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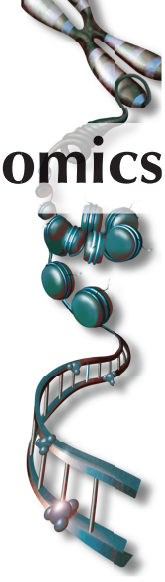

Fine mapping and subphenotyping implicates *ADRA1B* gene variants in psoriasis susceptibility in a Chinese population

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Aim: A genomic region on 5q33.3 lies between and encompasses the *IL12B* and *PTTG1* genes, and contains many potential psoriasis causal variants. We aimed to further examine the influence of variants in and around this region. **Materials & methods:** We used least absolute shrinkage and selection operator (LASSO)-based regression analysis to assess independent contributions of 2171 variants to psoriasis susceptibility and tested them for association with different clinical psoriasis subtypes. **Results:** We found that *ADRA1B* gene variants contribute to psoriasis in Chinese population. *ADRA1B* gene variants have a stronger association with moderate-to-severe disease group and an earlier age at onset of psoriasis than *IL-12B* and *PTTG1* variants. **Conclusion:** The association of variants in the *ADRA1B* gene with psoriasis could explain why variants in the *IL-12B*, *ADRA1B* and *PTTG1* gene regions are associated with psoriasis.

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Psoriasis is a chronic inflammatory and immune system-mediated disorder affecting the skin, joints and nails with an estimated prevalence of 2% worldwide [1]. Psoriasis exhibits a spectrum of clinical subtypes and can lead to wide variety of specific diagnoses [2]. Psoriasis has a strong genetic predisposition. In fact, to date more than 40 psoriasis susceptibility loci have been identified through genome-wide association studies (GWAS) [3–14]. The strongest susceptibility locus lies within the major histocompatibility complex (MHC) and is termed psoriasis susceptibility locus 1 (PSORS1). Other associated variants have been found to reside in the *IL12B*, *IL23R*, *IL23A*, *TNFAIP3*, *IL13*, *RNF114, TRAF3IP2, ERAP1* and *LCE* genes. However, it remains unclear which variants in and around these genes are causally related to psoriasis and not just in linkage disequilibrium (LD) with others and, ultimately, which of these variants should be combined to assess psoriasis risk because of their independent effects [15,16]. In addition, few, if any, studies have tried to associate these and other potential susceptibility variants to subclinical and clinical manifestations of psoriasis pathology to determine what aspects of psoriasis they may be most strongly associated with.

The *IL12* gene is a T-helper 1-type cytokine and is known to harbor variants associated with psoriasis [17]. In addition, *IL12B* gene expression has been shown to exhibit significantly increased levels in psoriatic skin by quantitative RT-PCR [18]. *PTTG1*, a transcription factor known to influence the regulation of many genes, has been shown to have increased levels of expression in the psoriatic epidermis [19]. Interestingly, *IL12* and *PTTG1*

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Table 1. Baseline characteristics of study participants for the study.

Variable	Data for cases	Data for controls
N	1139	1132
Age (years)	30.98 ± 12.3	35.09 ± 13.2
Male gender (%)	58.7	59.2
Age of onset (mean ± SD years)	22.31 ± 9.6	
Early onset psoriasis (≤20 years, %)	87.3	
PASI index (%):		
– PASI ≤10	28.7	
– PASI >10	71.3	
Clinical type:		
– Plaque	62.6	
– Guttate	37.4	

PASI: Psoriasis Area and Severity Index; SD: Standard deviation.

genes are located in the same region on 5q33.3, approximately 1.1 Mb away from each other, although they are not in the same LD block. In a GWAS pursued by our team [9,11], we found genome-wide-corrected statistical evidence confirming that the IL-12B gene harbors susceptibility variants, in particular for SNP rs2431697 which is located approximately 24 kb downstream of *PTTG1*. Since it is unknown whether variants in both IL-12B and *PTTG1* influence psoriasis susceptibility, we pursued a fine mapping study leveraging imputation methods, regularized regression techniques, as well as a subclinical phenotype association study, to determine if multiple variants in the *IL-12B* and *PTTG1* genes contribute to psoriasis susceptibility. We find that variants in the Adrenoceptor-α 1B *(ADRA1B*) gene, which lies between the *IL12B* and *PTTG1* genes, are most strongly associated with psoriasis as well as number of clinically relevant psoriasis phenotypes.

Materials & methods

Samples & clinical features

We leveraged 1139 psoriatic cases and 1132 healthy controls from a cohort used in one of our previous studies [11]. The psoriatic cases had clinical subphenotypes collected on them and the details of the clinical data on the patients are provided in Table 1. Two experienced dermatologists made the clinical diagnosis and classification of the patients. All the controls included in this study were screened to exclude the presence of psoriasis or any autoimmune disorders, as well as for the presence of family history of autoimmune disorders in first-, second- and third-degree relatives. To explore the deeper phenotypic impact of variants in the *IL12B* and *PTTG1* gene region in psoriasis, we used the clinical information to classify patients into different clinical groups. In this context, the Psoriasis Area and Severity Index (PASI) is used in the majority of international clinical trials designed to evaluate lesions. Patients were categorized into mild (PASI ≤10) and body surface area (BSA ≤10) and moderate to severe psoriasis (PASI >10 or body surface area [BSA >10]) [20]. Based on age of onset, psoriasis is usually divided into two subtypes: type I (before age 40 with peak onset at 16–22 years) and type II (after age 40 with peak onset at 57–60 years) [21]. Our study included 99.4% type I psoriatics, so we used an age of onset of 20 or less to define early onset in order to reduce any possible bias (Table 1). Written informed consent was obtained from each patient and control subject prior to enrollment in our study. The study was approved by the Ethics Committee of Institute of Dermatology, Anhui Medical University, and was conducted according to the Declaration of Helsinki principles.

Genotyping data & quality control

All 2271 participants in our study were of Chinese ancestry and genotyped with the Illumina Human 610-Quad Bead Chips as described previously [11]. For the genomic region of interest we studied total 211 variants (chr5: 158.7–159.9 Mb; using the GRCh37.p13 assembly; out of these 211 variants, 19 were in or around *PTTG1*, 18 variants were in or around *IL-12B* and 174 SNPs were between the two genes).

