Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity

We combined three existing GWAS data sets (referred to as Kiel\(^3\), the Collaborative Association Study of Psoriasis (CASP)\(^4\) and the Wellcome Trust Case Control Consortium 2 (WTCCC2)\(^5\)) with two independent case-control data sets of individuals of European descent genotyped on the Immunochip: the Psoriasis Association Genetics Extension (PAGE; 3,580 cases and 5,902 controls) and the Genetic Analysis of Psoriasis Consortium (GAPC; 2,997 cases and 9,183 controls) (data sets are described in Supplementary Tables 1 and 2). After quality control, the combined data set consisted of 10,588 individuals with psoriasis and 22,806 healthy controls. For each GWAS, we increased the SNP density through imputation, using European haplotype sequences generated by the 1000 Genomes Project (20100804 release) as templates. Overall, our analysis included 111,236 SNPs that were genotyped in both Immunochip data sets that also had good imputation quality in at least 2 of the 3 GWAS (Online Methods).

From meta-analysis of all five data sets, we confirmed associations at genome-wide significance for SNPs at 19 of the 21 known loci involved in psoriasis (Table 1, Supplementary Fig. 1 and Supplementary Table 3). We found nominal evidence of association for the remaining two loci in the combined analysis (ZMIZ1 and PRDX3, each with \(P < 3 \times 10^{-7}\)), as well as nominal evidence of association for all loci in separate analyses including only GWAS (all with \(P < 5 \times 10^{-7}\) or Immunochip data (all with \(P < 4 \times 10^{-8}\)). In addition, we identified 15 new risk loci associated at \(P < 5 \times 10^{-8}\) (Table 1, Supplementary Fig. 1 and Supplementary Table 3). Nine of the new signals were submitted, during design of the Immunochip, as genome-wide significant Immunochip loci by at least one other disease consortium (Supplementary Table 4, disease overlap), although we also submitted three of these (rs11121129, rs10865331 and rs9504361) on the basis of a preliminary meta-analysis of our GWAS data sets. Notably, of the remaining six signals, four were submitted for psoriasis (rs11795343, rs4561177, rs11652075 and rs545979). The strongest new association was observed for rs892085 at 19p13.2 near the ILF3 and CARM1 genes (combined \(P_{\text{combined}} = 3.0 \times 10^{-17}\); odds ratio (OR) = 1.17). Despite its proximity (< 500 kb away) to TYK2, conditional analysis showed that this SNP represents an independent signal (Supplementary Table 5). Other associated loci included 1p36.11 near RUNX3, 6p25.3 near EXOC2 and IRF4, 9p21.1 near DDX58, 11q22.3 near ZC3H12C, 11q24.3 in the ETS1 gene and 17q21.2 near STAT3, STAT5A and STAT5B. The functional characteristics of notable genes from the newly identified loci are summarized in Box 1, and regional association plots are shown in Supplementary Figure 2.

Psoriasis is a chronic, potentially disfiguring immune-mediated inflammatory disease of the skin with a prevalence of 0.2–2%, depending on the population of origin. Approximately one-quarter of indivi-
duals with psoriasis develop painful and debilitating arthritis, and there is increasing awareness of comorbidities, including metabolic syndromes and cardiovascular disease\(^1\). Current evidence suggests that a dysregulated cutaneous immune response, characterized by T-cell 1 (T H1) and 17 (T H17) activation, occurs in genetically sus-
ceptible individuals\(^1\). Recent large-scale association studies have identified 26 loci that are associated with psoriasis\(^3\)–\(^10\), 21 of which show association in individuals of European ancestry\(^3\)–\(^6\).\(^10\). Several of these loci overlap with those identified as being associated with other autoimmune diseases (for example, Crohn’s disease, ankylos-
ing spondylitis and celiac disease), particularly those near genes involved in T H17 differentiation and interleukin (IL)-17 responsiveness (for example, IL23R, IL12B, IL23A and TRAF3IP2)\(^11\). To accelerate the understanding of the genetic architecture of psoriasis, we helped design a custom SNP array—the Immunochip—with the aim of fine mapping previously identified genome-wide significant susceptibility loci (associated at \(P < 5 \times 10^{-8}\)) and exploring replication of thousands of SNPs representing additional promising signals\(^12\).\(^13\). In this study, we used Immunochip data to identify new susceptibility loci for psoriasis and to consider their association to other autoimmune disorders.

