厚生労働科学研究費補助金（創薬基盤推進研究事業）

分担研究報告書

血清ビタミンD値関連遺伝子多型と股関節骨折リスクとの関連に関する研究

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研究要旨：関節リウマチ（RA）患者は骨粗鬆症に離間する頻度が高く、骨折のハイリスク群に位置づけられている。特に股関節骨折は生命予後にも影響を及ぼすことが明らかとなっており、その予防は急務となっている。ビタミンDは骨粗鬆症治療薬の一つだが、血清ビタミンD値と関連する遺伝子多型の存在も報告されている。まず欧米人集団でのGWASによって特定された血清ビタミンD値関連遺伝子多型のバリデーションを日本人RA患者集団で行い、得られた遺伝子多型について10年間の前向き調査によって確認された骨折歴データを用いて、Coxハザードモデルによって骨折との関連を検討した。その結果、ビタミンDの輸送に関わるGC上のSNPが血清ビタミンD値に関連するとともに、骨折リスクとも関連することが明らかとなった。

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A. 研究目的

関節リウマチ（RA）罹患は骨粗鬆症の独立した危険因子であり、さらに骨粗鬆症の別の独立した危険因子であるコルチコステロイドを服用することも多いため、骨折罹患の危険性が健常人と比べ有意に高いことが知られている。骨折の中でも特に股関節の骨折は罹患すると日常生活に大きな影響を与え、さらに生命予後も悪化させることが知られている。ビタミンDは骨代謝に重要な役割を果たしており、欠乏すると骨粗鬆症につながるため、骨粗鬆症治療薬の一つにもなっている。これまで欧米の健常集団を用いたゲノムワイド関連解析（GWAS）によって、血清ビタミンD値と関連する遺伝子多型がいくつか報告されている。今回の研究の目的は大規模な日本人RA患者集団を用いてこの関連をバリデーションし、得られた血清ビタミンD値関連遺伝子多型が股関節骨折のリスクとなるかを検討することである。

B. 研究方法

本研究は東京女子医科大学で2000年より実施しているIORRAコホート研究の一貫として実施した。対象はIORRAコホート研究に参加している患者のうちDNA収集に同意した1957名である。このうち899名については2011年4月に血中総ビタミンD量を反映する指標である25(OH)Dというビタミンの主要代謝物の血中濃度を測定している。股関節骨折はIORRAデータベースに記録されている自己申告骨折のうちカルテ記載やレントゲンで確認できたものを記録として用いた。観察期間は最長10年であり、最初に生じた骨折で観察打ち切りとした。最終的に39例39骨折が含まれている。

既報のGWASの結果から、以下の5つの

SNP を選択した：rs2282679 (GC; group-specific component)、rs3829251、rs12785878、rs1790349 (DHCR7/NADSYN1; 7-dehydro- cholesterol reductase/ nicotinamide-adenine dinucleotide synthetase 1)、 rs10741657 (CYP2R1; cyto- chrome P450, family 2, subfamily R, polypeptide 1)。遺伝子多型同定にはTaqMan 法を用いた。

まず、血清 25(OH)D 値との各 SNP との関連について、IORRA コホートで既知の血清 25(OH)D 値関連因子(性、年齢、J-HAQ 身体機能評価指数、血清タンパク量、血清コレステロール値、血清 ALP 値、NSAIDs 使用の有無) で調整した重回帰分析で評価した。

次いで血清 25(OH)D 値と関連をバリデートできた SNP について、Cox ハザードモデルを用いて、既知の骨折リスク因子 (J-HAQ 身体機能評価指数、年齢、人工膝関節の既往、BMI) で調整した上で評価を行った。

(倫理面への配慮)

本研究で想定されている研究内容に関しては、「ヒトゲノム・遺伝子解析研究に関する倫理指針」、「疫学研究に関する倫理指針」、「臨床研究に関する倫理指針」など関連する指針などに基づいて妥当性を適切に判断している。また、東京女子医科大学遺伝子解析研究に関する倫理審査委員会において承認を得た上で、インフォームドコンセントのもとに書面による同意を得て実施している。

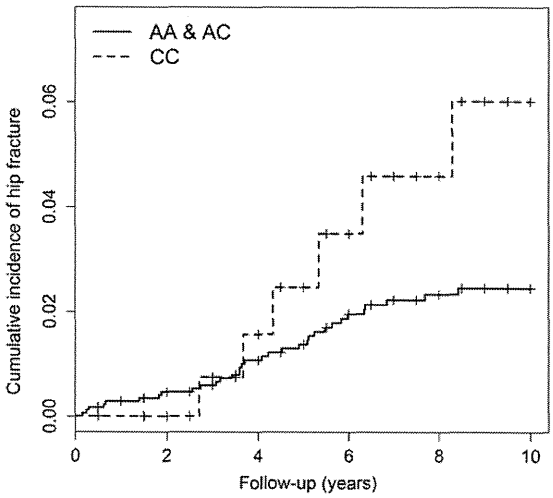
C. 研究結果

血清 25(OH)D 値と有意な関連を示した SNP は rs2282679 (GC; group-specific component)のみだった ($P = 8.1 \times 10^{-5}$、表 1)。ジェノタイプ毎の血清 25(OH)D 値それぞれ 16.1 (AA)、15.2(AC)、14.7(CC) であった。さらに Cox ハザードモデルによる解析により、rs2282679 は股関節骨折とも関連することが明らかとなった (ハザード比 2.52、図 2)。

表 1. 各 SNP 毎の多重回帰分析による血清 25(OH)D 値との関連

	SNP	β	P
GC	rs2282679	-0.13	8.1×10^{-5}
DHCR7	rs3829251	-0.0031	0.92
	rs12785878	0.0057	0.86
	rs1790349	-0.016	0.63
CYP2R1	rs10741657	0.035	0.30

図 1. rs2282679 (GC)の遺伝子型に基づく股関節骨折リスク



D. 考察

これまでに股関節骨折との関連を示す遺伝子多型の報告はいくつかあるが、rs2282679 と股関節骨折リスクとの関連の報告はない。これまで rs2282679 は血清 25(OH)D 値と有意な関連を示すことが報告されており、今回の検討でも日本人 RA 患者集団でその関連が確認できたが、今回の検討で股関節骨折のリスクとなることをはじめて示した。CC のジェノタイプを持つと継続的に血清ビタミン D 値が低値となることで、骨代謝に影響を与え、股関節骨折という結果につながると考えられる。

E. 結論

今後、rs2282679 の遺伝子型を調べる

ことでビタミンD製剤を必要とする患者群の同定が容易となり、股関節骨折のリスクを低減させ得る可能性があることを示した。

F. 健康危険情報

特になし。

G. 研究発表

1. 論文発表

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2. 学会発表

H. 知的財産権の出願・登録状況（予定も含む）

1. 特許取得

本研究と関連するものはなし。

2. 実用新案登録

本研究と関連するものはなし。

3. その他

本研究と関連するものはなし。

厚生労働科学研究費補助金（創薬基盤推進研究事業）

分担研究報告書

抗CCP抗体陰性関節リウマチの原因遺伝子検索に関する研究

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研究要旨：抗CCP抗体(ACPA)陰性関節リウマチ（RA）はRAの約20%を占め、関連するHLAアレルがACPA陽性RAと異なり、関節破壊も比較的軽症であることから、異なるRAサブセットであると考えられている。ACPA陰性RAの全ゲノム関連解析（GWAS）を多施設共同研究にて行い、いくつかの関連遺伝子が候補に挙がった。本年度はACPA陰性RAをさらにリウマトイド因子(RF)陽性と陰性のサブグループに分けて、ACPA陽性RAと関連遺伝子がどの程度共通しているかを中心に検討した。その結果、ACPA陽性RAの関連遺伝子とACPA陰性RAの関連遺伝子は相当数が共通しており、特にRF陽性サブグループとより共通していることが判明した。

