://orcid.org/0000-0003-0515-698X>
 Michael M Ward <http://orcid.org/0000-0003-1857-9367>
 Tae-Hwan Kim <http://orcid.org/0000-0002-3542-2276>
 John D Reveille <http://orcid.org/0000-0001-5950-0913>
 Bryan Paul Wordsworth <http://orcid.org/0000-0001-7512-3468>
 Huji Xu <http://orcid.org/0000-0002-8588-118X>
 Matthew A Brown <http://orcid.org/0000-0003-0538-8211>

REFERENCES

- Braun J, Listing J, Sieper J. Overestimation of the prevalence of ankylosing spondylitis in the Berlin study: comment on the article by Braun, et al. - Reply. *Arthritis Rheum-US* 2005;52:4049–50.
- Zeng QY, Chen R, Darmawan J, et al. Rheumatic diseases in China. *Arthritis Res Ther* 2008;10:R17.
- Rudwaleit M, Claudepierre P, Wordsworth P, et al. Effectiveness, safety, and predictors of good clinical response in 1250 patients treated with adalimumab for active ankylosing spondylitis. *J Rheumatol* 2009;36:801–8.
- Haroon N, Inman RD, Learch TJ, et al. The impact of tumor necrosis factor α inhibitors on radiographic progression in ankylosing spondylitis. *Arthritis Rheum* 2013;65:2645–54.
- Feldtkeller E, Khan MA, van der Heijde D, et al. Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. *Rheumatol Int* 2003;23:61–6.
- Reed MD, Dharmage S, Boers A, et al. Ankylosing spondylitis: an Australian experience. *Intern Med J* 2008;38:321–7.
- Ogdie A, Benjamin Nowell W, Reynolds R, et al. Real-world patient experience on the path to diagnosis of ankylosing spondylitis. *Rheumatol Ther* 2019;6:255–67.
- van der Linden S, Valkenburg H, Cats A. The risk of developing ankylosing spondylitis in HLA-B27 positive individuals: a family and population study. *Br J Rheumatol* 1983;22:18–19.
- Deodhar A, Mease PJ, Reveille JD, et al. Frequency of axial spondyloarthritis diagnosis among patients seen by US rheumatologists for evaluation of chronic back pain. *Arthritis Rheumatol* 2016;68:1669–76.
- Brown MA, Kennedy LG, MacGregor AJ, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum* 1997;40:1823–8.
- Pedersen OB, Svendsen AJ, Ejstrup L, et al. Ankylosing spondylitis in Danish and Norwegian twins: occurrence and the relative importance of genetic vs. environmental effectors in disease causation. *Scand J Rheumatol* 2008;37:120–6.
- Ellinghaus D, Jostins L, Spain SL, et al. Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. *Nat Genet* 2016;48:510–8.
- Wray NR, Yang J, Goddard ME, et al. The genetic interpretation of area under the ROC curve in genomic profiling. *PLoS Genet* 2010;6:e1000864.
- van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the new York criteria. *Arthritis Rheum* 1984;27:361–8.
- Zhou F, Cao H, Zuo X, et al. Deep sequencing of the MHC region in the Chinese population contributes to studies of complex disease. *Nat Genet* 2016;48:740–6.
- Jia X, Han B, Onengut-Gumuscu S, et al. Imputing amino acid polymorphisms in human leukocyte antigens. *PLoS One* 2013;8:e64683.
- Speed D, Balding DJ. MultiBLUP: improved SNP-based prediction for complex traits. *Genome Res* 2014;24:1550–7.
- Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011;12:77.
- Pencina MJ, D'Agostino RB, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 2011;30:11–21.
- Kundu S, Aulchenko YS, van Duijn CM, et al. PredictABEL: an R package for the assessment of risk prediction models. *Eur J Epidemiol* 2011;26:261–4.
- Rostami S, Hoff M, Brown MA. Prediction of ankylosing spondylitis in the population-based HUNT study by a genetic risk score combining 110 SNPs of genome-wide significance. *J Rheumatol* 2019.
