Journal of Dermatological Science 93 (2019) 8–13

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Original Article

Genetic polymorphism predicting Methotrexate efficacy in Chinese patients with psoriasis vulgaris

Ye hong Kuang[a,1], Yan Lu[a,1], Ke xiang Yan[b], Pan pan Liu[a], Wang qing Chen[a], Min xue Shen[a], Yi jing He[a], Li sha Wu[c], Qun Shi Qin[a], Xing chen Zhou[a], Jie Li[a], Juan Su[a], Cheng zhi Lv[d], Wu Zhu[a,*], Xiang Chen[a,*]

[a]Department of Dermatology, XiangYa Hospital, Hunan Key Laboratory of Skin Cancer and Psoriasis, Central South University, Changsha, Hunan, China
[b]Department of Dermatology, Hua Shan Hospital, Fu dan University, Shanghai, China
[c]Institute of medical science, Xiangya Hospital, Central South University, Changsha, Hunan, China
[d]Clinical Centre for Psoriasis, Dalian Skin Diseases Hospital, Dalian, Liaoning, China

ARTICLE INFO

Article history:
Received 25 December 2017
Received in revised form 4 June 2018
Accepted 24 June 2018

Keywords:
Methotrexate
Psoriasis
Efficacy
SNP
Whole exome sequencing
MassARRAY

ABSTRACT

Background: Methotrexate is the first systemic therapeutics of psoriasis. It is reported that 40% of the patients achieved a PASI75 after 12 weeks with a small dose of methotrexate (15 mg / w) treatment. So far there is not any large-scale exome sequencing been used to predict the efficacy of methotrexate in the treatment of psoriasis vulgaris.
Objective: To analyze the genetic polymorphism to predict methotrexate efficacy in Chinese patients with psoriasis vulgaris.
Methods: In this study, we used the whole exon high-throughput sequencing technology to detect the DNA sequence of 22 psoriasis vulgaris patients (11 responders, 11 non-responders) treated with methotrexate and captured approximately 236 variants with statistically significant in the whole exon sequencing, then in accordance with statistical differences and clinical relevance, we further selected 36 SNPs and 14 SNPs that have been reported in articles associated with the response of methotrexate. We used MassARRAY method to verify the 50 SNPs in 100 psoriatic patients treated with methotrexate.
Results: We found 3 SNPs, rs216195T > C in SMG6, rs1050301G > A in IMMT, rs2285421T > C in UPK1A which might associate with the drug response of methotrexate.
Conclusion: We have searched 3 new SNPs that could predict the efficacy of methotrexate in psoriasis vulgaris to some extent, providing a theoretical basis for precision medicine of methotrexate in future.

1. Introduce

Psoriasis is an immune-mediated chronic, relapsing, inflammatory, polygene genetic disease characterized as erythrocyte plaque, hyperplasia of keratinocytes and infiltration of lymphocytes [1–4]. Currently, the clinical drugs for psoriasis is increasing, the traditional drugs included methotrexate, retinoids, and cyclosporine A, etc [5]. There are a lot of new biological agents included TNF-α inhibitors, anti-IL-17, anti-IL-23 antibodies in clinical or under clinical studies. Although the treatment of psoriasis has entered a new era of biological agents, traditional medicines still hold an important position in the treatment of psoriasis. The individual treatment of psoriasis has been paid more and more attention in the modern society. The development of large-scale high-throughput sequencing technology provides a platform for the precise medication of psoriasis. Studies have been reported that the SNPs can predict the efficacy of biological agents [6,7]. Methotrexate is the most widely and effective used for the treatment of moderate-to-severe psoriasis in system medicine. Low dose methotrexate (15 mg / w) is used to cure all kinds of severe psoriasis and has the advantages of high efficacy, fast onset and good tolerance. Besides, methotrexate combined with biological agents can improve the efficacy of biological agents to reduce the generation of anti-antibodies and vascular events [8–10]. However, there are individual differences in the efficacy of methotrexate, the efficiency is 40% as reported, several SNPs has been reported to be associated with good response to methotrexate treatment in patients with psoriasis, it has been reported that the gene polymorphisms that influence the transporter and metabolic enzymes of methotrexate metabolic pathway affect

* Corresponding authors at: Department of Dermatology, XiangYa Hospital, Central South University, 87 XiangYa Road, Changsha, Hunan, 410008, China.
E-mail addresses: zhuwu70@hotmail.com (Z. Wu), chenxck@126.com (C. Xiang).
[1] The first two authors contributed equally to this work.

https://doi.org/10.1016/j.jdermsci.2018.06.009
0923-1811/

the clinical efficacy of methotrexate, but there is no whole exon sequencing method used to predict and analyze the SNPs associated with efficacy of methotrexate. At present, in the study, we selected 100 psoriasis patients treatment with methotrexate (63 responders, 37 non-responders) and found 3 SNPs might respond to methotrexate therapy in patients with psoriasis by MassARRAY method.