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研究協力者：寺尾　知可史
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A. 研究目的

ACPA 陰性 RA は RF 陽性と RF 陰性のサブグループに分類され、HLA アレルの usage が異なることを過去に報告した。今回我々は ACPA 陽性 RA と ACPA 陰性 RA サブグループがどの程度非 HLA 感受性遺伝子を共有しているかを検討した。

B. 研究方法

本研究班班員および、全国の協力病院から ACPA 陰性 RA の DNA 検体を収集し、RF 情報を収集した。一度でも RF が基準値を上回ったことがある患者は RF 陽性と判定、一度も RF が陽性となったことのない患者を RF 陰性と判定した。

抗 CCP 抗体陰性 RA 670 例とコントロール 16891 例を用いた GWAS データから、過去に日本人 RA を用いた GWAS で有意水準を満たした感受性遺伝子 21 個に関するデータを抽出し、ACPA(+)RA と ACPA(-)RA の odds ratio (OR)を比較。

次に ACPA(+)RA とコントロールの GWAS データから遺伝子を p 値にて階層化しそれぞれの階層内で ACPA(-)RA の OR vs ACPA(+)RA の OR、ACPA(-)RF(+)RA の OR vs ACPA(+)RA の OR、ACPA(-)RF(-)RA の OR vs ACPA(+)RA の OR の相関係数を求めて plot した。ACPA(-)RF(+)RA の OR vs ACPA(+)RF(-)RA の OR はそれぞれのサブグループの GWAS での p 値に基づいた相関係数を plot した。

（倫理面への配慮）

本研究は遺伝子情報を扱う臨床研究であることから「ヒトゲノム・遺伝子解析研究に関する倫理指針」「臨床研究に関する倫理指針」およびヘルシンキ宣言（2004 年改訂）を遵守する。本研究は京都大学医学部医の倫理委員会および各研究機関

においても倫理委員会で協議、承認されている。

C. 研究結果

抗 CCP 抗体陰性 RA 670 例 vs. コントロール 16891 例の GWAS メタ解析を行った。過去に日本人 RA を用いた GWAS で有意水準を満たした感受性遺伝子 21 個に関するデータを抽出し、ACPA(+)RA と ACPA(−)RA の odds ratio (OR)を比較した図を図 1 に示す。

図 1．ACPA 陽性 RA と関連を示す遺伝子に関して、ACPA 陽性 RA および ACPA 陰性 RA の Odds ratio 比較

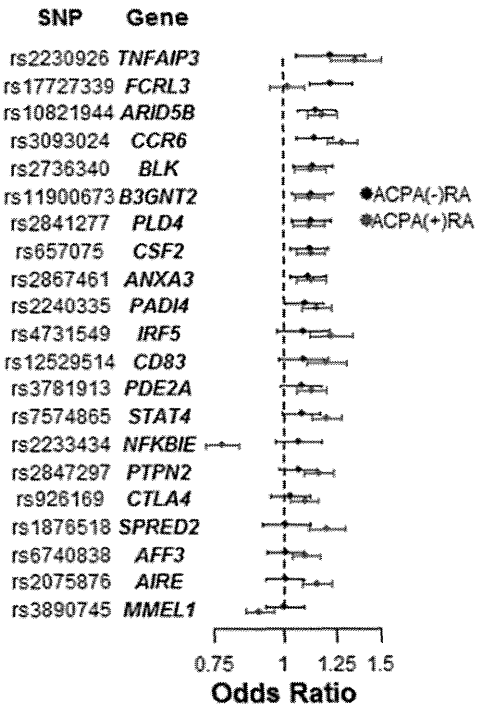


図 1 に示す通り、ACPA(−)RA では ACPA(+)RA の疾患感受性遺伝子をかなり共有していることが示された。

次に、ACPA(+)RA とコントロールの GWAS データから遺伝子を p 値にて階層化しそれぞれの階層内で ACPA(+)RA の OR vs ACPA(−)RF(+)RA の OR、ACPA(+)RA の OR vs ACPA(−)RF(−)RA の OR の相関係数を求めて plot した（図 2A、図 2B)。

図 2 ACPA(+)RA の関連遺伝子（候補）が示す OR と ACPA(−)RA サブグループでの OR の関連

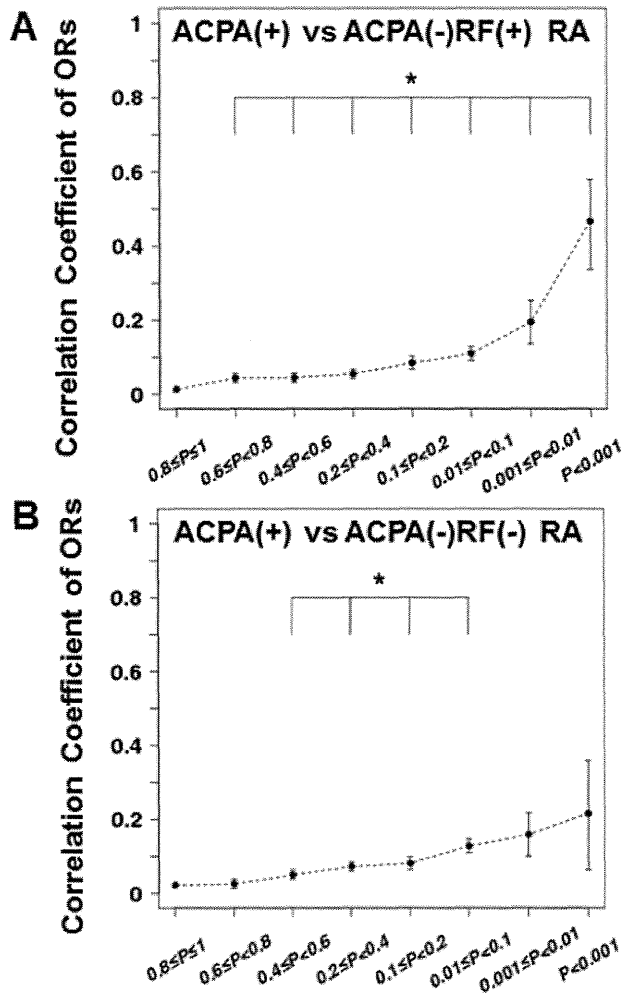


図 2 に示す通り、p 値が比較的低い（関連が強い）遺伝子が多く含まれる階層では ACPA(+)RA 遺伝子と ACPA(−)RF(+)RA および ACPA(−)RF(−)RA とも OR の相関が強く、ACPA(+)RA と関連する遺伝子は p 値の比較的低い遺伝子に関しても ACPA(−)RF(+)RA もしくは ACPA(−)RF(−)RA とも共通することが示唆された。また、その共通する程度は ACPA(−)RF(+)RA の方が ACPA(−)RF(−)RA よりも強いことも示唆された。