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Received 7 May; accepted 17 October; published online 11 November 2012; doi:10.1038/ng.2467
Table 1 Meta-analysis results for loci associated with psoriasis in a weighted analysis

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr.</th>
<th>Position (bp)</th>
<th>GWAS P value</th>
<th>Immunochip P value</th>
<th>Combined P value</th>
<th>Risk/ non-risk allele</th>
<th>RAf (case)</th>
<th>RAf (control)</th>
<th>OR*(meta)</th>
<th>Notable genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7552167</td>
<td>1</td>
<td>24518643</td>
<td>2.3 × 10⁻⁵</td>
<td>8.4 × 10⁻⁴</td>
<td>8.5 × 10⁻¹²</td>
<td>G/A</td>
<td>0.878</td>
<td>0.858</td>
<td>1.21</td>
<td>IL28RA</td>
</tr>
<tr>
<td>rs9988642</td>
<td>1</td>
<td>67726104</td>
<td>2.5 × 10⁻¹³</td>
<td>3.5 × 10⁻¹⁵</td>
<td>1.1 × 10⁻⁶</td>
<td>T/C</td>
<td>0.952</td>
<td>0.929</td>
<td>1.52</td>
<td>IL23R</td>
</tr>
<tr>
<td>rs6775951</td>
<td>1</td>
<td>152390187</td>
<td>1.1 × 10⁻¹⁵</td>
<td>2.7 × 10⁻²⁰</td>
<td>2.1 × 10⁻³⁴</td>
<td>T/C</td>
<td>0.689</td>
<td>0.640</td>
<td>1.26</td>
<td>LCE3B, LCE3D</td>
</tr>
<tr>
<td>rs62149416</td>
<td>2</td>
<td>61083506</td>
<td>3.4 × 10⁻¹⁰</td>
<td>3.2 × 10⁻⁹</td>
<td>1.8 × 10⁻¹⁷</td>
<td>T/C</td>
<td>0.671</td>
<td>0.635</td>
<td>1.17</td>
<td>FLJ16341, REL</td>
</tr>
<tr>
<td>rs171716942</td>
<td>2</td>
<td>163266091</td>
<td>4.1 × 10⁻⁹</td>
<td>1.0 × 10⁻¹⁰</td>
<td>3.3 × 10⁻¹⁸</td>
<td>T/C</td>
<td>0.891</td>
<td>0.863</td>
<td>1.27</td>
<td>KCNH7, IFIH1</td>
</tr>
<tr>
<td>rs27432</td>
<td>3</td>
<td>96119273</td>
<td>4.9 × 10⁻⁴</td>
<td>9.5 × 10⁻¹⁴</td>
<td>1.9 × 10⁻¹⁰</td>
<td>T/C</td>
<td>0.309</td>
<td>0.274</td>
<td>1.20</td>
<td>ERAP1</td>
</tr>
<tr>
<td>rs40433729</td>
<td>8</td>
<td>321960950</td>
<td>2.8 × 10⁻⁵</td>
<td>5.5 × 10⁻⁹</td>
<td>3.2 × 10⁻¹⁵</td>
<td>T/C</td>
<td>0.132</td>
<td>0.099</td>
<td>1.58</td>
<td>IL12B</td>
</tr>
<tr>
<td>rs892085</td>
<td>12</td>
<td>19818092</td>
<td>2.3 × 10⁻⁵</td>
<td>4.6 × 10⁻⁵</td>
<td>3.0 × 10⁻¹⁰</td>
<td>A/G</td>
<td>0.593</td>
<td>0.558</td>
<td>1.17</td>
<td>TNIP1</td>
</tr>
</tbody>
</table>

 Known susceptibility loci

rs892085           | 12    | 19818092      | 2.3 × 10⁻⁵   | 4.6 × 10⁻⁵         | 3.0 × 10⁻¹⁰      | A/G                   | 0.593      | 0.558         | 1.17       | TNIP1         |