We used the software Plink 1.07 [22] to perform basic quality control procedures. SNPs were excluded if the genotype call rate was <95%, the minor allele frequency (MAF) <1% or the Hardy–Weinberg equilibrium (HWE) p-value was $<10^{-7}$ in controls. We also removed individuals with a missing genotype rate >5%. The 211 SNPs

Table 2. Markers and samples used in original and imputation stages.

Data model	IL-12B†	PTTG1†	Between two genes†	Total†	Samples (case/control)
Two genes	18/2	19/1	0	37/3	1139/1132
Region	18/3	19/2	174/3	211/8	1123/1125
Imputation	188/7	167/12	1816/43	2171/62	1082/1083
Imputation + phenotype	188/7	167/12	1816/43	2171/62	1082/1083

†-/- SNPs used in initial GWAS and imputation study/SNPs selected by LASSO in this study.
GWAS: Genome-wide association study.

that passed the defined quality control thresholds were initially subjected to the single variant association tests using standard χ^2 analysis and then a regularized regression analysis as discussed in the Statistical Analysis section.

Statistical analysis

Imputation

To increase the density of variants in and around the *IL12B* and *PTTG1* gene regions we pursued imputation techniques to assign more variants to the regions [23]. This enabled us to obtain an abundance of SNPs, with both rare and common alleles, which might provide clues about the role of variants in the region in psoriasis susceptibility. Imputation was performed using the IMPUTE version 2 software suite [24] with the East Asia (ASN) haplotypes within the 1000 Genomes Project as a reference panel (August 2015 version). Our initial imputations yielded a total of 5027 SNPs in the region. The total genotyping rate in all individuals was 97.7%. Filters used for the imputation included a minor allele frequency (MAF) <0.01 and failed Hardy–Weinberg equilibrium (HWE) test ($p \leq 0.001$) and their use led to the removal of 2372 SNPs. An additional 484 SNPs were removed by a failed missingness test. After filtering, 2171 SNPs in 1082 cases and 1083 controls were used for further analysis (Table 2).

Best SNPs set selection by using least absolute shrinkage and selection operator (LASSO) model

We used a least absolute shrinkage and selection operator (LASSO) model [25] to identify the potential independently associated variants in the *IL12B* and *PTTG1* region, as implemented in the R package 'glmnet', analyzing all the imputed 2171 SNPs simultaneously. Regularized regression models, such as the LASSO, can accommodate LD between a large set of variants in even moderately sized samples. We split our samples into a training set and a test set by using k = 10-fold cross-validation in order to accurately estimate the test error that suffer neither from excessively high bias nor from very high variance. We fit a LASSO model on the training set and determined the optimal set of associated variants based on mean squared error (MSE) and then tested the resulting model on the test set using the best tuning parameter lambda (λ) value for which the overall mean cross-validation error was smallest [26]. In order to achieve a consistent and optimal value for the λ value, we used 1000 permutations and selected the median value. To validate the model we permutated case/control status and estimated the distribution of the test statistic empirically. Figure 1 shows the permutation test distribution. Finally, we refitted the LASSO model on the full dataset using the median λ from the 1000 permutations and determined the significance of the regression coefficients. This process resulted in the selection of 62 SNPs out of the 2171 total as independent, statistically significant predictors of psoriasis.

Genotype risk & prediction assessment

We fit logistic regression models with the 62 selected SNPs from the LASSO analyses and constructed receiver operating characteristic (ROC) curve to obtain the area under ROC curve (AUC). The AUC provides a reasonable measure of the discriminatory ability of the models. Different AUCs were evaluated and compared using DeLong's methods in the R package pROC [27]. To determine if the variants in the candidate genes *IL12B* and *PTTG1* could explain the overall strength of the associations for all the variants in the region, we assessed models predicting psoriasis with different SNP subsets in the overall region using logistic regression. We assessed four models: two gene-based models, including one with SNPs rs3213094, rs7709212 and rs2431697 located in the *IL12B* and *PTTG1* genes; a regional model including rs3181224, rs3213094, rs7709212, rs17056747, rs2421048, rs1592968, rs2431697 and rs2909733 which are located around the *IL12B* and *PTTG1* genes; a model that included all 62 imputed SNPs in the region identified in the LASSO regression models and a model with all 62 SNPs and age and gender as additional covariates. We compared the models using the overall sample odds ratios (ORs).

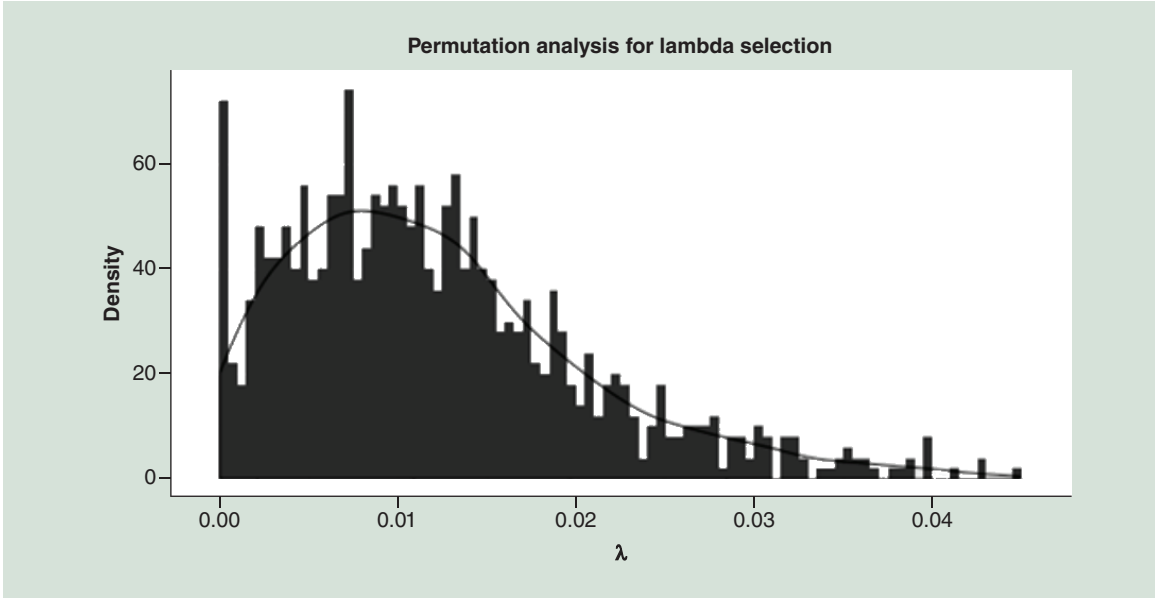

Figure 1. 1000 permutation test for lambda (λ) selection.