一方、図 3 に示すように ACPA(−)RF(+)RA と ACPA(−)RF(−)RA との比較においてはほとんど感受性遺伝子を共有していないことが示唆された。

図 3　ACPA(−)RF(+)RA と ACAP(−)RF(−)RA の感受性遺伝子（候補）の OR の関連

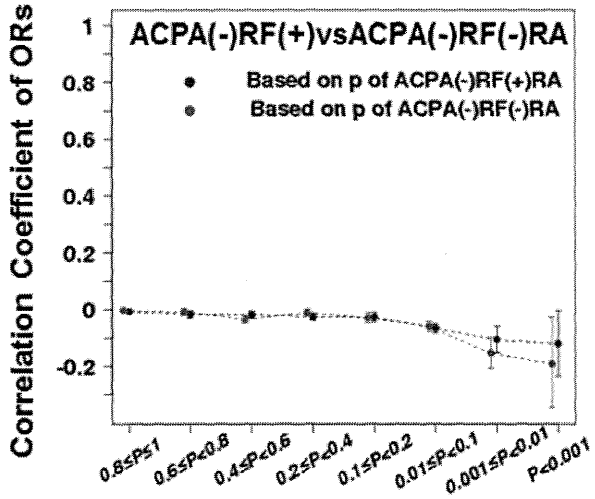
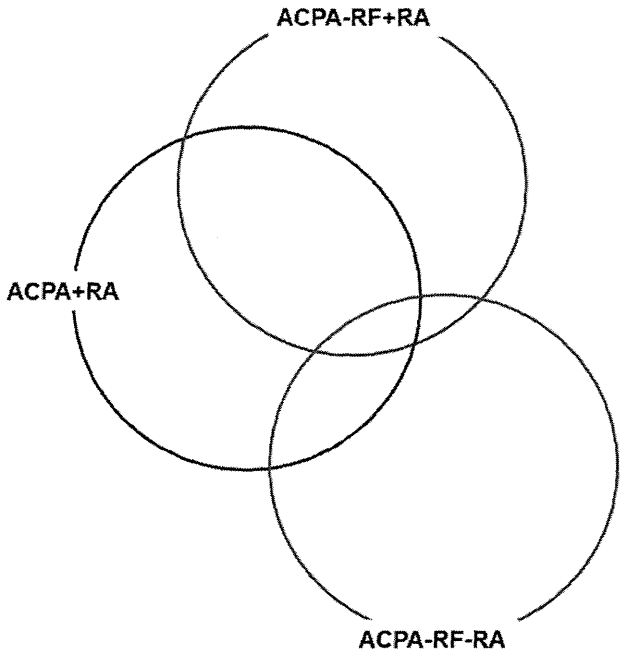


図 4　共有する感受性遺伝子の程度



D. 考察

ACPA 陰性 RA は heterogeneous な集団であり、RF 陽性と RF 陰性によって HLA の関連アレルが異なることから、遺伝的に異なる 2 つの subset である。しかしながら、そのサブセットによって GWAS を行った報告はこれまでに見られない。今回の我々の結果から、新たな感受性遺伝子が見つかったということではないが、ACPA 陽性 RA と関連する遺伝子の多くが ACPA 陰性 RA およびそのサブセットにおいても関連することが示された。このことは、ACPA 陰性 RA と ACPA 陽性 RA は HLA の関連が異なること以外は他の感受性遺伝子の多くは共通しており、これまで ACPA 陰性 RA 感受性遺伝子の報告が少ないのは単に検体数が少ないための検出力不足であることが示唆される。

図 4 に共有する感受性遺伝子の程度をイメージとして示している。

今回の結果は ACPA 陽性 RA もしくは RA 全体で検出された感受性遺伝子は、多くが ACPA 陰性 RA およびそのサブセットとも共有することが示され、今後、これらの感受性遺伝子を制御する創薬が行われた際は ACPA 陰性 RA でも効果が十分期待できることをも示唆している。

E. 結論

ACPA 陰性 RA と ACPA 陽性 RA は多くの非 HLA 感受性遺伝子を共有する。また、ACPA(−)RF(+)RA サブセットは ACPA(−)RF(−)RA と比較して、より ACPA(+)RA と遺伝的に近い関係にある。

F. 健康危険情報　特になし

G. 研究発表

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H. 知的財産権の出願・登録状況 (予定も含む)

1. 特許取得

本研究と関連するものはなし。

2. 実用新案登録

本研究と関連するものはなし。

3. その他

本研究と関連するものはなし。

厚生労働科学研究費補助金（創薬基盤推進研究事業）

分担研究報告書

疾患関連遺伝子の同定と臨床展開のための数理統計学的手法に関する研究

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研究要旨：疾患関連遺伝子に関する知見を臨床活用するにあたり、医療上の決断という視点に立った情報活用の統計学的・決断理論的位置づけを明確にし、多彩な遺伝的多様性情報の個別性と、情報の活用方法の個別性という２重の個別性を考慮した個別化医療に向けた理論的基礎が明らかになった。

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A. 研究目的

疾患感受性遺伝子や予後・亜型関連遺伝子の情報を臨床展開するにあたり、比較的低い総体危険度を有する座位から臨床的に意味のある情報を作成し、臨床現場に提供するために解決するべき課題を明らかにし、その課題解決のための方法論について考案することを目的とした。

B. 研究方法

疾患感受性遺伝子のジェノタイプ情報を活用した個別化医療の実践は、遺伝性の高いコモンディジーズ亜型（高遺伝性のある種の癌）と薬剤使い分けにおいて成功をおさめているが、それ以外の場合には困難な課題となっている。個別化医療展開に向けては、より曖昧なままの情報を臨床活用できるようにするといった、抜本的な方針展開が有用な可能性がある。その点に鑑み、統計学的検定によって支持されない情報を臨床活用するための確率・尤度的な基礎と決定理論的課題について、数理モデルを作成しシミュレーションを実施した。

（倫理面への配慮）

一般的な生殖細胞系列変異研究に活用される解析手法であることから、同研究にて用いられることを前提とした独立したアプリケーションとなるように配慮した。

C. 研究結果

統計学的検定によって支持されない情報の典型例であり、また、遺伝情報活用以外にも応用の可能性の大きい例として、２値型情報が少数標本に関して得られた場合をひな形とし、そのような情報を用いた判断戦略として、混合分布モデルとモンテカルロ推定とを基礎とする手法を考案し、そのパフォーマンス評価を実施し、戦略として成立する可能性があることを示した。

D. 考察

示した情報活用戦略を遺伝情報に基づいた個別化医療に展開するには、少なくとも２つの課題が残されていることが明らかである。１つは、単純化した決定過程での手法の構築にとどまっており、複雑な遺伝的多様性情報のモデル化に対応する必要がある。もう１つは戦略が臨床コミュニティにて受容されるかという点である。医療現場においては、0/1 型の情報による判断分岐が主流であり、確率・尤度の考え方をうまく持ち込めるかどうかは、依然として不透明である。し