- Diekhoff T, Hermann K-GA, Greese J, et al. Comparison of MRI with radiography for detecting structural lesions of the sacroiliac joint using CT as standard of reference: results from the SIMACT study. *Ann Rheum Dis* 2017;76:1502–8.
- Rudwaleit M, van der Heijde D, Khan MA, et al. How to diagnose axial spondyloarthritis early. *Ann Rheum Dis* 2004;63:535–43.
- Brandt HC, Spiller I, Song I-H, et al. Performance of referral recommendations in patients with chronic back pain and suspected axial spondyloarthritis. *Ann Rheum Dis* 2007;66:1479–84.
- Poddubnyy D, Vahldiek J, Spiller I, et al. Evaluation of 2 screening strategies for early identification of patients with axial spondyloarthritis in primary care. *J Rheumatol* 2011;38:2452–60.
- Moltó A, Paternotte S, Comet D, et al. Performances of the assessment of spondyloarthritis International Society axial spondyloarthritis criteria for diagnostic and classification purposes in patients visiting a rheumatologist because of chronic back pain: results from a multicenter, cross-sectional study. *Arthritis Care Res* 2013;65:1472–81.
- Brown MA, Pile KD, Kennedy LG, et al. HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. *Ann Rheum Dis* 1996;55:268–70.
- Braun J, Bollow M, Remlinger G, et al. Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. *Arthritis Rheum* 1998;41:58–67.
- Brown MA, Crane AM, Wordsworth BP. Genetic aspects of susceptibility, severity, and clinical expression in ankylosing spondylitis. *Curr Opin Rheumatol* 2002;14:354–60.
- UKB SNP-Heritability Browser, 2020. Available: https://nealelab.github.io/UKBB_ldsc/h2_summary_3571.html [Accessed cited 2020 17/04/2020].
- Suri P, Palmer MR, Tsepilov YA, et al. Genome-wide meta-analysis of 158,000 individuals of European ancestry identifies three loci associated with chronic back pain. *PLoS Genet* 2018;14:e1007601.
- Sampaio-Barros PD, Bortoluzzo AB, Conde RA, et al. Undifferentiated spondyloarthritis: a longterm followup. *J Rheumatol* 2010;37:1195–9.
- Bennett AN, McGonagle D, O'Connor P, et al. Severity of baseline magnetic resonance imaging-evident sacroiliitis and HLA-B27 status in early inflammatory back pain predict radiographically evident ankylosing spondylitis at eight years. *Arthritis Rheum* 2008;58:3413–8.
- Thomas GP, Willner D, Robinson PC, et al. Genetic diagnostic profiling in axial spondyloarthritis: a real world study. *Clin Exp Rheumatol* 2017;35:229–33.
- Robinson PC, Wordsworth BP, Reveille JD, et al. Axial spondyloarthritis: a new disease entity, not necessarily early ankylosing spondylitis. *Ann Rheum Dis* 2013;72:162–4.
- Videm V, Cortes A, Thomas R, et al. Current smoking is associated with incident ankylosing spondylitis -- the HUNT population-based Norwegian health study. *J Rheumatol* 2014;41:2041–8.
- Huang X-F, Li Z, De Guzman E, et al. Genomewide association study of acute anterior uveitis identifies new susceptibility loci. *Invest Ophthalmol Vis Sci* 2020;61:3.
- Martin N. How DNA companies like ancestry and 23andMe are using your genetic data, 2018. Available: <https://www.forbes.com/sites/nicolemartin/12018/12/05/how-dna-companies-like-ancestry-and-23andme-are-using-your-genetic-data/#9a55de261894>: Forbes
- Brown MA, Laval SH, Brophy S, et al. Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. *Ann Rheum Dis* 2000;59:883–6.
- Wilson PW, D'Agostino RB, Levy D, et al. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998;97:1837–47.
- Ye L, Liu Y, Xiao Q, et al. MRI compared with low-dose CT scanning in the diagnosis of axial spondyloarthritis. *Clin Rheumatol* 2020;39:1295–303.