Subphenotype analysis

We also considered the association of the 62 variants in the *IL12B* and *PTTG1* region with psoriasis subphenotypes and included age, gender, age of onset, clinical type and severity of psoriasis as covariates. The statistical significance of each variant in the models was determined if the p-value of the regression coefficient for a SNP was <0.05. To facilitate interpretation the OR for the SNPs have been reported relative to previous published GWAS psoriasis risk alleles. Welch two-sample t-tests were used to compare the estimates of the regression coefficients between different subphenotype models. All statistical association analyses were performed using Plink (v1.07) and R [22,28].

Polygenic inheritance analysis

The proportion of the heritable component of the liability to psoriasis was estimated using a linear mixed model, implemented via restricted maximum likelihood (REML) analysis. Our analyses were performed using the GCTA software [29]. Previously identified genome-wide significant loci that we included in these models were established through literature review. The start and end position for each locus was identified according to dbSNP 138.

Results

Cohort clinical characteristics

As noted, a total of 2271 individual were included in the study. The 1139 cases and 1132 controls were matched by gender and age. Several psoriasis clinical features were differentially distributed between different subphenotypes among these individuals (Table 1). There were 86.5% cases observed with positive family history of psoriasis, the cohort had a slightly higher male percentage (58.7% in cases and 59.2% in controls), a higher frequency (87.3%) of early age at onset ($\leq$ 20 years) of psoriasis, a lower percentage (28.7%) of mild psoriasis (PASI $\leq$10) and lower percentage (37.4%) of the guttate type of psoriasis.

LASSO models

For modeling allele and genotype effects at each SNP locus, we coded genotypes 0, 1, or 2 according to the number of minor alleles. For the LASSO model involving the original GWAS data, 203 of the estimated 211 genotype-related coefficients converged to zero, suggesting they were either redundant in their association with psoriasis (due to, e.g., LD with other SNPs in the model) or simply had minimal or no association with psoriasis. Only 8 of the 211 SNPs were found to be statistically significant and therefore assessed in additional models (Figure 2). The eight SNPs were in two LD blocks (Figure 3). For a LASSO model analysis involving the 2171 additional imputed SNPs,

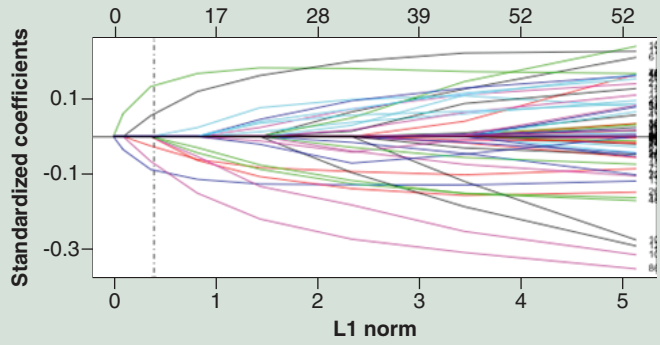

Figure 2. LASSO model for SNPs selection across IL-12B and PTTG1 region.

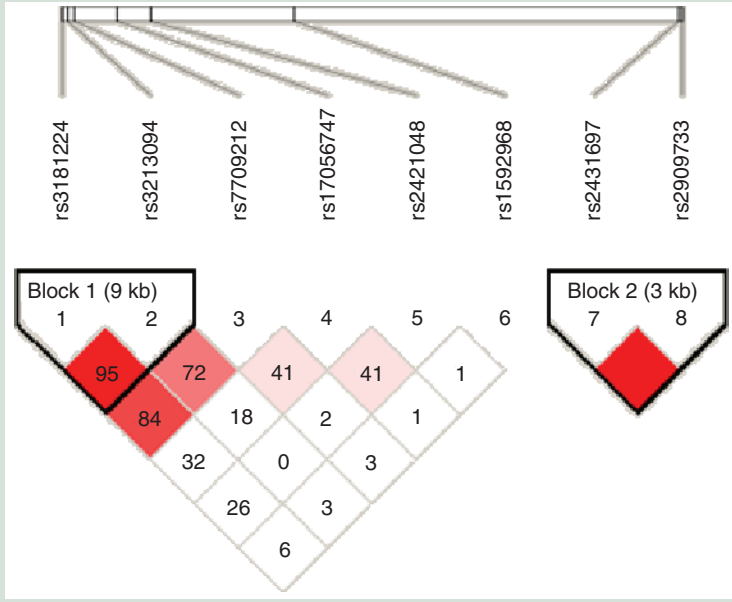

Figure 3. Linkage disequilibrium blocks across *IL-12B* and *PTTG1* region.

62 SNPs, 7 SNPs around *IL-12B*, 12 SNPs around *PTTG1* and 43 SNPs between the two genes, were found to be statistical significant (Table 2).

Prediction assessment

We fit logistic regression models using the SNPs identified from the LASSO regressions with different subsets of the SNPs to determine how important those SNPs were to psoriasis susceptibility. A model with SNPs within the *IL12B* and *PTTG1* genes that were selected by the LASSO model only included three SNPs, a model with SNPs in the regions of the two genes included eight SNPs, and the model with all the selected SNPs included 62 SNPs. Age and gender were added as covariates to these models as well. Table 2 presents the results of these model fits. The model with only SNPs in the *IL12B* and *PTTG1* genes or SNPs in the region of these genes only had modest predictive or discriminatory effects for psoriasis (DeLong's statistics for assessing model improvement: $p = 0.012$), suggesting that these SNPs confer only a small risk of psoriasis. However, the model with all 62 SNPs improved the predictive and discriminatory ability (AUC = 0.674; 95% CI: 0.652–0.696; $p = 3.8 \times 10^{-16}$), suggesting that adding more SNPs in the genomic region significantly improved the predictive ability of the models. The model