2. Material and methods

2.1. Patients

The research project was approved by Ethic Committee of XiangYa Hospital, the registrated number in Chinese Clinical Trial Registry online with the registration number is ChiCTR−OCH-14004518. The informed consent was obtained from all patients. All patients had received methotrexate for treatment of psoriasis for 12 weeks. Part of the patients were took oral folic acid as adjuvant therapy.

2.2. Evaluation of efficacy

Patients were stratified as: (i) 'responders'——those with clearly documented clinical improvement, using the Psoriasis Area and Severity Index (PASI), i.e. > 75% reduction in PASI from the start of methotrexate therapy; and (ii) 'non-responders'——those patients who showed no clear improvement, i.e. < 50% improvement in PASI while on therapy. In cases where the PASI score was not recorded, an explicit statement of response to therapy recorded in the clinical records was acceptable.

2.3. DNA extraction

DNA was extracted from the Whole blood cells (200 u L) using a QIAamp DNA Mini Kit (250) (Qiagen) according to manufacturer's instructions. All the blood samples were stored at −80 °C until used.

2.4. Whole exome sequencing

The qualified genomic DNA sample was randomly fragmented by Covaris technology and the size of the library fragments was mainly distributed between 200bp and 300bp. Then adapters were ligated to both ends of the resulting fragments. Extracted DNA was amplified by ligation-mediated PCR (LM-PCR), purified, and hybridized to the exome array for enrichment. Non-hybridized fragments were then washed out. Captured LM- PCR products were subjected to Agilent 2100 Bioanalyzer and quantitative PCR to estimate the magnitude of enrichment. Each qualified captured library was then loaded on Illumina Hiseq platforms, and we performed high-throughput sequencing for each captured library to ensure that each sample met the desired average sequencing coverage. Sequencing-derived raw image files were processed by Illumina basecalling Software for base-calling with default parameters and the sequence data of each individual was generated as paired-end reads, which was defined as "raw data" and stored in FASTQ format.

2.5. Sequenom MassArray analysis

SNPs were genotyped using Sequenom® MassARRAY™ (Sequenom Inc., San Diego, CA, U.S.A.) technology.

2.6. Data statistics and analysis

Unpaired two sample *t*-test was used to evaluate whether there are statistical relevance between the baseline of age, body mass index (BMI) and PASI with efficacy treated with methotrexate therapy respectively. $\chi 2$ test (Fisher's exact when the number is less than 40) was used to compare sex with efficacy treated with methotrexate therapy. Logistic regression analysis was to get OR95%CI and *p* value to text the relation between SNPs with efficacy after adjust for the baseline of age, BMI and PASI and sex. The statistical software is IBM SPSS Statistics 23.

3. Results

3.1. Clinical features of the psoriatic patients

In the study, all patients are Han Chinese, the baseline of age, BMI and sex of the patients had no significant difference between the responder and the non-responder group. But the baseline of PASI can influence the efficacy ($p < 0.05$), it will be corrected by multivariable logistic regression analysis. The details were seen in Table 1.

3.2. Whole exome sequencing analysis

In this study, we selected 22 patients for whole exome sequencing analysis to reconcile the clinical findings with molecular data in psoriatic patients, then we obtained 31, 522 variants. And the most significant SNPs rs483532 (Fisher. $p = 1.84 \times 10^{-4}$) as shown in Fig. 1. After all the variant base-calling, Fisher's exact-test and the Cochran–Armitage trend as well as different genetic models (dominant, recessive and general) analyses were used to identify variants that were significantly associated with drug response ($p < 0.05$). In fact, 236 variants were found to be associated with drug efficacy (Table S1). Moreover, according to the Fisher. p, mutation location and significance, 36 positive SNPs were selected and validated by MassArray in independent samples.

3.3. Univariate analysis of fifty positive SNPs

In this study, we selected 14 SNPs previously reported associated with response to methotrexate therapy, besides 36 SNPs, total 50 SNPs for validation by MassARRAY method. For quality control, only SNPs with a frequency above 5% and with a

Table 1
The Demographic data of the patients in two phase.

	Discovery Phase(n = 22)		p	Verification Phase(n = 100)		p
	PASI reduce<50%	PASI reduced>75%		PASI reduce<50%	PASI reduce>75%	
Age(mean ± SD)	44 ± 13	48 ± 15	0.569	53 ± 13	51 ± 14	0.803
Male	6(50.0%)	6(50.0%)	1.000[a]	26(39.4%)	40(60.6%)	0.490
Female	6(50.0%)	6(50.0%)		11(32.4%)	23(67.6%)	
BMI (mean ± SD)	23.25 ± 4.80	23.71 ± 3.05	0.793	23.90 ± 2.68	23.74 ± 3.24	0.805
0 W PASI (mean ± SD)	14.82 ± 5.75	14.76 ± 7.61	0.985	11.13 ± 7.62	15.72 ± 8.17	**0.007**

[a] Fisher's exact-test (n<40); PASI: Psoriasis Area and Severity Index; 0 W PASI: the baseline of PASI; BMI: Body Mass Index; SD: standard Deviation.

