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International genome-wide meta-analysis identifies new primary biliary cirrhosis risk loci and targetable pathogenic pathways

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Primary biliary cirrhosis (PBC) is a classical autoimmune liver disease for which effective immunomodulatory therapy is lacking. Here we perform meta-analyses of discovery data sets from genome-wide association studies of European subjects ($n = 2,764$ cases and 10,475 controls) followed by validation genotyping in an independent cohort ($n = 3,716$ cases and 4,261 controls). We discover and validate six previously unknown risk loci for PBC ($P_{\text{combined}} < 5 \times 10^{-8}$) and used pathway analysis to identify JAK-STAT/IL12/IL27 signalling and cytokine-cytokine pathways, for which relevant therapies exist.

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P primary biliary cirrhosis (PBC) is a rare cholestatic liver disease characterized by progressive autoimmune destruction of intrahepatic bile ducts, leading to cirrhosis and liver failure in a substantial proportion of cases¹. To date, four genome-wide association studies (GWAS) and two Illumina immunochip studies of PBC have confirmed associations at the human leukocyte antigen (HLA) locus and identified 27 non-HLA risk loci^{2–8}. Consistent with GWAS data for other autoimmune diseases, results of these studies implicate immune-related genes in disease pathogenesis, but in general fail to pinpoint the disease-causal variants within the identified risk loci. To identify risk alleles that may be relevant to disease biology and treatment and illuminate additional PBC risk loci, we undertook a genome-wide meta-analysis (GWMA) combining North American, Italian and UK PBC GWAS data sets^{2,4,5}. Functional annotation of the risk loci and pathway analyses were then performed to identify the alleles and pathways most relevant to disease cause and treatment.

Results

Discovery of new PBC risk loci. Following quality control, the combined discovery data set for GWMA consisted of 1,143,634 genotyped or imputed single-nucleotide polymorphisms (SNPs) in 2,764 cases and 10,475 controls (Supplementary Table 1). After genomic control correction and exclusion of known PBC risk loci from the final set of results, the inflation factor was $\lambda = 1.043$ (Supplementary Fig. 1). Meta-analysis of this data set identified 23 loci at genome-wide level of significance ($P < 5 \times 10^{-8}$, calculated using logistic regression of individual discovery data sets in ProbABEL followed by genomic control correction of individual discovery data sets in R and fixed-effects meta-analysis in META, see Methods). Of these, 22 had been detected in previous studies and the 23rd corresponded to a most-likely spurious signal from a single imputed SNP on chromosome 13 (Supplementary Fig. 2, Supplementary Table 2). However, we found suggestive evidence of association ($P < 2 \times 10^{-5}$ from fixed-effects meta-analysis in META) at 41 loci not previously known to be associated with PBC. The top-scoring SNPs (or close proxies in strong linkage disequilibrium with the top-scoring SNP) from these and nine other loci (including the likely spurious chromosome 13 signal) were taken forward for genotyping in an independent panel consisting of 3,716 PBC cases and 4,261 controls. In total, 120 SNPs at 50 independent loci were taken forward for validation, of which 114 were successfully genotyped (Supplementary Data 1).

In the validation analysis, we confirmed association with SNPs at six loci not previously known to be associated with PBC ($P < 4.4 \times 10^{-4}$, equivalent to $P = 0.05$ with a Bonferroni correction for 114 tests, calculated using logistic regression analysis of individual validation data sets in PLINK followed by meta-analysis in META, see Methods); meta-analysis of discovery and validation cohorts at these loci reached genome-wide levels of significance ($P_{\text{combined}} < 5 \times 10^{-8}$ from fixed-effect meta-analysis in META) (Table 1, Supplementary Figs 3 and 4). Furthermore, SNPs at two additional loci achieved P values suggestive of association ($P < 1 \times 10^{-3}$ from fixed-effect meta-analysis in META, equivalent to $P = 0.05$ with a Bonferroni correction for testing at 50 independent loci; Table 1, Supplementary Fig. 5). Newly identified PBC risk loci overlap with those of other autoimmune disorders and harbour several immunologically relevant candidate genes, most notably chemokine ligand 20 (*CCL20*) and interleukin 12B (*IL12B*; Table 1).

Discovery of candidate causal disease variants. In functional annotation of risk loci, we identified 199 candidate variants across

28 non-HLA risk loci with probabilistic identification of causal SNPs (PICS) probability > 0.0275 (ref. 9). At each risk locus, the most-likely causal variant was the index variant, with median PICS probability of 0.224 and values up to 0.998 for rs2546890 at 5q33.3 (Supplementary Data 2). Looking at all candidate variants across all risk loci, the majority were intronic, upstream or downstream gene variants with no predicted functional consequence (99/199, 40%). However, a substantial proportion (59/199, 30%) were regulatory region variants, defined as SNPs located within regulatory features, including enhancers, promoters, transcription factor-binding sites and open chromatin regions (Supplementary Data 3). Notably, candidate variants at 18 (64%) of the 28 annotated risk loci included at least one regulatory region variant. In contrast, only 5 of 199 candidates were missense variants (2.5%) (Supplementary Table 3a). However, these included rs2297067 in *EXOC3L4* at 14q32.32 and rs2304256 in *TYK2* at 19p13.2, both predicted by SIFT and/or PolyPhen to be deleterious or potentially damaging^{10,11}. Candidate variants included a single splice region variant, that is, rs17641524 at 1q31.3 that is predicted to affect splicing of *DENND1B* (Supplementary Table 3b).

We found that candidate variants at several risk loci are methylation quantitative trait loci (mQTLs), including mQTLs for *DENND1B*, *PLCL2*, *IRF5* and *TNFRSF1A*, all genes that are implicated in risk for other autoimmune diseases (Supplementary Data 4). We also found that candidate variants at several risk loci are expression quantitative trait loci (eQTLs) in lymphoblastoid and other cell lineages, including eQTLs for *CCL20*, *IL12A*, *IRF5* and *TYK2* (Supplementary Data 5).

At many risk loci, functional annotation highlighted a single candidate gene (Supplementary Data 2). However, most risk loci contained multiple compelling candidate variants. This complexity is well illustrated by the composite of candidate variants at the *PLCL2* gene and *MANBA* gene loci, which include multiple eQTL and mQTL SNPs. Thus, despite the presence of many candidate variants with regulatory or epigenetic roles within PBC risk loci, more direct biological experimental approaches are required to pinpoint the disease-causal variants at these loci.