かしながら、この点は、遺伝情報活用にとどまる課題ではなく、多方面からの要請となっていることに鑑み、本研究において解決するべき主たる課題ではないと考えられる。

E. 結論

疾患遺伝子情報の個別化医療展開のために、従来とは異なる情報利用手法の可能性を模索し、その道筋を明らかにすることができた。

F. 健康危険情報

該当なし。

G. 研究発表

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H. 知的財産権の出願・登録状況（予定も含む）

1. 特許取得

本研究と関連するものはなし。

2. 実用新案登録

本研究と関連するものはなし。

3. その他

本研究と関連するものはなし。

III. 研究成果の刊行に関する一覧表

雑誌

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Ⅳ．研究成果の刊行物・別刷

LETTER

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Genetics of rheumatoid arthritis contributes to biology and drug discovery

A list of authors and their affiliations appears at the end of the paper

A major challenge in human genetics is to devise a systematic strategy to integrate disease-associated variants with diverse genomic and biological data sets to provide insight into disease pathogenesis and guide drug discovery for complex traits such as rheumatoid arthritis (RA)[1]. Here we performed a genome-wide association study meta-analysis in a total of >100,000 subjects of European and Asian ancestries (29,880 RA cases and 73,758 controls), by evaluating ~10 million single-nucleotide polymorphisms. We discovered 42 novel RA risk loci at a genome-wide level of significance, bringing the total to 101 (refs 2–4). We devised an *in silico* pipeline using established bioinformatics methods based on functional annotation[5], *cis*-acting expression quantitative trait loci[6] and pathway analyses[7–9]—as well as novel methods based on genetic overlap with human primary immunodeficiency, haematological cancer somatic mutations and knockout mouse phenotypes—to identify 98 biological candidate genes at these 101 risk loci. We demonstrate that these genes are the targets of approved therapies for RA, and further suggest that drugs approved for other indications may be repurposed for the treatment of RA. Together, this comprehensive genetic study sheds light on fundamental genes, pathways and cell types that contribute to RA pathogenesis, and provides empirical evidence that the genetics of RA can provide important information for drug discovery.

We conducted a three-stage trans-ethnic meta-analysis (Extended Data Fig. 1). On the basis of the polygenic architecture of RA[10] and shared genetic risk among different ancestry[3,4], we proposed that combining a genome-wide association study (GWAS) of European and Asian ancestry would increase power to detect novel risk loci. In stage 1, we combined 22 GWAS for 19,234 cases and 61,565 controls of European and Asian ancestry[2–4]. We performed trans-ethnic, European-specific and Asian-specific GWAS meta-analysis by evaluating ~10 million single-nucleotide polymorphisms (SNPs)[11]. Characteristics of the cohorts, genotyping platforms and quality control criteria are described in Extended Data Table 1 (overall genomic control inflation factor $\lambda_{GC} < 1.075$).

Stage 1 meta-analysis identified 57 loci that satisfied a genome-wide significance threshold of $P < 5.0 \times 10^{-8}$, including 17 novel loci (Extended Data Fig. 2). We then conducted a two-step replication study (stage 2 for *in silico* and stage 3 for *de novo*) in 10,646 RA cases and 12,193 controls for the loci with $P < 5.0 \times 10^{-6}$ in stage 1. In a combined analysis of stages 1–3, we identified 42 novel loci with $P < 5.0 \times 10^{-8}$ in any of the trans-ethnic, European or Asian meta-analyses. This increases the total number of RA risk loci to 101 (Table 1 and Supplementary Table 1).

Comparison of 101 RA risk loci revealed significant correlations of risk allele frequencies (RAFs) and odds ratios (ORs) between Europeans and Asians (Extended Data Fig. 3a–c; Spearman's $\rho = 0.67$ for RAF and 0.76 for OR; $P < 1.0 \times 10^{-13}$), although five loci demonstrated population-specific associations ($P < 5.0 \times 10^{-8}$ in one population but $P > 0.05$ in the other population without overlap of the 95% confidence intervals (95% CIs) of the ORs). In the population-specific genetic risk model, the 100 RA risk loci outside of the major histocompatibility complex (MHC) region[12] explained 5.5% and 4.7% of heritability in Europeans and Asians, respectively, with 1.6% of the heritability explained by the novel loci. The trans-ethnic genetic risk model, based on the RAF from one population but the OR from the other population, could explain the majority (>80%) of the known heritability in each population (4.7% for Europeans and 3.8% for Asians). These observations support our hypothesis that the genetic risk of RA is shared, in general, among Asians and Europeans.

We assessed enrichment of 100 non-MHC RA risk loci in epigenetic chromatin marks[13] (Extended Data Fig. 3d). Of 34 cell types investigated, we observed significant enrichment of RA risk alleles with trimethylation of histone H3 at lysine 4 (H3K4me3) peaks in primary $CD4^+$ regulatory T cells (T_{reg} cells; $P < 1.0 \times 10^{-5}$). For the RA risk loci enriched with T_{reg} H3K4me3 peaks, we incorporated the epigenetic annotations along with trans-ethnic differences in patterns of linkage disequilibrium to fine-map putative causal risk alleles (Extended Data Fig. 3e, f).

We found that approximately two-thirds of RA risk loci demonstrated pleiotropy with other human phenotypes (Extended Data Fig. 4), including immune-related diseases (for example, vitiligo, primary biliary cirrhosis), inflammation-related or haematological biomarkers (for example, fibrinogen, neutrophil counts) and other complex traits (for example, cardiovascular diseases).

Each of 100 non-MHC RA risk loci contains on average ~4 genes in the region of linkage disequilibrium (in total 377 genes). To prioritize systematically the most likely biological candidate gene, we devised an *in silico* bioinformatics pipeline. In addition to the published methods that integrate data across associated loci[7,8], we evaluated several biological data sets to test for enrichment of RA risk genes, which helps to pinpoint a specific gene in each loci (Extended Data Figs 5, 6 and Supplementary Tables 2–4).

We first conducted functional annotation of RA risk SNPs. Sixteen per cent of SNPs were in linkage disequilibrium with missense SNPs ($r^2 > 0.80$; Extended Data Fig. 5a, b). The proportion of missense RA risk SNPs was higher compared with a set of genome-wide common SNPs (8.0%), and relatively much higher in the explained heritability (~26.8%). Using *cis*-acting expression quantitative trait loci (*cis*-eQTL) data obtained from peripheral blood mononuclear cells (5,311 individuals)[6] and from $CD4^+$ T cells and $CD14^+CD16^-$ monocytes (212 individuals), we found that RA risk SNPs in 44 loci showed *cis*-eQTL effects (false discovery rate (FDR) q or permutation $P < 0.05$; Extended Data Table 2).

Second, we evaluated whether genes from RA risk loci overlapped with human primary immunodeficiency (PID) genes[14], and observed significant overlap (14/194 = 7.2%, $P = 1.2 \times 10^{-4}$; Fig. 1a and Extended Data Fig. 5c). Classification categories of PID genes showed different patterns of overlap: the highest proportion of overlap was in 'immune dysregulation' (4/21 = 19.0%, $P = 0.0033$) but there was no overlap in 'innate immunity'.