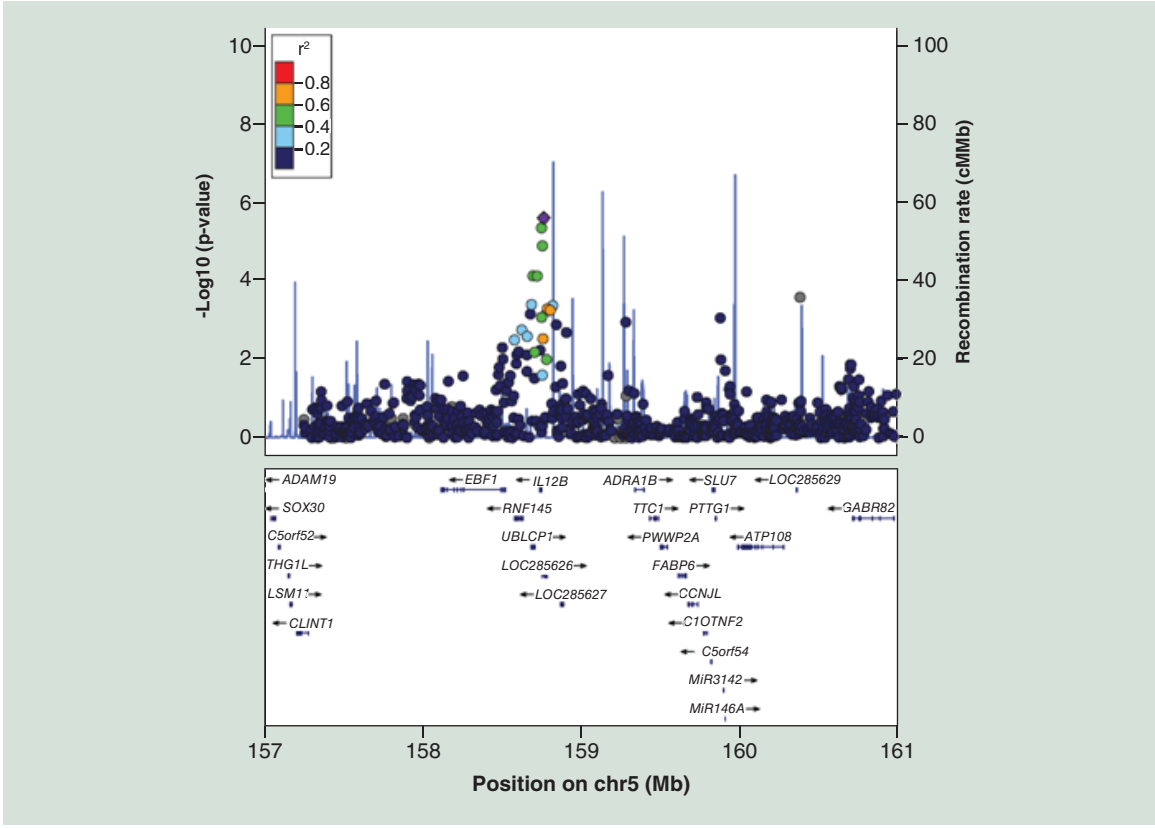

Figure 4. Association scatter plots for psoriasis susceptibility loci in *IL-12B* and *PTTG1* region. The p-values of SNPs (shown as -log10 values in y-axis, from the previous genome-wide single-marker association analysis using the Cochran–Armitage trend test) were plotted against their map positions (x-axis). The color of each SNP spot reflects its r2 with the top SNP (large red diamond) within each association locus, changing from red to white. Estimated recombination rates (based on the combined CHB and JPT samples from the HapMap project) were plotted in light blue. Gene annotations were adapted from the University of California at Santa Cruz Genome Browser (http://genome.ucsc.edu/).
CHB: Han Chinese in Beijing, China; JPT: Japanese in Tokyo, Japan.

with the 62 SNPs and sex and age as covariates exhibited the largest discriminatory ability (AUC = 0.701; 95% CI: 0.685–0.718; $p = 2.2 \times 10^{-16}$) (Figure 4).

Individual locus association analysis in case–control cohort

We tested the association between all 62 SNPs and the risk of psoriasis (Supplementary Table 1) individually through simple single SNP-based logistic-regression models. The allele frequencies and ORs resulting from the 62 logistic regressions were generally consistent with previous GWAS published data [11]. A total of 16 SNPs exhibited significant ($p < 0.05$) associations with psoriasis in our case–control cohort. Among these SNPs, three of them were considered in our previous GWAS analysis [11]. For these three SNPs, we found consistent evidence of association with psoriasis for SNP rs3181224, which is near the *IL12B* gene (OR = 1.72; p = 0.003) evaluated in our previous GWAS as well as this study. The two other SNPs, rs185289 located in the *ADRA1B* gene and rs2431097 located near *PTTG1*, exhibit significant associations in this study (p = 0.048 and p = 0.020, respectively), but not in our original GWAS. The other 13 SNPs that showed evidence for association in our current study were all imputed SNPs, and included SNPs located in the gene regions *LOC285627, CCNJL, C1QTNF2* and the *C5orf54* gene, respectively (rs12374547, rs12658758, rs137997397 and rs80174134), two SNPs located in the *LOC285626* gene (rs117043229 and rs186544545), two SNPs located in the *SLU7* gene (rs148818078 and rs2961945), two SNPs located in the *PTTG1* gene (rs2961914 and rs147918059) and four SNPs located in the *ADRA1B* gene (rs72808152, rs57791007, rs185289 and rs72810112). The most statistically significant associations among these 16 SNPs (p = 0.002) are all located in *ADRA1B* gene region.

Association with plaque & guttate type of psoriasis

We pursued a multiple logistic regression analysis focusing on 713 plaque psoriasis cases against all of our controls for the 62 SNPs. We identified 12 loci that exhibited statistically significant associations with this subphenotype (Table 3). We performed the same analysis for the individuals in our sample with the guttate psoriasis diagnosis (n = 416), and we found significant associations for nine loci. A total of three loci were found to be significant in both the plaque and guttate groups (Table 3). SNPs in the *IL12B, ADRA1B* (three SNPs), *CCNJL* and *MIR3142* were significant in the plaque subgroup but not in the guttate subgroup. SNPs in the *LOC285627* (three SNPs), and *TTC1* genes were significant only in the guttate subgroup. We directly tested the differences in allelic distribution between both plaque and guttate subgroups, and only the SNP in the *ADRA1B* locus was significantly different between the two groups (p = 0.0341).