For previously identified susceptibility loci, the most significantly associated SNP within 500 kb (3 Mb for the MHC region) of the previously published SNP is shown. rs34536443 was the most significantly associated SNP in the 19q13.2 region. The strongest conditional signal in the 19q13.2 region was rs12720356 (OR = 1.25; minor allele frequency (MAF) in controls = 0.09; P = 3.2 × 10⁻¹⁰). The association of this SNP with psoriasis has been previously reported and is independent of the major histocompatibility (MHC) region near the MICA gene (rs13437088: P = 3.1 × 10⁻⁴⁰; OR = 1.32), in agreement with previous conditional analysis. The conditional signal at 5q15 was in the ERAP2 gene (rs2910686: P = 2.0 × 10⁻⁸), which did not show any evidence of association in the unconditioned analysis (P = 0.46). Further investigation showed that the risk-increasing alleles at ERAP1 and the risk-decreasing alleles at ERAP2 preferentially appear on the same haplotype, and the signal near ERAP2 was thus masked by ERAP1 before conditional analysis (Supplementary Note).

To identify independent secondary signals, we performed conditional analysis, using as covariates the strongest signals from the 34 loci achieving genome-wide significance in this study. We identified secondary signals in five loci: 2q24.2, 5q15, 5q33.3, 6p21.33 and 19q13.2 (Supplementary Figs. 3 and 4 and Supplementary Tables 6 and 7). The strongest signal from the conditional analysis mapped to the major histocompatibility (MHC) region near the MICA gene (rs13437088: P = 3.1 × 10⁻⁴⁰; OR = 1.32), in agreement with a previous conditional analysis. The conditional signal at 5q15 was in the ERAP2 gene (rs2910686: P = 2.0 × 10⁻⁸), which did not show any evidence of association in the unconditioned analysis (P = 0.46). Further investigation showed that the risk-increasing alleles at ERAP1 and the risk-decreasing alleles at ERAP2 preferentially appear on the same haplotype, and the signal near ERAP2 was thus masked by ERAP1 before conditional analysis (Supplementary Note). The strongest conditional signal in the 19q13.2 region was rs12720356 in the TYK2 gene (OR = 1.25; minor allele frequency (MAF) in controls = 0.09; P = 3.2 × 10⁻¹⁰). The association of this SNP with psoriasis has been previously reported and is independent of the major histocompatibility (MHC) region near the MICA gene (rs13437088: P = 3.1 × 10⁻⁴⁰; OR = 1.32), in agreement with previous conditional analysis. The conditional signal at 5q15 was in the ERAP2 gene (rs2910686: P = 2.0 × 10⁻⁸), which did not show any evidence of association in the unconditioned analysis (P = 0.46). Further investigation showed that the risk-increasing alleles at ERAP1 and the risk-decreasing alleles at ERAP2 preferentially appear on the same haplotype, and the signal near ERAP2 was thus masked by ERAP1 before conditional analysis (Supplementary Note).
These interactions confirm the results of previous studies. We found ten potentially causal SNPs, which encode members of the STAT family of transcriptional activators. STAT3 participates in signaling downstream of the IL-2 family of cytokines, including IL-2, IL-7, IL-15 and IL-21. Both proteins contribute to the development of regulatory T (Treg) cells and inhibit the differentiation of TH17 cells.

Box 1 Annotated functions of notable candidate genes within newly identified psoriasis susceptibility loci

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL17A</td>
<td>Encodes a transcription factor that regulates IL17A promoter activity and controls RORyt-dependent TH17-mediated colitis in vivo.</td>
</tr>
<tr>
<td>IL23R</td>
<td>Encodes a member of the RIG-I innate antiviral receptor, which recognizes cytosolic double-stranded RNA and is induced by IFN-γ.</td>
</tr>
<tr>
<td>CARD14</td>
<td>Encodes a member of the CARD family of proteins that mediates recruitment and activation of the NF-κB pathway.</td>
</tr>
<tr>
<td>STAT5A</td>
<td>Encodes a transcription factor that regulates IL-2 promoter activity and stabilizes mRNA.</td>
</tr>
</tbody>
</table>