Third, we evaluated overlap with cancer somatic mutation genes[15], under the hypothesis that genes with cell growth advantages may contribute to RA development. Among 444 genes with registered cancer somatic mutations[15], we observed significant overlap with genes implicated in haematological cancers (17/251 = 6.8%, $P = 1.2 \times 10^{-4}$; Fig. 1b and Extended Data Fig. 5d), but not with genes implicated in non-haematological cancers (6/221 = 2.7%, $P = 0.56$).

Table 1 | Novel rheumatoid arthritis risk loci identified by trans-ethnic GWAS meta-analysis in >100,000 subjects

SNP	Chr	Genes	A1/A2 (+)	Trans-ethnic OR (95% CI)	Trans-ethnic P	European OR (95% CI)	European P	Asian OR (95% CI)	Asian P
rs227163	1	*TNFRSF9*	C/T	1.04 (1.02–1.06)	3.9×10^{-4}	1.00 (0.97–1.03)	9.3×10^{-1}	1.11 (1.08–1.16)*	3.1×10^{-9}*
rs28411352	1	*MTF1-INPP5B*	T/C	1.11 (1.08–1.14)*	2.8×10^{-12}*	1.10 (1.07–1.14)*	5.9×10^{-9}*	1.12 (1.06–1.19)	7.8×10^{-5}
rs2105325	1	*LOC100506023*	C/A	1.12 (1.08–1.15)*	6.9×10^{-13}*	1.12 (1.08–1.15)*	3.3×10^{-11}*	1.13 (1.04–1.23)	5.2×10^{-3}
rs10175798	2	*LBH*	A/G	1.08 (1.06–1.11)*	1.1×10^{-9}*	1.09 (1.06–1.12)*	4.2×10^{-8}*	1.07 (1.02–1.13)	6.4×10^{-3}
rs6732565	2	*ACOXL*	A/G	1.07 (1.05–1.10)*	2.7×10^{-8}*	1.10 (1.07–1.14)*	9.4×10^{-9}*	1.04 (1.00–1.08)	4.0×10^{-2}
rs6715284	2	*CFLAR-CASP8*	G/C	1.15 (1.10–1.20)*	1.8×10^{-9}*	1.15 (1.10–1.20)*	2.5×10^{-9}*	-	-
rs4452313	3	*PLCL2*	T/A	1.09 (1.06–1.12)*	1.6×10^{-10}*	1.11 (1.08–1.15)*	5.2×10^{-11}*	1.04 (0.99–1.09)	9.2×10^{-2}
rs3806624	3	*EOMES*	G/A	1.08 (1.05–1.11)*	8.6×10^{-9}*	1.08 (1.05–1.12)*	2.8×10^{-8}*	1.06 (0.99–1.14)	1.0×10^{-1}
rs9826828	3	*IL20RB*	A/G	1.44 (1.28–1.61)*	8.6×10^{-10}*	1.44 (1.28–1.61)*	8.7×10^{-10}*	-	-
rs13142500	4	*CLNK*	C/T	1.10 (1.07–1.13)*	3.0×10^{-9}*	1.10 (1.06–1.15)	2.4×10^{-6}	1.10 (1.04–1.15)	2.8×10^{-4}
rs2664035	4	*TEC*	A/G	1.07 (1.04–1.10)	9.5×10^{-8}	1.08 (1.05–1.11)*	3.3×10^{-8}*	1.03 (0.97–1.08)	3.3×10^{-1}
rs9378815	6	*IRF4*	C/G	1.09 (1.06–1.12)*	1.7×10^{-10}*	1.09 (1.05–1.12)	1.4×10^{-7}	1.10 (1.04–1.15)	2.3×10^{-4}
rs2234067	6	*ETV7*	C/A	1.15 (1.10–1.20)*	1.6×10^{-9}*	1.14 (1.09–1.19)*	4.1×10^{-8}*	1.22 (1.06–1.41)	7.0×10^{-3}
rs9373594	6	*PPIL4*	T/C	1.09 (1.06–1.12)*	3.0×10^{-9}*	1.07 (1.02–1.12)	6.5×10^{-3}	1.11 (1.07–1.15)*	4.8×10^{-8}*
rs67250450	7	*JAZF1*	T/C	1.10 (1.07–1.14)*	3.7×10^{-9}*	1.11 (1.07–1.14)*	2.6×10^{-9}*	1.02 (0.84–1.23)	8.5×10^{-1}
rs4272	7	*CDK6*	G/A	1.10 (1.06–1.13)*	5.0×10^{-9}*	1.10 (1.07–1.14)*	1.2×10^{-8}*	1.06 (0.98–1.15)	1.3×10^{-1}
rs998731	8	*TPD52*	T/C	1.08 (1.05–1.11)*	1.9×10^{-8}*	1.09 (1.06–1.12)*	6.6×10^{-9}*	1.02 (0.96–1.10)	4.9×10^{-1}
rs678347	8	*GRHL2*	G/A	1.08 (1.05–1.11)*	1.6×10^{-8}*	1.10 (1.06–1.13)*	7.3×10^{-9}*	1.03 (0.98–1.10)	2.6×10^{-1}
rs1516971	8	*PVT1*	T/C	1.15 (1.10–1.20)*	1.3×10^{-10}*	1.16 (1.11–1.21)*	3.2×10^{-11}*	-	-
rs12413578	10	10p14	C/T	1.20 (1.13–1.29)*	4.8×10^{-8}*	1.20 (1.12–1.29)	7.5×10^{-8}	-	-
rs793108	10	*ZNF438*	T/C	1.08 (1.05–1.10)*	1.3×10^{-9}*	1.07 (1.04–1.10)	6.1×10^{-7}	1.09 (1.04–1.14)	4.4×10^{-4}
rs2671692	10	*WDFY4*	A/G	1.07 (1.05–1.10)*	2.8×10^{-9}*	1.06 (1.03–1.09)	2.6×10^{-5}	1.10 (1.05–1.14)	9.9×10^{-6}
rs726288	10	*SFTPD*	T/C	1.14 (1.07–1.20)	1.6×10^{-5}	0.96 (0.86–1.06)	4.1×10^{-1}	1.22 (1.14–1.31)*	8.8×10^{-9}*
rs968567	11	*FADS1-FADS2-FADS3*	C/T	1.12 (1.07–1.16)*	1.8×10^{-8}*	1.12 (1.07–1.16)*	1.8×10^{-8}*	-	-
rs4409785	11	*CEP57*	C/T	1.12 (1.09–1.16)*	1.2×10^{-11}*	1.12 (1.08–1.16)*	3.6×10^{-9}*	1.16 (1.07–1.27)	4.3×10^{-4}
chr11:107967350	11	*ATM*	A/G	1.21 (1.13–1.29)*	1.4×10^{-8}*	1.21 (1.13–1.29)*	1.1×10^{-8}*	-	-
rs73013527	11	*ETS1*	C/T	1.09 (1.06–1.12)*	1.2×10^{-10}*	1.08 (1.05–1.11)	1.0×10^{-6}	1.14 (1.08–1.21)	4.1×10^{-6}
rs773125	12	*CDK2*	A/G	1.09 (1.06–1.12)*	1.1×10^{-10}*	1.09 (1.06–1.12)*	2.1×10^{-8}*	1.10 (1.04–1.17)	1.1×10^{-3}
rs10774624	12	*SH2B3-PTPN11*	G/A	1.09 (1.06–1.13)*	6.8×10^{-9}*	1.09 (1.06–1.13)*	6.9×10^{-9}*	-	-
rs9603616	13	*COG6*	C/T	1.10 (1.07–1.13)*	1.6×10^{-12}*	1.11 (1.07–1.14)*	2.8×10^{-11}*	1.08 (1.02–1.14)	1.0×10^{-2}
rs3783782	14	*PRKCH*	A/G	1.14 (1.09–1.18)*	2.2×10^{-9}*	1.12 (0.96–1.31)	1.4×10^{-1}	1.14 (1.09–1.19)*	4.4×10^{-9}*
rs1950897	14	*RAD51B*	T/C	1.10 (1.07–1.13)*	8.2×10^{-11}*	1.09 (1.06–1.12)*	5.0×10^{-8}*	1.16 (1.08–1.25)	1.1×10^{-4}
rs4780401	16	*TXNDC11*	T/G	1.07 (1.05–1.10)*	4.1×10^{-8}*	1.09 (1.06–1.13)*	8.7×10^{-9}*	1.03 (0.98–1.08)	2.5×10^{-1}
rs72634030	17	*C1QBP*	A/C	1.12 (1.08–1.17)*	1.5×10^{-9}*	1.12 (1.06–1.19)	2.9×10^{-5}	1.12 (1.07–1.18)	9.6×10^{-6}
rs1877030	17	*MED1*	C/T	1.09 (1.06–1.12)*	1.9×10^{-8}*	1.09 (1.05–1.13)	1.3×10^{-5}	1.09 (1.04–1.14)	3.2×10^{-4}
rs2469434	18	*CD226*	C/T	1.07 (1.05–1.10)*	8.9×10^{-10}*	1.05 (1.02–1.08)	6.7×10^{-4}	1.11 (1.07–1.15)*	1.2×10^{-8}*
chr19:10771941	19	*ILF3*	C/T	1.47 (1.30–1.67)*	8.6×10^{-10}*	1.47 (1.30–1.67)*	8.8×10^{-10}*	-	-
rs73194058	21	*IFNGR2*	C/A	1.08 (1.05–1.12)	1.2×10^{-6}	1.13 (1.08–1.18)*	2.6×10^{-8}*	1.03 (0.98–1.08)	2.9×10^{-1}
rs1893592	21	*UBASH3A*	A/C	1.11 (1.08–1.14)*	7.2×10^{-12}*	1.11 (1.07–1.15)*	9.8×10^{-9}*	1.11 (1.05–1.18)	1.3×10^{-4}
rs11089637	22	*UBE2L3-YDJC*	C/T	1.08 (1.05–1.11)*	2.1×10^{-9}*	1.10 (1.06–1.15)	2.0×10^{-7}	1.06 (1.02–1.10)	8.9×10^{-4}
rs909685	22	*SYNGR1*	A/T	1.13 (1.10–1.16)*	1.4×10^{-16}*	1.11 (1.08–1.15)*	6.4×10^{-12}*	1.23 (1.14–1.33)	2.0×10^{-7}
chrX:78464616	X	*P2RY10*	A/C	1.11 (1.07–1.15)*	3.5×10^{-8}*	1.16 (0.78–1.75)	4.6×10^{-1}	1.11 (1.07–1.15)*	3.6×10^{-8}*