We also applied functional GWAS (FGWAS) and its associated annotation file¹² to our full set of discovery GWMA results and thereby identified 75 annotations with enrichment ($P < 0.01$ from FGWAS) of GWMA association signals (Supplementary Data 6). After a stepwise selection approach similar to that of Pickrell¹², the best-fitting model included six annotations highlighting negative enrichment of repressed chromatin regions in a lymphoblastoid cell line, and positive enrichment of DNase-I-hypersensitive sites in a variety of cell types, in particular CD20+ and Th1 T cells (Supplementary Table 4).

Identification of candidate targetable biological pathways.

To identify biological pathways involved in development of PBC, we conducted pathway analysis using GCTA¹³ followed by i-GSEA4GWAS¹⁴. We identified several immunoregulatory pathways associated with PBC, in particular, IL-12 and other cytokines as well as T-cell signalling pathways. To account for bias that might result from the strong HLA association with PBC, we repeated this analysis with SNPs/genes in the HLA region excluded. Notably, IL-12, IL-27 and JAK-STAT signalling pathways were still associated with PBC, even after their HLA contribution had been removed (Table 2).

We identified molecules that targeted these pathways by overlaying the Drug Gene Interaction database¹⁵ and calculating a pathway specificity score and Jaccard index of each drug for each of the pathways that remained associated with PBC after the

Table 1 | PBC risk loci identified in the current study.

a. Confirmed risk loci (validation $P < 4.4 \times 10^{-4}$ resulting in combined $P < 5 \times 10^{-8}$)										
Locus	SNP	Position (build 38)	A1/A2	Discovery <i>P</i>	Validation <i>P</i>	Joint <i>P</i>	OR (95% CI)	Region (build 38)	Nearby genes and functional annotation*	Autoimmune overlap
2q12.1	rs12712133	102,249,813	A/G	1.62×10^{-5}	7.94×10^{-5}	5.19×10^{-9}	1.14 (1.07-1.21)	102,118,975-102,438,307	IL1R1, IL1RL2 [‡] , FAM183DP, IL1RL1 [‡] , IL18R1, LOC100422339, IL18RAP, MIR4772	CD, CeD
2q36.3	rs4973341	227,795,646	C/T	6.48×10^{-7}	7.73×10^{-5}	2.34×10^{-10}	0.82 (0.74-0.90)	227,747,828-227,815,647	RNA5SP121, SNRPGP8, LOC100533842, CCL20 ^{‡,§}	
4p16.3	rs11724804	971,991	A/G	3.67×10^{-7}	4.25×10^{-6}	9.01×10^{-12}	1.22 (1.12-1.33)	853,681-1,014,424	GAK, TMEM175, DGKQ [‡] , SLC26A1a, IDUA, FGFR1	
5q21.1	rs526231	103,345,680	T/C	3.10×10^{-5}	9.39×10^{-5}	1.14×10^{-8}	0.87 (0.81-0.93)	102,939,698-103,416,571	PAM [§] , EIF3KP1, GIN1, PPIP5K2, CSorf30 ^{‡,§}	RA
5q33.3	rs2546890	159,332,892	G/A	1.20×10^{-6}	1.89×10^{-5}	1.06×10^{-10}	0.87 (0.82-0.93)	159,117,927-159,414,310	RNF145, UBLCP1, RNU4ATAC2P, IL12B, LOC285626 [‡]	Pso, MS, CD
6q23.3	rs6933404	137,638,098	C/T	9.47×10^{-7}	2.84×10^{-5}	1.27×10^{-10}	1.18 (1.09-1.27)	137,571,557-137,803,754	LOC102723649, LOC442263, OLIG3 [‡] , TNFAIP3 [‡]	RA, SLE, SjS, CeD, UC, MS
b. Suggestive risk loci (validation $P < 1 \times 10^{-3}$)										
Locus	SNP	Position (build 38)	A1/A2	Discovery <i>P</i>	Validation <i>P</i>	Joint <i>P</i>	OR for A1 (95% CI)	Region (build 38)	Nearby genes and functional annotation*	Autoimmune overlap
5q23.1	rs2434360	116,057,393	T/G	3.20×10^{-3}	9.94×10^{-4}	1.04×10^{-5}	1.14 (1.05-1.23)	116,032,882-116,163,459	RPS25P6, ARL14EPL, COMMD10	
16p11	rs1859308	27,386,677	T/C	7.72×10^{-5}	5.37×10^{-4}	1.63×10^{-7}	0.85 (0.77-0.93)	27,359,133-27,434,733	IL4R, IL21R	
<p>A1, tested allele; CD, Crohn disease; CeD, coeliac disease; CI, confidence interval; MS, multiple sclerosis; OR, odds ratio in validation cohorts; Pso, psoriasis; RA, rheumatoid arthritis; SjS, Sjogren syndrome; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism; UC, ulcerative colitis.</p> <p>PBC risk loci identified in the current study. SNPs were taken forward for validation based on having a discovery <i>P</i> value $< 2 \times 10^{-5}$ (or, in the case of rs526231 and rs2434360, based on acting as a proxy for a SNP with a <i>P</i> value $< 2 \times 10^{-5}$). Discovery <i>P</i> values were calculated using logistic regression of individual discovery data sets in ProbABEL followed by genomic control correction of individual discovery data sets in R and fixed-effects meta-analysis in META; validation <i>P</i> values were calculated using logistic regression of individual data sets in PLINK followed by fixed-effect meta-analysis in META; joint <i>P</i> values were calculated using fixed-effect meta-analysis of discovery and validation data sets in META; see Methods. Autoimmune overlap refers to overlap between risk loci for PBC and those of other autoimmune conditions.</p> <p>*Functional annotation.</p> <p>‡Regulatory variants: The index SNP or variants in strong linkage disequilibrium (LD, $r^2 \geq 0.8$) with the index SNP at this locus overlap regulatory elements that are related to the annotated gene (Supplementary Table 3).</p> <p>‡mQTLs: The index SNP or variants in strong LD are correlated to methylation related to the annotated gene (Supplementary Data 4).</p> <p>§eQTLs: The index SNP or variants in strong LD are correlated to expression of the annotated gene (see Supplementary Data 3).</p>										

HLA contribution had been removed (Table 2, Supplementary Data 7). This combined analysis identified pathways and immunomodulatory agents that represent promising leads for further study in models of PBC.