For the newly identified loci, the index SNP from CARD14, a gene that harbors Mendelian variants predisposing to psoriasis, was also a common and damaging variant, as described elsewhere. For the remaining loci, we could account for essentially all index SNP signals by conditioning on nearby missense SNPs, consistent with the possibility that they are causal. Notable nonsynonymous variants included the protective polymorphism encoding a p.Arg381Gln alteration in IL23R, a SNP in the PRSS53 gene, which is also the most highly overexpressed gene in psoriatic skin in this locus, and a variant in YDJC that also increases risk for celiac disease, rheumatoid arthritis and Crohn's disease.
Using the results of a large-scale study of gene expression in psoriatic versus normal skin\textsuperscript{34}, we found 14 upregulated genes (IL12RB2, LCE3D, REL, PUS10, CDSN, PRSS53, PRSS8, NOS2, DDX58, ZC3H12C, SOCS1, STAT3, CARD14 and IFI1H1) and 4 downregulated genes (MICA, RNF114, PTRF and POLI) in the 34 associated regions (false discovery rate (FDR) of <0.05; fold change of >1.5 or <0.67; Supplementary Table 9). The number of differentially expressed genes in psoriasis susceptibility loci was not greater than expected by chance (P = 0.39). None of the 34 top SNPs met the Bonferroni-corrected significance threshold (P < 1 x 10\textsuperscript{-7}) for expression quantitative trait loci (eQTLs) in skin tissue, as assessed by microarray analysis of mRNA levels\textsuperscript{25}. However, rs2910686, one of the five SNPs identified by conditional analysis, was a causal SNP of expression. Consistent with this function, it possesses a diverse and well-conserved set of innate immune mechanisms\textsuperscript{35},\textsuperscript{36} that emerged long before the development of adaptive immunity\textsuperscript{34}. In this context, we found it noteworthy that five of the six newly identified loci that are thus far uniquely associated with psoriasis are involved in innate immune responses (DDX58, KLFL4, ZC3H12C, CARD14 and CARM1; Box 1 and Supplementary Table 4). Among all confirmed psoriasis-susceptibility loci, 11 out of 14 psoriasis-specific loci (the 5 new loci involved in the innate immune response along with IL28RA, LCE3D, NOS2, FBXL19, NFKBIA and RNF114) encode plausible regulators of innate host defense\textsuperscript{1,2,35}. Conversely, only 6 out of 20 loci shared with other autoimmune diseases contain genes that contribute to innate immunity (REL, IFI1H1, TNIP1, TNAIP3, IRF4 and ELMO1). These provisional comparisons further illustrate the insights that can be gained by developing and comparing complete and well-annotated sets of risk loci for autoimmune disorders.

The known and newly identified psoriasis susceptibility loci implicated by this study encode several proteins engaged in the TNF, IL-23 and IL-17 signaling pathways, which are targeted by highly effective biological therapies\textsuperscript{36}. Notably, our strongest non-MHC signal directly implicates TYK2, a drugable target that contributes to several autoimmune diseases. Agents targeting the closely related JAK kinases are showing encouraging results in clinical trials\textsuperscript{37}. Our findings will help prioritize and interpret the results of sequencing and gene expression studies. Further genomic studies are needed to identify the underlying causal variants in these psoriasis susceptibility loci and to bring increased understanding of pathogenetic mechanisms.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**ACKNOWLEDGMENTS**

Major support for this study was provided by the US National Institutes of Health, the Wellcome Trust and the German Research Foundation. We thank J.C. Barrett for contribution to the design of the Immunochip and helpful analytical discussion, as well as E. Gray, S. Bumpstead, D. Simpkin and the staff of the Wellcome Trust Sanger Institute Sample Management and Genotyping teams for their genotyping and analytical contributions. We acknowledge use of the British 1958 Birth Cohort DNA collection, funded by the UK Medical Research Council (G0000934) and the
The authors declare no competing financial interests.


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ONLINE METHODS
Sample collections. The samples used in the three GWAS data sets (Kiel, CASP and WTCCC2) were previously described\(^5\). Samples in the PAGE and GAPC data sets (Supplementary Tables 1 and 2) were collected from subjects of European descent at the participating institutions after obtaining informed consent. Enrollment of human subjects for this study was approved by the ethics boards of the participating institutions in adherence with the Declaration of Helsinki Principles. DNA was isolated from blood or Epstein-Barr virus (EBV)-immortalized lymphoblastoid cell lines using standard methods.