SNPs newly associated with $P < 5.0 \times 10^{-8}$ in the combined study of the stage 1 GWAS meta-analysis and the stages 2 and 3 replication studies of trans-ethnic (Europeans and Asians), European or Asian ancestry are indicated. SNPs, positions and alleles are based on the positive (+) strand of NCBI build 37. A1 represents an RA risk allele. Chr, chromosome; OR, odds ratio; 95% CI, 95% confidence interval. Full results of the studies are available in Supplementary Table 1. Hyphens between gene names indicate that several candidate RA risk genes were included in the region.
*Association results with $P < 5.0 \times 10^{-8}$.

Fourth, we evaluated overlap with genes implicated in knockout mouse phenotypes[16]. Among the 30 categories of phenotypes[16], we observed 3 categories significantly enriched with RA risk genes ($P < 0.05/30 = 0.0017$): 'haematopoietic system phenotype', 'immune system phenotype', and 'cellular phenotype' (Extended Data Fig. 5e).

Last, we conducted molecular pathway enrichment analysis (Fig. 1c and Extended Data Fig. 5f). We observed enrichment (FDR $q < 0.05$) for T-cell-related pathways, consistent with cell-specific epigenetic marks, as well as enrichment for B-cell and cytokine signalling pathways (for example, interleukin (IL)-10, interferon, granulocyte–macrophage colony-stimulating factor (GM-CSF)). For comparison, our previous RA GWAS meta-analysis[2] did not identify the B-cell and cytokine signalling pathways, thereby indicating that as more loci are discovered, further biological pathways are identified.

On the basis of these new findings, we adopted the following 8 criteria to prioritize each of the 377 genes from the 100 non-MHC RA risk loci (Fig. 2 and Extended Data Fig. 6a–c): (1) genes with RA risk missense variant ($n = 19$); (2) *cis*-eQTL genes ($n = 51$); (3) genes prioritized by PubMed text mining[7] ($n = 90$); (4) genes prioritized by protein–protein interaction (PPI)[8] ($n = 63$); (5) PID genes ($n = 15$); (6) haematological cancer somatic mutation genes ($n = 17$); (7) genes prioritized by associated knockout mouse phenotypes ($n = 86$); and (8) genes prioritized by molecular pathway analysis[9] ($n = 35$).

Ninety-eight genes (26.0%) had a score ≥2, which we defined as 'candidate biological RA risk genes'. Nineteen loci included multiple biological RA risk genes (for example, *IL3* and *CSF2* at chromosome 5q31), whereas no biological gene was selected from 40 loci (Supplementary Table 5).

To provide empirical evidence of the pipeline, we evaluated relationships of the gene scores to independent genomic or epigenetic information. Genes with higher biological scores were more likely to be the nearest gene to the risk SNP (18.6% for gene score <2 and 49.0% for gene score ≥2; $P = 2.1 \times 10^{-8}$), and also to be included in the region where RA risk SNPs were overlapping with H3K4me3 T_{reg} peaks (41.9% for gene score <2 and 57.1% for gene score ≥2; $P = 0.034$). Further, T_{reg} cells demonstrated the largest increase in overlapping proportions with H3K4me3 peaks for increase of biological gene scores compared with other cell types (Extended Data Fig. 6d).