Discussion

The current study adds to our knowledge of the genetic architecture of PBC. Notably, our data identify CCL20 as a candidate risk gene for PBC. Chemokine ligand 20 (CCL20) and its chemokine receptor CCR6 contribute to the formation and function of mucosal lymphoid tissues and are notably, in the context of the immune-mediated lymphocytic cholangitis characteristic of PBC, involved in the localization of Th17 cells and CD8 effector T cells to cholangiocytes and the periductal area in portal tracts¹⁶. This study also reinforces the importance of IL-12 and JAK-STAT signalling in this disease.

The functional annotation of risk loci has helped to assign priority to the candidate genes at newly identified and established risk loci. Furthermore, the identification of disease-associated regulatory variants at multiple risk loci emphasizes the potential importance of gene regulation in the pathogenesis of PBC (and presumably other complex disorders). This possibility is corroborated by the finding of numerous risk loci wherein the index and/or closely related SNPs that appear to represent regulatory, mQTL and/or eQTLs variants related to the nearby gene. Via the FGWAS analysis, this study also suggests particular importance of CD20 + B cells and Th1 cells in the pathogenesis of PBC. However, both the cell types and the specific gene

variants most relevant to PBC require further investigation and in particular exploration of the tissue-specific functional effects of the disease-associated variants.

By looking for drug-gene interactions, we have identified candidate drugs targeting specific, PBC-associated pathways, creating new opportunities to re-purpose available drugs for targeted immune therapy. Despite the speculative nature of this analysis, the data provide a start point in the search for novel therapies that are urgently needed to improve outcomes for PBC patients.

Methods

Study samples and genotyping. The use of human subjects for this study was approved by the University Health Network Research Ethics Board, The Mayo Clinic Institutional Review Board, Etico Indipendente IRCCS Istituto Clinico Humanitas, UC Davis Institutional Review Board and the Oxford Research Ethics Committee.

All PBC cases included in the Canadian-US, Italian and UK discovery and validation cohorts fulfilled the American Association for the Study of Liver Diseases criteria for PBC.

The Canadian-US discovery cohort included 499 PBC cases who were self-reported whites of European descent and 390 healthy Canadian controls, all genotyped using the Illumina HumanHap370 BeadChip. Additional controls included in this cohort were 1,094 control subjects provided from the Prostate Cancer Genetics Markers Susceptibility (CGEMS), 1,142 controls from the Breast CGEMS studies and 1,748 controls from the New York Cancer Project, all of whom who were genotyped on an Illumina 550 K bead array⁴. Following all quality control (QC) procedures, the final Canadian-US discovery set included 499 PBC cases and 4,374 controls.

The PBC cases included in the Italian discovery cohort were self-reported whites of Italian descent genotyped using the Illumina Human610-Quad BeadChip. Controls in this cohort were healthy Italians genotyped using the Illumina 1M-duo

Table 2 | Results from pathway analysis in iGSEA4GWAS.

Gene set	Source	FDR (with HLA)	FDR (without HLA)
NO2-dependent IL-12 pathway in NK cells	Biocarta	6.7×10^{-4}	
JAK-STAT signalling pathway ^{*,†}	KEGG	0.001	0.013
IL-12 mediated signalling events	PID	0.001	
IL-12- and Stat4-dependent signalling in Th1 development ^{*,†}	Biocarta		<0.001
Interferon signalling	REACTOME	0.001	
PD-1 signalling	REACTOME	0.001	
Phosphorylation of CD3 and TCR- ζ chains	REACTOME	0.001	
IL-27-mediated signalling events ^{*,†}	PID	0.001	<0.001
Cytokine-cytokine receptor interaction ^{‡,§, ,¶,*,†,††,‡‡}	KEGG	0.002	0.010
IFN- γ signalling	REACTOME	0.002	
MHC class II antigen presentation	REACTOME	0.003	
Cytokine signalling in immune system	REACTOME	0.004	
Antigen processing and presentation	KEGG	0.004	
Intestinal immune network for IgA production	KEGG	0.004	
Co-stimulation by the CD28 family	REACTOME	0.005	
IL-2 mediated signalling events	PID	0.008	
TCR signalling	REACTOME	0.008	
Downstream TCR signalling	REACTOME	0.008	
Cell adhesion molecules	KEGG	0.015	
Th1, Th2 differentiation [†]	Biocarta	0.019	0.012
IL-2 receptor beta chain in T-cell activation	Biocarta	0.021	
Interferon α/β signalling	REACTOME	0.035	
IL-23-mediated signalling events	PID	0.039	

FDR, false discovery rate; IFN, interferon; IL, interleukin; PD, programmed cell death; TCR, T cell antigen receptor; NK, natural killer.

Gene sets with FDR <0.05 are listed. Results are shown with or without inclusion in the analysis of SNPs within the HLA region. The top 10 hits from our drug-positioning analysis using a combined pathway from the HLA excluded set are indicated by symbols for the associated pathways that they affect.

^{*}Tofacitinib.

[†]Glatiramer acetate.

[‡]Axitinib.

[§]Pazopanib.

^{||}Vatalanib.

[¶]Cediranib.

[#]X-82.

^{**}Telatinib.

^{††}Linifanib.

^{‡‡}Tandutinib.

array. Following QC procedures, the final Italian discovery set comprised 449 cases and 940 controls.

The PBC cases included in the UK discovery cohort were self-reported whites of British descent, genotyped using the Illumina Human-660 W Quad array. Controls in this cohort were 5,163 population controls genotyped on the Illumina 1M-Duo array as part of the Wellcome Trust Case Control Consortium 2 project. Following QC procedures, the UK discovery set comprised 1,816 cases and 5,161 controls.