Association with severity of psoriasis

We explored the association between the SNPs and severity of psoriasis, and observed a strong association between a SNP in the *ADRA1B* locus (p = 0.002; Supplementary Table 1) with severity of psoriasis. For this locus, *ADRA1B* exhibited a stronger association with the moderate-to-severe skin disease group than in the mild disease group (p = 0.005 vs p = 0.025). The OR was >1, supporting the role of this psoriasis risk allele in the development of more severe forms of skin disease. We also found a significant association of IL-12B and PTTG1 locus with severity (p = 0.003 and p = 0.005, respectively).

Association with age at onset of psoriasis

Many of the cases (99.4%) in our psoriasis cohort are type I psoriatics (i.e., age of onset <40 years). We therefore considered an analysis to explore the SNP associations in the *IL12B* and *PTTG1* region with age of onset defined by <20 years old. We found a significant association between SNPs in the *ADRA1B* gene and an earlier age at onset of psoriasis among all psoriatics (p = 0.002). Direct tests of the differences in the association strength of SNPs between the earlier and older subgroups suggested that SNPs in the *IL-12B* and *PTTG1* genes were significantly different between the two groups (p = 0.017 and p = 0.012, respectively) (Supplementary Table 2).

Polygenic inheritance analysis

We considered the prevalence of psoriasis in Chinese Han population to be 0.47% based on a previous reports [30]. In combination, using GCTA, the four independent associated loci (rs72808152, rs57791007, rs185289 and rs72810112) in the *ADRA1B* gene in total explain 46.5% of the phenotype variance of psoriasis under the assumption of a disease prevalence of 0.47%.

Discussion

It is well known that the genetic region harboring variants that confer the greatest risk for psoriasis susceptibility in both European and Chinese populations is the MHC class 1 region. Outside of the MHC region, the *IL12B* gene has been shown to harbor variants that exhibit strong association with type I psoriasis in Caucasian and Chinese populations [4,12,17,18]. Several recent studies have suggested that Chinese populations have a number of unique genetic variants that are associated with psoriasis (for example, *PTTG1*, *NFKB1*, *MTHFR* and *CCDC129*) [9,14]. From their chromosomal locations, both *IL-12B* and *PTTG1* are located in the chromosome 5 region 5q33.3 and very near each other (in fact, ~1.1 Mb away from each other). However, as noted, variants in the *PTTG1* gene have shown independent associations with psoriasis in our previous study [9]. This suggests that more than one gene in the broader *IL12B*/*PTTG1* region might be associated with psoriasis. We are interested in the question of whether or not there are many independent SNPs in this region that are associated with psoriasis. In addition, consistent with additional questions surrounding the 'common disease–common variant' (CD-CV) hypothesis, we are also interested in the question of whether or not the common variants in the region have individual small effects, are there rare variants that have larger effects, and whether or not the SNPs in the region are associated with different subtypes and phenotypic correlates of psoriasis.

To address these questions, we first explored the relationship of variants in the region with psoriasis using an extension of our previously described cohort [11]. We leveraged penalized regression methods to assess the contribution of all SNPs, which included both directly genotyped and imputed SNPs, to psoriasis simultaneously. It is known the penalized regression methods can outperform single marker analysis in selecting the subset of markers most associated with disease among a broader set and their use can significantly improve predictions by

Table 3. Association study of 62 loci used in present studies to be associated with plaque and guttate type of psoriasis in 2271 individual case–control cohort.