Finally, we evaluated the potential role of RA genetics in drug discovery. We proposed that if human genetics is useful for drug target validation, then it should identify existing approved drugs for RA. To test this 'therapeutic hypothesis'[1], we obtained 871 drug target genes corresponding to approved, in clinical trials or experimental drugs for human diseases[17,18] (Supplementary Table 6). We evaluated whether any of the protein products from the identified biological RA risk genes, or any genes from a direct PPI network with such protein products

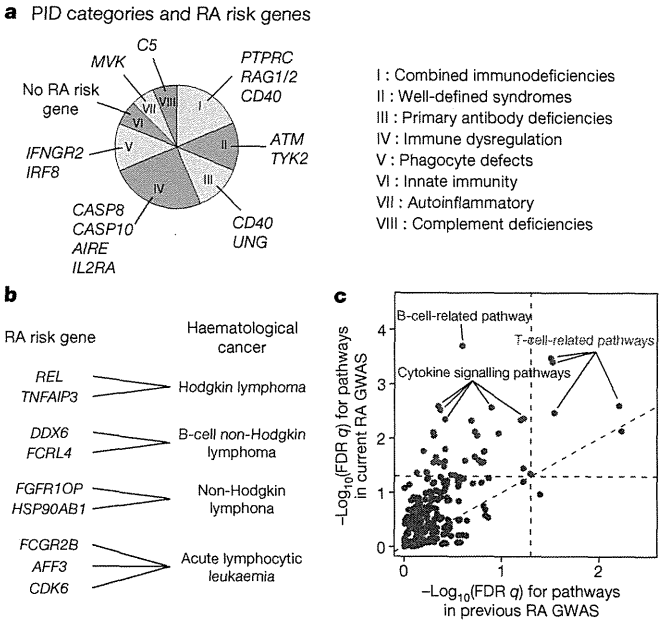

Figure 1 | Overlap of RA risk loci with PID genes, haematological cancer somatic mutations and molecular pathways. **a**, Overlap of RA risk genes with PID genes, subdivided by PID categories (I–VIII). **b**, Examples of overlap of haematological cancer somatic mutation genes with RA risk genes. **c**, Comparisons of molecular pathway analysis results between the current trans-ethnic meta-analysis (y-axis) and the previous meta-analysis for RA (x-axis)[2]. Each dot represents a molecular pathway. Dotted line represents FDR $q = 0.05$ or $y = x$.

(Fig. 3a), are the pharmacologically active targets of approved RA drugs (Extended Data Fig. 7a).

Twenty-seven drug target genes of approved RA drugs demonstrated significant overlap with 98 biological RA risk genes and 2,332 genes from the expanded PPI network (18 genes overlapped; 3.7-fold enrichment by permutation analysis, $P < 1.0 \times 10^{-5}$; Fig. 3b). For comparison, all drug target genes (regardless of disease indication) overlapped with 247 genes, which is 1.7-fold more enrichment than expected by chance, but less than 2.2-fold enrichment compared with overlap of the target genes of RA drugs ($P = 0.0035$). Examples of approved RA therapies identified by this analysis include tocilizumab[19,20] (anti-IL6R), tofacitinib[21] (JAK3 inhibitor) and abatacept[21] (CTLA4–immunoglobulin; Fig. 3c and Extended Data Fig. 8).

We also assessed how approved drugs for other diseases might be connected to biological RA risk genes. We highlight *CDK6* and *CDK4*, targets of three approved drugs for different types of cancer[22] (Fig. 3d).

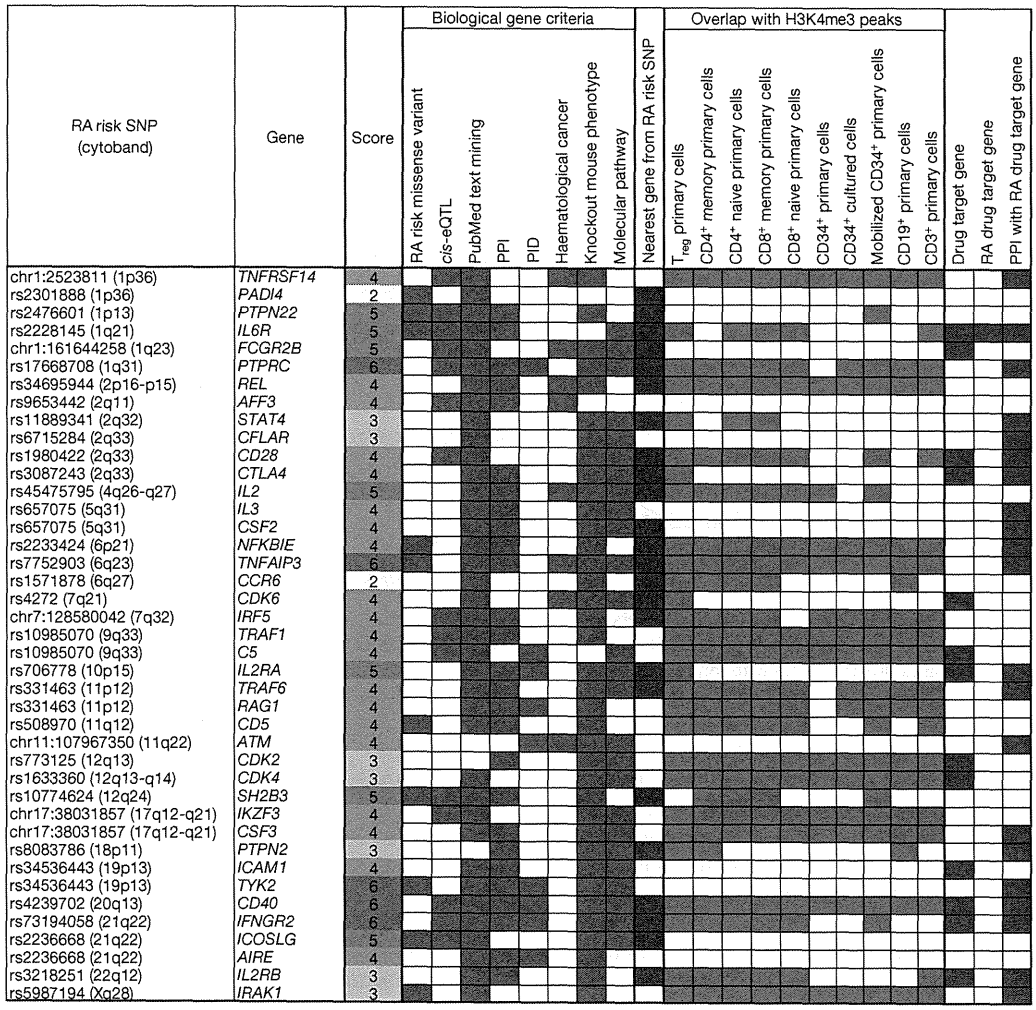

Figure 2 | Prioritized biological RA risk genes. Representative biological RA risk genes. We list the summary gene score derived from individual criteria (filled red box indicates criterion satisfied; 98 genes with a score ≥2 out of 377 genes included in the RA risk loci were defined as 'biological candidate genes'; see Extended Data Fig. 6). Filled blue boxes indicate the nearest gene to the RA risk SNP. Filled green boxes indicate overlap with H3K4me3 peaks in immune-related cells. Filled purple boxes indicate overlap with drug target genes. For full results, see Supplementary Table 5.