The ‘Canadian’ 903 PBC cases and 834 controls included in the validation studies were self-reported whites of European descent recruited from Canada, Europe and the United States to an ongoing PBC genetics study based in Toronto. The 721 ‘US’ PBC cases and 294 controls included in the validation studies were self-reported whites of European descent enrolled in the Mayo Clinic PBC Genetic Epidemiology registry and biorepository based at the Mayo Clinic in Rochester (<https://clinicaltrials.gov/ct2/show/NCT01161953?term=pbcrank=5>). The Italian PBC cases and controls included in the validation studies were self-reported whites of Italian descent recruited to the Italian PBC Genetics study based at Istituto Humanitas in Milan. The Italian controls were obtained from Ospedale Alessandro Manzoni, Lecco, Italy and were unrelated healthy volunteers with no known non-Italian heritage. Cases and controls from the Canadian, Italian and the US cohorts were genotyped at the University Health Network/Mount Sinai Hospital Clinical Genomics Centre using a Sequenom iPLEX Gold assay. Following QC procedures, the final validation set included 903 cases and 834 controls from Canada; 300 cases and 618 controls from Italy; and 721 cases and 294 controls from the United States (Supplementary Table 1).

The ‘UK’ PBC cases included in the validation studies were self-reported whites of British descent recruited to the UK-PBC project via the UK-PBC Consortium (<http://www.uk-pbc.com/>). Cases were genotyped using Sequenom iPLEX Gold assay at the Wellcome Trust Sanger Institute Genotyping Facility (<http://www.sanger.ac.uk/>). The UK validation control data were obtained from the TwinsUK resource, an adult twin registry comprising 12,000 (predominantly female) British twins. Genotype data for 3,512 twin individuals (genotyped using the Illumina HumanHap610 array) were obtained from the Department of Twin Research and Genetic Epidemiology at King’s College London. One twin from each genotyped pair was included in the current study, amounting to 2,603 unrelated individuals. Following QC procedures, the final UK validation set comprised 1,792 PBC cases and 2,515 TwinsUK controls (Supplementary Table 1).

Quality control. We implemented a standard QC pipeline across all three discovery data sets, over-and-above QC procedures carried out in the respective primary analyses^{2,4,5}. QC checks were carried out using the software package PLINK¹⁷. Within each discovery data set we removed SNPs with a genotype call rate <95%; minor allele frequency <0.05; significant deviation from Hardy Weinberg Equilibrium in controls ($P < 10^{-5}$) or a large difference (>5%) in the proportion of missing genotypes in cases versus controls. We removed samples showing high rates of missing data (>90%); whole-genome heterozygosity > six s.d. from the mean; estimated proportion of identity by descent (IBD) sharing with another sample >0.1, or apparent gender discrepancies (based on X-chromosomal heterozygosity >0.2 for men and <0.2 for women). Principal component analysis (based on a subset of 32,000 highly informative SNPs) was carried out using the ‘smartpca’ routine of the EIGENSOFT package¹⁸ to identify population outliers for exclusion and to identify principal components that differed between cases and controls; these principal components were used as covariates in subsequent association analyses.

Genome-wide imputation. We used the SNPs and samples passing QC to carry out genome-wide imputation within each of our cohorts using the software package MaCH¹⁹ with HapMap3 CEU + TSI samples as reference data sets. Within each cohort we used approximately the same set of genotyped SNPs in cases and controls to ensure similar levels of informativity. Following imputation, we retained only those SNPs displaying minor allele frequency >0.005 and imputation quality score $R^2 > 0.5$ in all three cohorts.

Statistical analysis of discovery cohorts. Within each cohort we carried out association analysis of the genome-wide imputed data allowing for imputation uncertainty using the software package ProbABEL²⁰. We performed logistic regression of disease phenotype on allele dosage; principal components that differed between cases and controls were included as covariates to help correct for population stratification. Quantile–quantile plots of the genome-wide set of test statistics were examined and genomic control correction was carried out within each cohort by multiplying the standard error of the estimated log odds ratio for each SNP by the square root of the genomic control inflation factor λ (ref. 21). The resulting log odds ratios and adjusted standard errors from all three cohorts were

meta-analysed using the software package META to produce the final set of genome-wide discovery results²².

Validation analysis. We selected loci for validation if they achieved suggestive level of significance in the discovery analysis (minimum $P < 2 \times 10^{-5}$) and were not already known to be associated with PBC. We also selected loci for validation if they had achieved genome-wide significant association in one previous study but had never been validated in an independent cohort. We selected approximately two validation SNPs per locus; for loci displaying extended patterns of linkage disequilibrium or harbouring several putative independent association signals we attempted to select two validation SNPs within each subregion.

Within each locus chosen for validation we assigned priority to SNPs according to whether they had been genotyped in the TwinsUK cohort (which was used as a validation cohort for the UK validation cases). One SNP selected for validation (rs2297067) did not have genotype data available in TwinsUK and was therefore imputed within TwinsUK based on genotyped SNPs in the surrounding 5-Mb region using the software packages SHAPEIT²³ and IMPUTE2 (ref. 24), with 1,000 Genomes (Phase I version 3 integrated data, released on March 2012) used as a reference sample. The TwinsUK cohort was subjected to a variety of additional QC checks as described previously²⁵; the 2,515 controls used here correspond to the 2,520 controls used previously with an additional five exclusions due to discrepant gender²⁵.

Within each validation cohort we carried out case/control association analysis of those SNPs that were successfully genotyped using logistic regression in PLINK. Results from the four validation cohorts (or from the combined discovery and validation cohorts) were combined via meta-analysis in META.

Imputation to 1,000 Genomes within validated loci. Imputation within the discovery cohorts was carried out at the six validated loci using the software packages SHAPEIT²³ and IMPUTE2 (ref. 24) with the 1,000 Genomes (Phase I integrated variant set, release December and June 2013) used as a reference panel. The same genotyped SNPs that had been used to inform HapMap3 imputation for the discovery analysis were used for the 1,000 Genomes imputation within these targeted regions. Association analysis of SNPs passing post-imputation QC ('info' score > 0.5) was carried out separately within each cohort; the results were genomic control corrected by multiplying the standard error of the estimated log odds ratio for each SNP by the square root of the previously estimated genomic control inflation factor λ for each cohort, and results were combined across the cohorts via meta-analysis in META. This confirmed the findings from our original (HapMap3) imputation experiment but did not identify any substantially stronger associations or candidate causal variants than we had already found.