Locus	SNP	pos_hg38	A1	Ref. OR	Frq. ctrl.	OR (95% CI)	p-value	OR plaque (95% CI)	P-plaque	OR guttate (95% CI)	P-guttate
UBLCP1	rs117788486	159267648	A		0.019	0.77 (0.46–1.26)	0.297	0.91 (0.51–1.57)	0.738	0.52 (0.23–1.09)	0.103
IL12B	rs3181224	159313842	G	1.42	0.050	1.57 (1.16–2.12)	0.003	1.72 (1.23–2.42)	0.002	1.37 (0.90–2.07)	0.141
IL12B	rs3181216	159325970	T		0.313	1.18 (0.95–1.46)	0.133	1.26 (0.99–1.62)	0.065	1.12 (0.83–1.50)	0.451
IL12B	rs3212219	159327453	A		0.445	0.89 (0.71–1.11)	0.306	0.97 (0.75–1.25)	0.788	0.81 (0.60–1.11)	0.185
LOC285626	rs7709212	159337169	C	0.75	0.451	1.04 (0.84–1.28)	0.723	1.01 (0.79–1.28)	0.949	1.09 (0.82–1.44)	0.572
LOC285626	rs117043229	159352321	C		0.028	0.60 (0.38–0.94)	0.028	0.42 (0.23–0.74)	0.003	0.92 (0.51–1.62)	0.783
LOC285626	rs186544545	159366780	T		0.008	2.04 (1.05–4.04)	0.037	1.92 (0.92–4.02)	0.083	2.22 (0.87–5.46)	0.087
LOC285626	rs2042254	159392273	T		0.284	1.00 (0.82–1.23)	0.987	0.98 (0.77–1.23)	0.837	0.99 (0.74–1.31)	0.920
LOC285627	rs12374547	159406527	A		0.328	0.84 (0.73–0.98)	0.026	0.86 (0.72–1.01)	0.074	0.80 (0.65–0.99)	0.040
LOC285627	rs17056747	159416380	G	1.37	0.088	1.05 (0.36–3.21)	0.927	1.41 (0.45–4.47)	0.542	0.36 (0.02–2.49)	0.376
LOC285627	rs62377649	159417981	A		0.086	1.39 (0.45–4.17)	0.556	1.05 (0.33–3.36)	0.933	3.84 (0.54–80.2)	0.249
LOC285627	rs7702827	159452861	C		0.381	1.19 (0.26–5.40)	0.813	1.69 (0.33–9.55)	0.526	0.83 (0.08–9.28)	0.868
LOC285627	rs4921229	159453332	G		0.381	0.73 (0.16–3.35)	0.676	0.57(0.10–2.88)	0.497	0.90 (0.08–9.03)	0.924
LOC285627	rs6859054	159475993	G		0.233	1.09 (0.92–1.30)	0.315	1.10 (0.91–1.34)	0.334	1.08 (0.85–1.37)	0.538
LOC285627	rs187699618	159478920	T		0.014	0.62 (0.29–1.26)	0.196	0.79 (0.35–1.67)	0.548	0.21 (0.03–0.78)	0.045
LOC285627	rs2421048	159480008	C	0.83	0.450	0.89 (0.72–1.10)	0.274	0.80 (0.62–1.02)	0.074	1.08 (0.81–1.44)	0.588
LOC285627	rs192881597	159503959	C		0.009	1.47 (0.80–2.79)	0.222	1.46 (0.75–2.90)	0.274	1.40 (0.60–3.22)	0.423
LOC285627	rs78337385	159537368	C		0.062	0.79 (0.49–1.26)	0.313	1.04 (0.61–1.81)	0.876	0.52 (0.27–1.00)	0.049
LOC285627	rs73313654	159572660	T		0.076	0.92 (0.60–1.40)	0.688	0.71 (0.43–1.16)	0.181	1.27 (0.72–2.20)	0.398
LOC285627	rs4921510	159573926	G		0.436	0.94 (0.80–1.11)	0.485	0.94 (0.78–1.14)	0.541	0.95 (0.76–1.18)	0.625
LOC285627	rs6556429	159590152	C		0.111	1.13 (0.91–1.41)	0.263	1.14 (0.89–1.46)	0.312	1.21 (0.89–1.63)	0.223
LOC285627	rs151167212	159627774	T		0.015	0.74 (0.35–1.53)	0.420	0.69 (0.29–1.58)	0.389	0.96 (0.32–2.65)	0.938
LOC285627	rs116600175	159670773	A		0.029	0.82 (0.53–1.26)	0.368	0.60 (0.34–1.01)	0.058	1.17 (0.67–2.02)	0.568
LOC285627	rs66845594	159681500	T		0.030	0.88 (0.55–1.41)	0.610	0.93 (0.54–1.57)	0.796	0.86 (0.42–1.67)	0.666
ADRA1B	rs6895322	159697830	G		0.146	1.14 (0.94–1.38)	0.197	1.24 (1.00–1.55)	0.049	0.97 (0.74–1.27)	0.838
ADRA1B	rs77261928	159706927	G		0.112	0.87 (0.68–1.11)	0.273	0.85 (0.64–1.13)	0.264	0.88 (0.63–1.22)	0.461
ADRA1B	rs1966491	159724028	C		0.212	1.17 (0.69–1.99)	0.549	1.04 (0.57–1.87)	0.904	1.39 (0.66–2.93)	0.379
ADRA1B	rs918516	159728241	C		0.279	0.95 (0.81–1.10)	0.475	0.97 (0.81–1.15)	0.695	0.94 (0.76–1.16)	0.546
ADRA1B	rs1118982	159740340	A		0.215	0.96 (0.57–1.63)	0.877	1.06 (0.58–1.91)	0.858	0.85 (0.40–1.80)	0.668
ADRA1B	rs111352246	159742063	T		0.051	1.28 (0.96–1.71)	0.087	1.37 (1.00–1.89)	0.052	1.13 (0.75–1.68)	0.551
ADRA1B	rs31680	159846205	A		0.173	0.84 (0.70–1.00)	0.050	0.82 (0.67–1.00)	0.051	0.88 (0.68–1.12)	0.298
ADRA1B	rs72808152	159875269	T		0.167	0.74 (0.61–0.90)	0.003	0.73 (0.58–0.91)	0.005	0.78 (0.60–1.02)	0.078
ADRA1B	rs142034556	159897095	A		0.017	0.63 (0.33–1.18)	0.156	0.79 (0.39–1.56)	0.502	0.40 (0.13–1.06)	0.088
ADRA1B	rs57791007	159904343	T		0.016	0.53 (0.29–0.97)	0.041	0.56 (0.27–1.12)	0.114	0.51 (0.21–1.13)	0.115
ADRA1B	rs185289	159908845	G	1.15	0.170	1.19 (1.00–1.41)	0.048	1.27 (1.05–1.54)	0.016	1.09 (0.85–1.38)	0.492
ADRA1B	rs72810112	159952668	A		0.123	1.39 (1.14–1.72)	0.002	1.31 (1.03–1.66)	0.027	1.48 (1.12–1.95)	0.005
TTC1	rs11135089	160058219	T		0.201	1.16 (0.99–1.36)	0.073	1.05 (0.87–1.26)	0.599	1.30 (1.05–1.62)	0.017

A1: Minor allele; GWAS: Genome-wide association study; OR: Odds ratio; p-value: p-values for the coefficients estimated from additive logistic-regression models adjusted for age and gender; Ref. OR: Effect size estimate calculated from the reference GWAS data; OR estimates are expressed relative to the minor allele.

Table 3. Association study of 62 loci used in present studies to be associated with plaque and guttate type of psoriasis in 2271 individual case–control cohort (cont.).