The collections used in the GAPC and PAGE Immunochip studies are described in Supplementary Table 2.

The samples from GAPC substantially overlapped with those described as replication data sets in Strange et al.\(^5\). All cases had been diagnosed as having psoriasis vulgaris. The GAPC cases and the Irish and Spanish controls were genotyped at the Wellcome Trust Sanger Institute, and all samples were provided by the relevant groups listed in Supplementary Table 2 and by the members of the GAP Consortium listed in the Supplementary Note. The UK controls were the WTCCC common control samples that did not overlap with the samples included in the original GWAS (the data set consisted of 6,740 1958 British Birth cohort and 2,900 UK Blood Service samples genotyped at the Wellcome Trust Sanger Institute and the University of Virginia). The German controls were obtained from the PopGen Biobank and genotyped at the Institute of Clinical Molecular Biology at Christian-Albrechts-University of Kiel. The Finland control data were from the DILGOM collection\(^70\). The Irish controls were provided by the Irish Blood Transfusion Service/Trinity College Dublin Biobank, and Irish cases were collected with the aid of the Dublin Centre for Clinical Research. We did not include specific controls from Austria or Sweden, but principal-components analysis suggested that the cases from these cohorts were well matched to the controls from The Netherlands and Germany.

For the PAGE Immunochip study, samples also substantially overlapped with previously published replication data sets. The German cases (described as a replication data set in Ellinghaus et al.\(^5\)) and all samples from the United States and Canada, as well as 439 Estonian cases from the University of Tartu, were genotyped at the Institute of Clinical Molecular Biology at Christian-Albrechts-University of Kiel. The respective samples were provided by the groups listed in Supplementary Table 2 and by the members of PAGE listed in the Supplementary Note. The German controls were obtained from a population-based sample from the general population living in the region of Augsburg in southern Germany (KORA S4/F4)\(^71\), which was genotyped at the Helmholtz Zentrum Munich, and from the population-based epidemiological HNR study, for which genotyping was performed at the Life and Brain Center at the University Clinic in Bonn. The remaining Estonian samples were obtained from and genotyped at the Estonian Genome Center University of Tartu (EGCUT).

Genotyping panel and SNPs. The Immunochip is a custom Illumina Infinium high-density array consisting of 196,524 variants (after Illumina quality control) compiled largely from variants identified in previous GWAS of 12 different immune-mediated inflammatory diseases, including psoriasis\(^5\). The main aims of the Immunochip were deeper replication and fine mapping of genome-wide significant loci, as well as increasing power to promote promising, but less significant SNPs to genome-wide significance. For fine mapping, SNPs within 0.2 cM on either side of the GWAS top SNPs at 186 loci were selected from 1000 Genomes Project\(^72\) low-coverage pilot Utah residents of Northern and Western European ancestry (CEU) sequencing data as well as additional variants identified by resequencing from groups involved in the chip design. For promotion of promising signals and those not quite reaching genome-wide significance, each disease-focused group was allowed to submit approximately 3,000 additional SNPs. We submitted 17 of the 19 confirmed regions associated with psoriasis at genome-wide significance (Table 1) for fine mapping on the basis of a preliminary meta-analysis of our data, and 1 of the confirmed signals (IL28RA) and 9 of the new psoriasis-associated signals (Supplementary Table 5, disease overlap) were submitted for fine-mapping by groups studying other diseases (although we also submitted 3 of these as part of our additional SNP allocation: rs11121129, rs10865331 and rs9504361). Six additional signals were detected on the basis of additional SNP allocation in individual groups; four of these (rs11795343, rs4561177, rs11652075 and rs549797) were submitted by our group. All Immunochip samples were genotyped as described in Illumina’s protocols.

Genotype calling. For the PAGE data set, genotype calling was performed using Illumina’s GenomeStudio Data Analysis software and the custom-generated cluster file of Trynka et al.\(^13\), which was generated by initial clustering of 2,000 UK samples with the GenTrain2.0 algorithm and subsequent manual readjustment and quality control). Genotype calling for the GAPC data set was performed using GenoSNP\(^73\) from allele intensities, except for the German, Irish, and Finnish controls, which were called using the same method described for the PAGE data set.