Functional annotation of validated loci. Left and right boundaries for each associated region were defined by finding a 0.1-cM interval either side of the most strongly associated SNP where no SNP has $P < 1 \times 10^{-5}$. We looked for overlap between PBC risk loci and confirmed risk loci for other autoimmune conditions using ImmunoBase, a web-based resource focused on the genetics and genomics of immunologically related human diseases (<http://www.immunobase.org/>). To assign priority to candidate genes and candidate variants at risk loci, we used the online PICS (Probabilistic Identification of Causal SNPs) algorithm to identify candidate variants at each risk locus with a PICS probability > 0.0275 (<http://www.broadinstitute.org/pubs/finemapping/?q=pics>)². We adopted this threshold to be consistent with Farh *et al.*⁹ in their paper describing the approach. Given an index SNP corresponding to the most associated SNP in a locus, the PICS algorithm calculates (based on the known linkage disequilibrium pattern in the region, as measured in a large ImmunoChip or 1000 Genomes reference sample) a score for each SNP in the region, representing the extent to which that SNP could, in fact, be the true causal SNP, allowing for statistical sampling variation.

We then used the Ensembl Variant Effect Predictor web tool to annotate candidate variants for their predicted functional consequences (<http://www.ensembl.org/info/docs/tools/vep/index.html>). We used Genevar to evaluate the measured effects of these variants on DNA methylation in tissue collected from 856 healthy female twins of the MuTHER resource (<http://www.sanger.ac.uk/resources/software/genevar/>)^{26,27}. We used Genevar²⁶, seeQTL (http://www.bios.unc.edu/research/genomic_software/seeQTL/)²⁸ and the University of Chicago eQTL browser (<http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/>) to identify eQTLs amongst candidate variants.

We also used the FGWAS software and its associated annotation file (containing 450 genomic annotations of various types), applied to our full set of GWMA results, to investigate the extent to which genetic variants associated with PBC were enriched within specific annotation categories¹². Testing each annotation individually, we found 75 annotations that showed enrichment ($P < 0.01$) of GWMA association signals; as many of these annotations are correlated with one another we used a stepwise selection approach followed by cross-validation to mitigate overfitting (similar to the procedure performed by Pickrell¹²) on these 75 annotations to identify a final best-fitting model that included 6 annotations. Annotation information used by FGWAS was derived from a variety of sources including Maurano *et al.*²⁹, Thurman *et al.*³⁰ and Hffman *et al.*³¹ (see Appendix of Pickrell¹² for details).

Pathway analysis. Using summary results from the GWMA (effect size, standard error and allele frequency) along with SNP linkage disequilibrium estimated from the Italian GWAS individual-level genotype data, we performed approximate conditional analysis using the software GCTA¹³. Only the independently associated signals with conditional P value and P_{GWMA} both < 0.001 were retained for further consideration. We submitted the rsIDs and P_{GWMA} of these SNPs as well as gene sets from BioCarta, KEGG, PID and Reactome curated by MSigDB (as of 26 March 2014) to the i-GSEA4GWAS web server¹⁴. This programme identified genes within 20 kb of the SNPs and represented each gene by the greatest $-\log P_{\text{GWMA}}$ of the SNP(s) mapped to it. Gene sets were then assessed for enrichment with significant genes while SNP label permutations were conducted to correct for bias from variations in gene size and gene set size. False discovery rate was used to correct for multiple testing based on the distributions of enrichment scores generated by permutation.

Drug-pathway analysis. To identify drugs that affected the pathways associated with PBC (when the HLA locus was excluded), we first identified the genes participating in each pathway. We then downloaded drug-gene associations from the Drug Gene Interaction database¹⁵ and scored each drug by the proportion of each its targets that were in each pathway, which we termed as the drug's pathway specificity. As a secondary scoring metric, we evaluated the proportion of each pathway affected by the drug using the Jaccard index on the respective sets of pathway genes and targeted genes. To identify promising drug candidates, we ranked drugs first by our primary specificity metric and then by the secondary Jaccard index.

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This study was initially conceived and designed by H.J.C., G.F.M., C.A.A., M.F.S., R.N.S., C.I.A. and K.A.S.; the collection and processing of samples for the study were supervised and coordinated by G.F.M., G.M.H., D.P., A.L., D.C., M.E.G., P.I., K.N.L., M.F.S., R.N.S. and K.A.S.; lab work was supervised by G.X. and the statistical analyses of the data were performed by H.J.C., G.F.M., G.M.H., C.S.G., C.I.A. and K.A.S.; the paper was written primarily by H.J.C., G.F.M., G.M.H., C.S.G., C.I.A. and K.A.S. and critically reviewed and revised by all of the above authors.

Additional information

Supplementary Information accompanies this paper at <http://www.nature.com/naturecommunications>