Locus	SNP	pos_hg38	A1	Ref. OR	Frq. ctrl.	OR (95% CI)	p-value	OR plaque (95% CI)	P-plaque	OR guttate (95% CI)	P-guttate
PWWP2A	rs73817277	160089101	G		0.034	0.80 (0.55–1.16)	0.231	0.79 (0.51–1.20)	0.279	0.81 (0.46–1.37)	0.445
FABP6	rs10041333	160227102	C	1.13	0.095	1.17 (0.94–1.45)	0.155	1.15 (0.90–1.47)	0.264	1.29 (0.96–1.72)	0.086
CCNJL	rs17057577	160267383	G		0.017	1.31 (0.76–2.28)	0.336	1.16 (0.62–2.13)	0.642	1.54 (0.69–3.42)	0.289
CCNJL	rs12658758	160272105	G		0.480	0.83 (0.72–0.96)	0.011	0.83 (0.71–0.97)	0.022	0.83 (0.69–1.01)	0.066
CCNJL	rs117802788	160294235	C		0.016	0.62 (0.34–1.10)	0.106	0.69 (0.34–1.34)	0.290	0.48 (0.19–1.09)	0.094
CCNJL	rs13180487	160306706	C	1.3	0.030	1.34 (0.82–2.20)	0.246	1.40 (0.81–2.42)	0.231	1.31 (0.62–2.69)	0.465
CCNJL	rs6556490	160311702	G		0.118	1.02 (0.80–1.29)	0.888	1.05 (0.80–1.36)	0.746	0.97 (0.69–1.35)	0.858
C1QTNF2	rs139083494	160334145	T		0.012	0.60 (0.29–1.20)	0.151	0.62 (0.27–1.34)	0.230	0.52 (0.17–1.38)	0.212
C1QTNF2	rs6878970	160344746	T		0.165	1.28 (0.98–1.67)	0.075	1.35 (1.00–1.84)	0.054	1.20 (0.82–1.76)	0.338
C1QTNF2	rs137997397	160358060	T		0.019	0.45 (0.25–0.80)	0.007	0.50 (0.25–0.97)	0.044	0.39 (0.17–0.85)	0.023
C1QTNF2	rs72814353	160358410	A		0.155	1.24 (0.93–1.65)	0.146	1.10 (0.79–1.53)	0.559	1.41 (0.95–2.10)	0.090
C1QTNF2	rs143456165	160366168	C		0.037	0.66 (0.42–1.03)	0.070	0.81 (0.49–1.32)	0.405	0.49 (0.24–0.93)	0.037
C1QTNF2	rs2910184	160368047	T		0.208	1.02 (0.83–1.27)	0.837	0.96 (0.75–1.23)	0.772	1.07 (0.80–1.44)	0.644
C5orf54	rs80174134	160385936	T		0.018	0.50 (0.26–0.94)	0.034	0.55 (0.25–1.11)	0.105	0.47 (0.18–1.12)	0.106
SLU7	rs76499425	160400850	T		0.015	1.26 (0.72–2.25)	0.422	1.15 (0.59–2.21)	0.681	1.28 (0.59–2.74)	0.532
SLU7	rs148818078	160410541	C		0.038	1.68 (1.18–2.42)	0.005	1.75 (1.17–2.61)	0.006	1.44 (0.85–2.38)	0.166
SLU7	rs2961945	160410567	T		0.480	0.75 (0.62–0.91)	0.003	0.75 (0.61–0.93)	0.009	0.76 (0.58–0.98)	0.035
SLU7	rs3811999	160419865	T	1.09	0.163	1.21 (0.98–1.48)	0.072	1.16 (0.92–1.46)	0.211	1.29 (0.97–1.70)	0.079
PTTG1	rs12054773	160421280	T		0.020	0.68 (0.41–1.14)	0.148	0.80 (0.45–1.38)	0.425	0.41 (0.15–0.93)	0.049
PTTG1	rs2961914	160441951	A		0.165	1.47 (1.06–2.06)	0.023	1.42 (0.97–2.09)	0.072	1.38 (0.87–2.20)	0.172
PTTG1	rs147918059	160451401	G		0.008	2.51 (1.33–4.88)	0.005	2.50 (1.21–5.21)	0.013	2.31 (1.00–5.26)	0.047
MIR3142	rs2431697	160452971	C	1.28	0.182	0.94 (0.68–1.28)	0.689	1.01 (0.70–1.45)	0.949	0.91 (0.58–1.40)	0.664
MIR3142	rs2909733	160456645	T	0.83	0.233	1.08 (0.90–1.30)	0.418	1.10 (0.88–1.36)	0.405	1.01 (0.78–1.31)	0.949
MIR3142	rs2431097	160463878	T	1.09	0.209	1.24 (1.03–1.48)	0.020	1.28 (1.04–1.57)	0.019	1.13 (0.88–1.45)	0.327
MIR3142	rs76535237	160466772	C		0.054	1.27 (0.93–1.72)	0.128	1.26 (0.89–1.79)	0.191	1.40 (0.93–2.10)	0.109

A1: Minor allele; GWAS: Genome-wide association study; OR: Odds ratio; p-value: p-values for the coefficients estimated from additive logistic-regression models adjusted for age and gender; Ref. OR: Effect size estimate calculated from the reference GWAS data; OR estimates are expressed relative to the minor allele.