Imputation. To increase the number of overlapping SNPs between data sets, we performed imputation on the 3 GWAS data sets using minicmap\(^24\) (Kiel and CASP) and IMPUTE2 (refs. 75,76) (WTCCC2) using data from CEU reference haplotypes from the 1000 Genomes Project\(^27\) (December 2010 version of the 10/08/04 sequence and alignment release containing 629 individuals of European descent). SNPs with low imputation quality ($r^2 \leq 0.3$ for minicmap and info score < 0.5 for IMPUTE2) were removed. For all 3 data sets, cases and controls were imputed together.

Sample and genotype quality control. For the Immunochip data sets, we first excluded SNPs with a call rate below 95% or with a Hardy-Weinberg equilibrium $P$ value of $<1 \times 10^{-6}$. Samples with less than SNP call rates below 98% were then excluded. Because the Immunochip includes a large proportion of fine-mapping SNPs that are associated with autoimmune disease, we used a set of independent SNPs that have $P$ values $>0.5$ from the meta-analysis of the three GWAS data sets as a quality control tool for each individual Immunochip data set. Using the HapMap 3 samples as reference\(^77\), we performed principal-component analysis to identify and remove samples with non-European ancestry. We also removed samples with extreme inbreeding coefficients or heterozygosity values computed by PLINK\(^78\).

To assess possible stratification in the data sets, principal-components analysis was also performed in each of the Immunochip data sets separately (excluding HapMap). There was no evidence of stratification between the cases and controls of each sample group. However, as expected, the top principal components did separate the samples well by country of origin. The use of the top ten eigenvectors as covariates in the analysis did not completely correct for stratification, and, therefore, a linear mixed-model method (efficient mixed-model association expedited (EMMAX)) was instead used for the association analysis. These methods have been shown to outperform principal components in correcting for this type of population stratification and cryptic relatedness\(^79\), which is becoming more common as sample sizes increase and studies comprise more collaborative efforts.

To identify duplicate pairs or highly related individuals among data sets, we used a panel of 873 independent SNPs that were genotyped in both the GWAS and Immunochip samples and performed pairwise comparisons using the genome function in PLINK\(^79\), requiring Pi-HAT of 20.5. We identified 1,142 (885 from GAPC and 257 from PAGE) related sample pairs (mostly duplicates) and removed one sample from each pair. We also removed 4,828 controls from the UK common Immunochip controls because of duplication in the WTCCC2 GWAS sample. For GWAS samples that were duplicated in the Immunochip data sets (the majority of duplicates), we removed the samples from the Immunochip data sets to keep the previously published data sets intact.

The GWAS data sets underwent quality control as previously described and were analyzed for association using the top principal components from the previous analyses as covariates\(^5\). We visually inspected the signal intensity cluster plots for all SNPs with associations reaching genome-wide significance to confirm high-quality genotype calling.

Genomic control. Genomic control inflation factors for the five data sets were 1.09 (Kiel), 1.06 (CASP), 1.04 (WTCCC2), 0.99 (PAGE) and 0.96 (GAPC), indicating that population structure and cryptic relatedness were adequately controlled for in these data sets. Because the Immunochip was designed for deep replication and fine mapping of loci associated with autoimmune...
diseases\textsuperscript{12}, using all independent SNPs from the chip would not give an accurate estimate of the genomic control\textsuperscript{80} value ($\lambda_{GC}$). Therefore, we selected common SNPs (with minor allele frequency (MAF) of >0.05) from the Immunochip that had association $P$-values of >0.5 on the basis of a meta-analysis combining the Kiel, CASP and WTCCC2 GWAS, and then performed LD pruning to identify an independent SNP set to compute $\lambda_{GC}$ for the association results from the Immunochip data sets. As a result of SNP selection bias, the genomic control value for the final meta-analysis was computed using a set of independent SNPs associated with reading and writing ability (J.C. Barrett, personal communication). We further removed SNPs that were within 500 kb of previously detected psoriasis-associated loci (within 3 Mb in the MHC region), and the remaining 1,426 SNPs yielded $\lambda_{GC}$ of 1.11 for the meta-analysis overall. Using $\lambda_{1000}$ (ref. 81), the genomic control inflation factor for an equivalent study of 1,000 cases and 1,000 controls, the rescaled $\lambda$ equaled 1.01.