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University Hospital, Lewsey Road, Luton LU4 0DZ, UK. ¹⁸⁰Maidstone and Tunbridge Wells NHS Trust, Maidstone Hospital, Hermitage Lane, Maidstone ME16 9QQ, UK. ¹⁸¹Maidstone and Tunbridge Wells NHS Trust, Tunbridge Wells Hospital, Tonbridge Road, Pembury, Tunbridge Wells TN2 4QJ, UK. ¹⁸²Medway NHS Foundation Trust, Medway Maritime Hospital, Windmill Road, Gillingham ME7 5NY, UK. ¹⁸³Mid Cheshire Hospitals NHS Foundation Trust, Leighton Hospital, Middlewich Road, CW1 4QJ, UK. ¹⁸⁴Mid Essex Hospital Services NHS Trust, Broomfield Hospital, Court Road, Chelmsford CM1 7ET, UK. ¹⁸⁵Mid Essex Hospital Services NHS Trust, St Peters Hospital, Spital Road, Maldon CM9 6EG, UK. ¹⁸⁶Mid Yorkshire Hospitals NHS Trust, Dewsbury and District Hospital, Halifax Road, Dewsbury WF13 4HS, UK. ¹⁸⁷Milton Keynes Hospital NHS Foundation Trust, Milton Keynes Hospital, Standing Way, Milton Keynes MK6 5LD, UK. ¹⁸⁸Newcastle upon Tyne Hospitals NHS Foundation Trust, Freeman Hospital, Freeman Road, High Heaton, Newcastle upon Tyne NE7 7DN, UK. ¹⁸⁹NHS Ayrshire & Arran, University Hospital Crosshouse, Kilmarnock Road, Kilmarnock KA2 0BE, UK. ¹⁹⁰NHS Borders, Borders General Hospital, Melrose TD6 9BS, UK. ¹⁹¹NHS Dumfries & Galloway, Dumfries and Galloway Royal Infirmary, Bankend Road, Dumfries DG1 4AP, UK. ¹⁹²NHS Fife, Queen Margaret Hospital, Whitefield Road, Dunfermline KY12 0SU, UK. ¹⁹³NHS Fife, Victoria Hospital, Hayfield Road, Kirkcaldy KY2 5AH, UK. ¹⁹⁴NHS Forth Valley, Forth Valley Royal Hospital, Stirling Road, Larbert FK5 4WR, UK. ¹⁹⁵NHS Forth Valley, Stirling Community Hospital, Livlands, Stirling FK8 2AU, UK. ¹⁹⁶NHS Grampian, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB25 2ZN, UK. ¹⁹⁷NHS Grampian, Dr Gray's Hospital, Elgin IV30 1SN, UK. ¹⁹⁸NHS Grampian, Woolmanhill Hospital, Skene Street, Aberdeen AB25 1LD, UK. ¹⁹⁹NHS Greater Glasgow and Clyde, Gartnavel General Hospital, 1053 Great Western Road, Glasgow G12 0YN, UK. ²⁰⁰NHS Greater Glasgow and Clyde, Glasgow Royal Infirmary, 84 Castle Street, Glasgow G4 0SF, UK. ²⁰¹NHS Greater Glasgow and Clyde, Inverclyde Royal Hospital, Larkfield Road, Greenock PA16 0XN, UK. ²⁰²NHS Greater Glasgow and Clyde, Royal Alexandra Hospital, Corsebar Road, Paisley PA2 9PN, UK. ²⁰³NHS Greater Glasgow and Clyde, Southern General Hospital, 1345 Govan Road, Glasgow G51 4TF, UK. ²⁰⁴NHS Greater Glasgow and Clyde, Victoria Infirmary, Langside Road, Glasgow G42 9TY, UK. ²⁰⁵NHS Highland, Caithness General Hospital, Bankhead Road, Wick KW1 5NS, UK. ²⁰⁶NHS Highland, Raigmore Hospital, Old Perth Road, Inverness IV2 3UJ, UK. ²⁰⁷NHS Lanarkshire, Hairmyres Hospital, Eaglesham Road, East Kilbride G75 8RG, UK. ²⁰⁸NHS Lanarkshire, Monklands Hospital, Monkscourt Avenue, Airdrie ML6 0JS, UK. ²⁰⁹NHS Lanarkshire, Wishaw General Hospital, 50 Netherton Street, Wishaw ML2 0DP, UK. ²¹⁰NHS Lothian, Royal Infirmary of Edinburgh, 51 Little France Crescent, Old Dalkeith Road, Edinburgh EH16 4SA, UK. ²¹¹NHS Lothian, St John's Hospital, Howden Road West, Howden, Livingston EH54 6PP, UK. ²¹²NHS Lothian, Western General Hospital, Crewe Road South, Edinburgh EH4 2XU, UK. ²¹³NHS Tayside, Perth Royal Infirmary, Taymount Terrace, Perth PH1 1NX, UK. ²¹⁴NHS Tayside, Ninewells Hospital, Dundee DD1 9SY, UK. ²¹⁵Norfolk and Norwich University Hospitals NHS Foundation Trust, Norfolk and Norwich University Hospital, Colney Lane, Norwich NR4 7UY, UK. ²¹⁶North Bristol NHS Trust, Frenchay Hospital, Frenchay Park Road, Bristol BS16 1LE, UK. ²¹⁷North Cumbria University Hospitals NHS Foundation Trust, Cumberland Infirmary, Newtown Road, Carlisle CA2 7HY, UK. ²¹⁸North Cumbria University Hospitals NHS Foundation Trust, West Cumberland Hospital, Hensingham, Whitehaven CA28 8JG, UK. ²¹⁹North Tees and Hartlepool NHS Foundation Trust, University Hospital of Hartlepool, Holdforth Road, Hartlepool TS24 9AH, UK. ²²⁰North Tees and Hartlepool NHS Foundation Trust, University Hospital of North Tees, Hardwick, Stockton on Tees TS19 8PE, UK. ²²¹Northampton General Hospital NHS Trust, Northampton General Hospital, Cliftonville, Northampton NN1 5BD, UK. ²²²North Devon Healthcare NHS Trust, North Devon District Hospital, Raleigh Park, Barnstaple EX31 4JB, UK. ²²³Northern Health and Social Care Trust, Whiteabbey Hospital, Doagh Road, Newtownabbey BT37 9RH, UK. ²²⁴Northern Lincolnshire and Goole NHS Foundation Trust, Diana, Princess of Wales Hospital, Scartho Road, Grimsby DN33 2BA, UK. ²²⁵Northern Lincolnshire and Goole NHS Foundation Trust, Goole and District Hospital, Woodland Avenue, Goole DN14 6RX, UK. ²²⁶Northern Lincolnshire and Goole NHS Foundation Trust, Scunthorpe General Hospital, Cliff Gardens, Scunthorpe DN15 7BH, UK. ²²⁷Northumbria Healthcare NHS Foundation Trust, Hexham General Hospital, Corbridge Road, Hexham NE46 1QJ, UK. ²²⁸Northumbria Healthcare NHS Foundation Trust, North Tyneside Hospital, Rake Lane, North Shields NE29 8NH, UK. ²²⁹Nottingham University Hospitals NHS Trust, Nottingham City Hospital, Hucknall Road, Nottingham NG5 1PB, UK. ²³⁰Nottingham University Hospitals NHS Trust, Queen's Medical Centre, Derby Road, Nottingham NG7 2UH, UK. ²³¹Oxford University Hospitals NHS Trust, John Radcliffe Hospital, Headley Way, Headington,