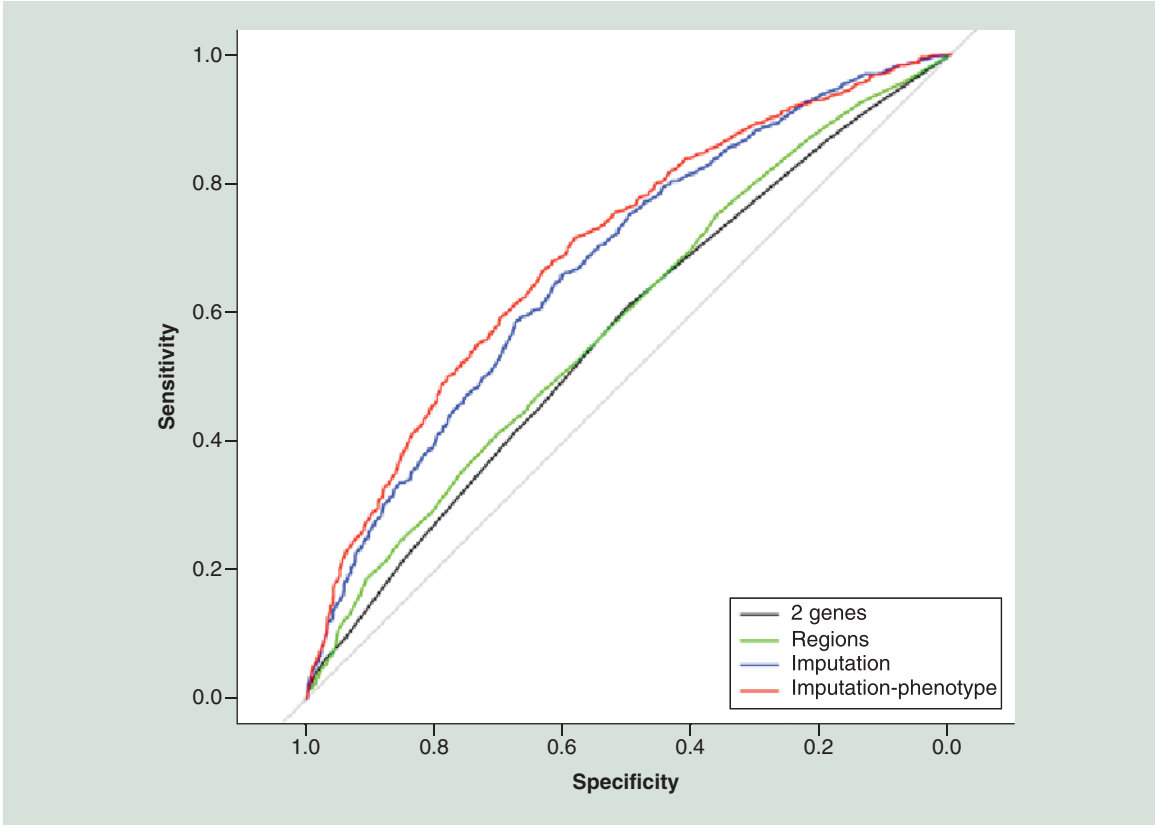

Figure 5. Receiver operating characteristic curve of for predictive models in different SNP groups.

accommodating correlations between the SNPs (due to, e.g., LD) by producing a sparse model despite testing many predictors or SNPs in the model [31,32]. Penalized methods, such as the LASSO method we chose, are even more particular, selecting only one or several variables per causal locus.

Direct individual SNP association analysis applied to our entire cohort of cases and controls, suggested that only eight SNPs of 211 total SNPs emerged as statistically significantly associated with psoriasis. Three selected SNPs in the *IL12B* gene locus were significantly associated, two of which (rs3213094 and rs7709212) had been identified as significantly associated with psoriasis in our previous study [11]. One selected SNP rs2431697 around *PTTG1* locus was also identified as significantly associated with psoriasis. Including 2171 imputed SNPs in a LASSO model resulted in 62 SNPs being selected as associated with psoriasis. The 62 SNPs are located between *IL12B* and *PTTG1* gene region, which covers a total of 13 genes. As shown in Figure 4, these SNPs were in or near all 13 genes. Strongly associated SNPs with psoriasis were within *IL12B* region as in our previous GWAS study, consistent with our analysis of psoriasis using our imputed dataset.

The most significant locus and largest number of associated SNPs were all located in *ADRA1B* gene in our current analyses (which is between *IL-12B* and *PTTG1*), suggesting that other SNPs may play a role as well. The benefits of our LASSO model are that by shrinking the size of the coefficients reflecting the strength of the associations with the SNPs and pushing the coefficients toward zero. For SNPS with little or no apparent effect on a trait toward zero, only SNPs that are likely to have independent yet simultaneous effects on psoriasis are identified [25,31]. Multiple testing issues are also accommodated [33].

In addition, we also consider whether different sets SNPs exhibited stronger associations with psoriasis onsets. We compared a number of SNP sets associated with psoriasis (Figure 5) and found that a logistic-regression model using all the 62 imputed SNPs and age and gender as covariates was significantly better than models that included subsets of SNPs. We also explored whether any sets of SNPs were uniquely associated with clinical psoriasis-related phenotypes. We found that the SNPs in the *ADRA1B* gene were most significantly associated with the plaque psoriasis subgroup but not in the guttate subgroup. *ADRA1B* SNPs also showed a stronger association with

moderate-to-severe skin disease group and an earlier age at onset of psoriasis, highlighting the contribution of *ADRA1B* to psoriasis in the Chinese population and implies that psoriasis is an extremely heterogeneous disease with various clinical features. In addition, in GCTA analysis, four independent associated loci in the *ADRA1B* gene estimated explain 46.5% of the phenotype variance of psoriasis. It still yet needs to be substantiated through further studies especially in different ethnic population.

It has been shown that *IL-12B* and *PTTG1* harbor SNPs associated with psoriasis and both genes are involved in the *IL-23* pathway. Our finding that SNPs in the *ADRA1B* gene are the most strongly associated with psoriasis and aspects of psoriasis pathobiology are of great interest, since the *ADRA1B* gene is one subtype of G-protein-coupled receptor superfamily of α-1-AR that encodes the α-1B-adrenergic receptor which activates mitogenic responses and regulates growth and proliferation of many cells. *ADRA1B* also plays a role in related pathways involved in signaling by GPCR and activation of cAMP-dependent PKA that could have biological roles in psoriasis pathobiology.

Future perspective

In short, our analyses revealed some differences between the whole genome, psoriasis diagnosis-defined case–control analysis and our subphenotypes analysis. *ADRA1B* is preferentially associated with psoriasis in Chinese population and might contribute to the complexity of psoriasis clinical features. We have found some specific genotypes of SNPs associated with plaque-type, moderate-to-severe and an earlier age at onset psoriasis. Surprisingly, we did not find very strong evidence to support *IL12B* and *PTTG1* with onset of psoriasis. The role of variant alleles in the *ADRA1B* gene needs replication studies to evaluate their ultimate contribution to psoriasis, psoriasis onset and psoriasis-related clinical features.

Summary points

- Direct individual SNP analysis including 2171 imputed SNPs in a least absolute shrinkage and selection operator (LASSO) model resulted in 62 SNPs being selected as associated with psoriasis.
- The 62 SNPs are located between *IL12B* and *PTTG1* gene region.
- Slightly different subsets of these SNPs exhibited associations with these subphenotypes.
- The most significant locus and largest number of associated SNPs were all located in *ADRA1B* gene, which is between the *IL-12B* and *PTTG1* genes in the 5q33.3 region.
- SNPs in the *ADRA1B* gene were most significantly associated with the plaque psoriasis subgroup.
- SNPs in the *ADRA1B* gene were most significantly associated with the moderate-to-severe skin disease group.
- SNPs in the *ADRA1B* gene were most significantly associated an earlier age at onset of psoriasis.
- *ADRA1B* is preferentially associated with psoriasis in Chinese population and might contribute to the complexity of psoriasis clinical features.
- The association of variants in the *ADRA1B* gene with psoriasis could explain why variants in the broader *IL-12B*, *ADRA1B* and *PTTG1* gene region were associated with psoriasis previously.

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