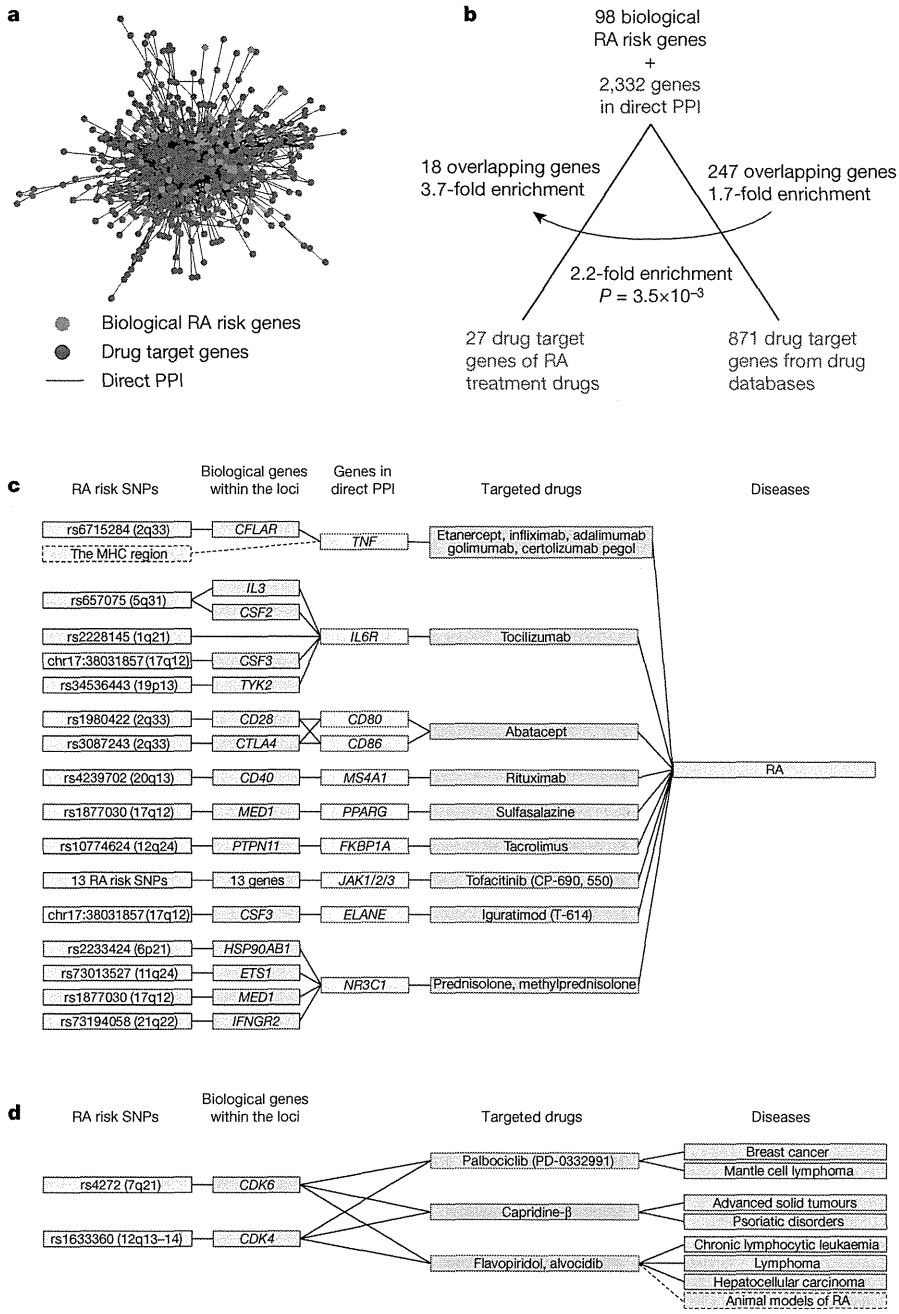

Figure 3 | Connection of biological RA risk genes to drug targets. **a**, PPI network of biological RA risk genes and drug target genes. **b**, Overlap and relative enrichment of 98 biological RA risk genes with targets of approved RA drugs and with all drug target genes. Enrichment was more apparent than that from all 377 RA risk genes (Extended Data Fig. 7c). **c**, Connections between RA risk SNPs (blue), biological genes (purple), genes from PPI (green) and approved RA drugs (orange). For full results, see Extended Data Fig. 8. **d**, Connections between RA genes and drugs indicated for other diseases.

In support for repurposing, one *CDK6/CDK4* inhibitor, flavopiridol, has been shown to ameliorate disease activity in animal models of RA[22]. Further, the biology is plausible, as several approved RA drugs were initially developed for cancer treatment and then repurposed for RA (for example, rituximab). Although further investigations are necessary, we propose that target genes/drugs selected by this approach could represent promising candidates for novel drug discovery for RA treatment.

We note that a non-random distribution of drug-to-disease indications in the databases could potentially bias our results. Namely, because RA risk genes are enriched for genes with immune function, spurious enrichment with drug targets could occur if the majority of drug indications in databases were for immune-mediated diseases or immune-related target genes. However, such enrichment was not evident in our analysis (~11% for drug indications and ~9% for target genes; Extended Data Fig. 7b).

Through a comprehensive genetic study with >100,000 subjects, we identified 42 novel RA risk loci and provided novel insight into RA pathogenesis. We particularly highlight the role of genetics for drug discovery. Although there have been anecdotal examples of this[1,23], our study provides a systematic approach by which human genetic data can be efficiently integrated with other biological information to derive biological insights and drive drug discovery.

METHODS SUMMARY

Details can be found in Methods, Extended Data Fig. 1, Extended Data Table 1 and Supplementary Information, including (1) information about the patient collections;

(2) genotyping, quality control and genotype imputation of GWAS data; (3) genome-wide meta-analysis (stage 1); (4) *in silico* and *de novo* replication studies (stages 2 and 3); (5) trans-ethnic and functional annotations of RA risk SNPs; (6) prioritization of biological candidate genes; and (7) drug target gene enrichment analysis.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions Y.O. carried out the primary data analyses. D.W. managed drug target gene data. G.T. conducted histone mark analysis. T.R., H.-J.W., T.E., A.M., B.E.S., P.L.D. and L.F. conducted eQTL analysis. C.T., K.I., Y.K., K.O., A.S., S.Y., G.X., E.K. and K.A.S. conducted the *de novo* replication study. R.R.G., A.M., W.O., T.B., T.W.B., L.J., J. Yin, L.Y., D.-F.S., J. Yang, P.M.V., M.A.B. and H.X. conducted the *in silico* replication study. E.A.S., D.D., J.C., T.K., R.Y. and A.T. managed GWAS data. All other authors, as well as the members of the RACI and GARNET consortia, contributed to additional analyses and genotype and clinical data enrolments. Y.O. and R.M.P. designed the study and wrote the manuscript, with contributions from all authors on the final version of the manuscript.

Author Information Summary statistics from the GWAS meta-analysis, source codes, and data sources used in this study are available at http://plaza.umin.ac.jp/~yokada/datasource/software.htm. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to R.M.P. (robert.plenge@merck.com) or Y.O. (yokada.brc@tmd.ac.jp).

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