Oxford OX3 9DU, UK. ²³²Pennine Acute Hospitals NHS Trust, Fairfield General Hospital, Rochdale Old Road, Bury BL9 7TD, UK. ²³³Pennine Acute Hospitals NHS Trust, North Manchester General Hospital, Delaunays Road, Crumpsall M8 5RB, UK. ²³⁴Pennine Acute Hospitals NHS Trust, Rochdale Infirmary, Whitehall Street, Rochdale OL12 0NB, UK. ²³⁵Pennine Acute Hospitals NHS Trust, The Royal Oldham Hospital, Rochdale Road, Oldham OL1 2JH, UK. ²³⁶Peterborough and Stamford Hospitals NHS Foundation Trust, Peterborough City Hospital, Edith Cavell Campus, Bretton Gate, Peterborough PE3 9GZ, UK. ²³⁷Peterborough and Stamford Hospitals NHS Foundation Trust, Stamford & Rutland Hospital, Ryhall Road, Stamford PE9 1UA, UK. ²³⁸Plymouth Hospitals NHS Trust, Derriford Hospital, Derriford Road, Plymouth PL6 8DH, UK. ²³⁹Poole Hospital NHS Foundation Trust, Poole Hospital, Longfleet Road, Poole BH15 2JB, UK. ²⁴⁰Portsmouth Hospitals NHS Trust, Queen Alexandra Hospital, Cosham, Portsmouth PO6 3LY, UK. ²⁴¹Princess Alexandra Hospital NHS Trust, St Margaret's Hospital, The Plain, Epping CM16 6TN, UK. ²⁴²Princess Alexandra Hospital NHS Trust, The Princess Alexandra Hospital, Hamstel Road, Harlow CM20 1QX, UK. ²⁴³Queen Elizabeth Hospital King's Lynn NHS Foundation Trust, The Queen Elizabeth Hospital King's Lynn, Gayton Road, King's Lynn PE30 4ET, UK. ²⁴⁴Rotherham NHS Foundation Trust, Rotherham Hospital, Moorgate Road, Rotherham S60 2UD, UK. ²⁴⁵Royal Berkshire NHS Foundation Trust, Royal Berkshire Hospital, Craven Road, Reading RG1 5AN, UK. ²⁴⁶Royal Bournemouth and Christchurch Hospitals NHS Foundation Trust, Royal Bournemouth Hospital, Castle Lane East, Bournemouth BH7 7DW, UK. ²⁴⁷Royal Cornwall Hospitals NHS Trust, Royal Cornwall Hospital, Treliste, Truro TR1 3LJ, UK. ²⁴⁸Royal Devon and Exeter NHS Foundation Trust, Royal Devon and Exeter Hospital, Barrack Road, Exeter EX2 5DW, UK. ²⁴⁹Royal Free London NHS Foundation Trust, The Royal Free Hospital, Pond Street, London NW3 2QG, UK. ²⁵⁰Royal Free London NHS Foundation Trust, Barnet Hospital, Wellhouse Lane, Barnet EN5 3DJ, UK. ²⁵¹Royal Free London NHS Foundation Trust, Chase Farm Hospital, The Ridgeway, Enfield EN2 8JL, UK. ²⁵²Royal Liverpool and Broadgreen University Hospitals NHS Trust, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP, UK. ²⁵³Royal United Hospitals Bath NHS Foundation Trust, Royal United Bath Hospital, Combe Park, Bath BA1 3NG, UK. ²⁵⁴Royal Wolverhampton Hospitals NHS Trust, New Cross Hospital, Wolverhampton Road, Wolverhampton WV10 0QP, UK. ²⁵⁵Royal Wolverhampton Hospitals NHS Trust, Cannock Chase Hospital, Brunswick Road, Cannock WS11 5XY, UK. ²⁵⁶University Hospitals of North Midlands NHS Trust, County Hospital, Weston Road, Stafford ST16 3SA, UK. ²⁵⁷Salisbury NHS Foundation Trust, Salisbury District Hospital, Salisbury SP2 8BJ, UK. ²⁵⁸Sandwell and West Birmingham Hospitals NHS Trust, Sandwell General Hospital, Lyndon, West Bromwich B71 4HJ, UK. ²⁵⁹Sheffield Teaching Hospitals NHS Foundation Trust, Northern General Hospital, Herries Road, Sheffield S5 7AU, UK. ²⁶⁰Sheffield Teaching Hospitals NHS Foundation Trust, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF, UK. ²⁶¹Sherwood Forest Hospitals NHS Foundation Trust, King's Mill Hospital, Mansfield Road, Sutton in Ashfield NG17 4JL, UK. ²⁶²Sherwood Forest Hospitals NHS Foundation Trust, Newark Hospital, Boundary Road, Newark NG24 4DE, UK. ²⁶³Shrewsbury and Telford Hospital NHS Trust, Princess Royal Hospital, Apley Castle, Telford TF1 6TF, UK. ²⁶⁴Shrewsbury and Telford Hospital NHS Trust, Royal Shrewsbury Hospital, Mytton Oak Road, Shrewsbury SY3 8XQ, UK. ²⁶⁵South Devon Healthcare NHS Foundation Trust, Torbay Hospital, Lowes Bridge, Torquay TQ2 7AA, UK. ²⁶⁶South Eastern Health and Social Care Trust, Lagan Valley Hospital, 39 Hillsborough Road, Lisburn BT28 1JP, UK. ²⁶⁷South Eastern Health and Social Care Trust, Ulster Hospital, Upper Newtownards Road, Dundonald, Belfast BT16 1RH, UK. ²⁶⁸South Tees Hospitals NHS Foundation Trust, The James Cook University Hospital, Marton Road, Middlesbrough TS4 3BW, UK. ²⁶⁹South Tees Hospitals NHS Foundation Trust, Friarage Hospital, Northallerton DL6 1JG, UK. ²⁷⁰South Tyneside NHS Foundation Trust, South Tyneside District Hospital, Harton Lane, South Shields NE34 0PL, UK. ²⁷¹South Warwickshire NHS Foundation Trust, Warwick Hospital, Lakin Road, Warwick CV34 5BW, UK. ²⁷²Southend University Hospital NHS Foundation Trust, Southend Hospital, Prittlewell Chase, Westcliff-on-Sea SS0 0RY, UK. ²⁷³Southport & Ormskirk Hospital NHS Trust, Ormskirk District General Hospital, Wigan Road, Ormskirk L39 2AZ, UK. ²⁷⁴Southport & Ormskirk Hospital NHS Trust, Southport and Formby District General Hospital, Town Lane, Kew, Southport PR8 6PN, UK. ²⁷⁵St George's University Hospitals NHS Foundation Trust, St George's Hospital, Blackshaw Road, Tooting, London SW17 0QT, UK. ²⁷⁶St Helens and Knowsley Teaching Hospitals NHS Trust, St Helens Hospital, Marshalls Cross Road, St Helens WA9 3DA, UK. ²⁷⁷St Helens and Knowsley Teaching Hospitals NHS Trust, Whiston Hospital, Warrington Road, Prescot L35 5DR, UK. ²⁷⁸Stockport NHS Foundation Trust, Stepping Hill Hospital, Poplar Grove, Hazel Grove, Stockport SK2 7JE, UK. ²⁷⁹Surrey and Sussex Healthcare NHS Trust, East Surrey Hospital, Canada Avenue, Redhill RH1 5RH, UK. ²⁸⁰Tameside Hospital NHS Foundation Trust, Tameside General Hospital, Fountain Street, Ashton-under-Lyne OL6 9RW, UK. ²⁸¹United Lincolnshire Hospitals NHS Trust, Lincoln County Hospital, Greetwell Road, Lincoln LN2 5QY, UK. ²⁸²United Lincolnshire Hospitals NHS Trust, Grantham and District Hospital, ¹⁰¹ Manthorpe Road, Grantham NG31 8DG, UK. ²⁸³United Lincolnshire Hospitals NHS Trust, Pilgrim Hospital Boston, Sibsey Road, Boston PE21 9QS, UK. ²⁸⁴University College London Hospitals NHS Foundation Trust, University College Hospital, 235 Euston Road, London NW1 2BU, UK. ²⁸⁵University Hospital of South Manchester NHS Foundation Trust, Wythenshawe Hospital, Southmoor Road, Wythenshawe, Manchester M23 9LT, UK. ²⁸⁶University Hospital Southampton NHS Foundation Trust, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK. ²⁸⁷University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital, Mindelsohn Way, Edgbaston, Birmingham B15 2GW, UK. ²⁸⁸University Hospitals Bristol NHS Foundation Trust, Bristol Royal Infirmary, Upper Maudlin Street, Bristol BS2 8HW, UK. ²⁸⁹University Hospitals Coventry and Warwickshire NHS Trust, University Hospital, Clifford Bridge Road, Coventry CV2 2DX, UK. ²⁹⁰University Hospitals of Leicester NHS Trust, Glenfield Hospital, Groby Road, Leicester LE3 9QP, UK. ²⁹¹University Hospitals of Leicester NHS Trust, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW, UK. ²⁹²University Hospitals of Leicester NHS Trust, Leicester Royal Infirmary, Infirmary Square, Leicester LE1 5WW, UK. ²⁹³University Hospitals of Morecambe Bay NHS Foundation Trust, Royal Lancaster Infirmary, Ashton Road, Lancaster LA1 4RP, UK. ²⁹⁴University Hospitals of North Midlands NHS Trust, Royal Stoke University Hospital, Newcastle Road, Stoke-on-Trent ST4 6QG, UK. ²⁹⁵Walsall Healthcare NHS Trust, Walsall Manor Hospital, Moat Road, Walsall WS2 9PS, UK. ²⁹⁶Warrington and Halton Hospitals NHS Foundation Trust, Warrington Hospital, Lovely Lane, Warrington WA5 1QG, UK. ²⁹⁷West Hertfordshire Hospitals NHS Trust, Hemel Hempstead General Hospital, Hillfield Road, Hemel Hempstead HP2 4AD, UK. ²⁹⁸West Hertfordshire Hospitals NHS Trust, St Albans City Hospital, Waverley Road, St Albans AL3 5PN, UK. ²⁹⁹West Hertfordshire Hospitals NHS Trust, Watford General Hospital, Vicarage Road, Watford WD18 0HB, UK. ³⁰⁰West Middlesex University NHS Trust, West Middlesex University Hospital, Twickenham Road, Isleworth TW7 6AF. ³⁰¹West Suffolk NHS Foundation Trust, Walnut Tree Hospital, Walnut Tree Lane, Sudbury CO10 1BE, UK. ³⁰²West Suffolk NHS Foundation Trust, West Suffolk Hospital, Hardwick Lane, Bury St Edmunds IP33 2QZ, UK. ³⁰³Western Sussex Hospitals NHS Foundation Trust, Worthing Hospital, Lyndhurst Road, Worthing BN11 2DH, UK. ³⁰⁴Western Sussex Hospitals NHS Foundation Trust, St Richard's Hospital, Spitalfield Lane, Chichester PO19 6SE, UK. ³⁰⁵Weston Area Health NHS Trust, Weston General Hospital, Grange Road, Uphill, Weston super Mare BS23 4TQ, UK. ³⁰⁶Whittington Hospital NHS Trust, The Whittington Hospital, Magdala Avenue, London N19 5NF, UK. ³⁰⁷Wirral University Teaching Hospital NHS Foundation Trust, Arrowe Park Hospital, Upton CH49 5PE, UK. ³⁰⁸Wirral University Teaching Hospital NHS Foundation Trust, Victoria Central Hospital, Mill Lane, Wallasey CH44 5UF, UK. ³⁰⁹Worcestershire Acute Hospitals NHS Trust, Alexandra Hospital, Woodrow Drive, Redditch B98 7UB, UK. ³¹⁰Worcestershire Acute Hospitals NHS Trust, Kidderminster Hospital and Treatment Centre, Bewdley Road, Kidderminster DY11 6RJ, UK. ³¹¹Worcestershire Acute Hospitals NHS Trust, Worcestershire Royal Hospital, Charles Hastings Way, Worcester WR5 1DD, UK. ³¹²Wrightington, Wigan And Leigh NHS Trust, Royal Albert Edward Infirmary, Wigan Lane, Wigan WN1 2NN, UK. ³¹³Wye Valley NHS Trust, The County Hospital, Stonebow Road, Hereford HR1 2BN, UK. ³¹⁴Yeovil District Hospital NHS Foundation Trust, Yeovil District Hospital, Higher Kingston, Yeovil BA21 4AT, UK. ³¹⁵York Teaching Hospital NHS Foundation Trust, Bridlington Hospital, Bessingby Road, Bridlington YO16 4QP, UK. ³¹⁶York Teaching Hospital NHS Foundation Trust, Scarborough Hospital, Woodlands Drive, Scarborough YO12 6QL, UK. ³¹⁷York Teaching Hospital NHS Foundation Trust, The York Hospital, Wigginton Road, York YO31 8HE, UK. ³¹⁸Great Western Hospitals NHS Foundation Trust, Marlborough Road, Swindon, Wiltshire SN3 6BB, UK.