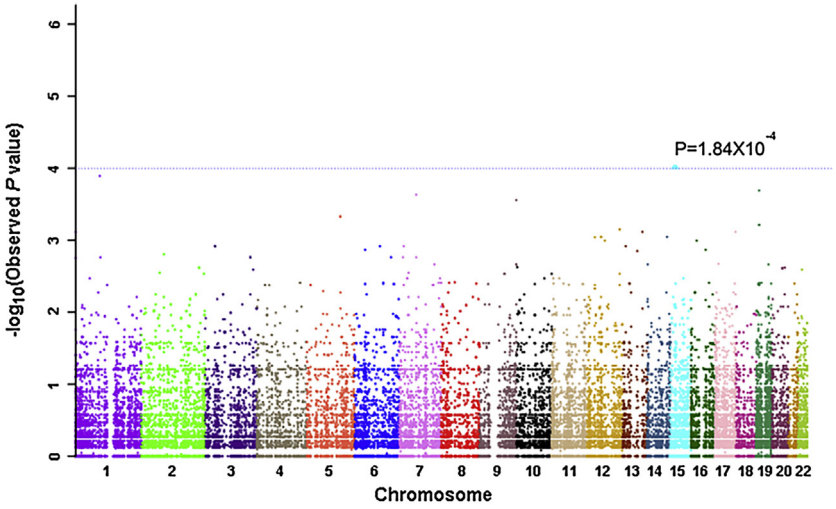

Fig. 1. Manhattan plot of allele association tests of all SNPs (single nucleotide polymorphisms). The different colors mean different chromosomes. Fisher's exac *t*-test, the most significant SNPs rs483532, Fisher, $p = 1.84 \times 10^{-4}$.

genotyping rate ≥95% and agreed with the Hardy–Weinberg equilibrium ($p > 0.05$) were included in the final statistical analysis. So a total of 42 SNPs were qualified. Among other 8 unqualified SNPs, 7 SNPs were only not agreed with the Hardy–Weinberg equilibrium, 1 SNPs were rejected with a genotyping rate <95% and not agreed with the Hardy–Weinberg equilibrium too. The details were seen in Table 2.

3.4. Binary logistic regression analysis of 50 SNPs

First, we performed single-factor binary logistic regression analysis for the 50 SNPs respectively and got a crude P value. Then, in order to adjust for the baseline of age, BMI and PASI and sex of onset of psoriasis, we introduced these factors together with the SNP into independent variables for binary logistic regression analysis and got another adjusted P value. Finally, we got the 6 SNPs ($P < 0.05$). The result that $P < 0.05$ were seen in Table 3, the others were seen in Table S3. Although these differences became non-significant ($P > 0.001$) with Bonferroni correction ($n = 50$), the variation of these SNPs is different between responders and non-responders to MTX treatment. So we then excluded the last two SNPs rs2838956 and rs3744786, because they did not meet gene dose dependence, and we conducted a multivariate binary logistic regression analysis using the other 4 SNPs together with factors such as the baseline of, age, BMI and PASI and sex of onset of psoriasis as independent variables in the next paragraph.

3.5. Multivariable logistic regression analysis of SMG6 rs216195T > G, IMMT rs1050301 g > a and UPK1A rs228521 t > c

As mentioned in the previous paragraph, We used multivariable logistic regression analysis to evaluate the 4 SNPs with adjustment for the baseline of age, BMI and PASI and sex to analysis the data and found 3 SNPs ($p < 0.05$). For SMG6 rs216195T > G variation, 12 patients carried the SMG6 rs216195TT genotype, 51 patients carried the SMG6 rs216195 GT genotype, and 36 patients carried the SMG6 rs216195GG genotype. SMG6 rs216195GT genotype (OR = 5.574, 95% CI = 1.1035–30.032, p = 0.046) and SMG6 rs216195GG genotype (OR = 6.147, 95% CI = 1.114–33.922, p = 0.037) were all probably associated with the effective response compared to the TT genotype respectively, For IMMT rs1050301 G > A variation, 28 patients carried the IMMT rs1050301GG genotype, 44 patients carried the IMMT rs1050301GA genotype, and 27 patients carried the IMMT rs1050301AA genotype. IMMT rs1050301AA (OR = 0.246, 95% CI = 0.061-0.990, p = 0.048) was probably associated with the ineffective response compared to the GG genotype, For UPK1A rs2285421T > C variation, 20 patients carried the UPK1A rs2285421TT genotype, 45 patients carried the UPK1A rs2285421TC genotype and 35 patients carried the UPK1A rs2285421CC genotype. UPK1A rs2285421TC genotype (OR = 4.420, 95% CI = 1.175–16.630, p = 0.028) and rs2285421CC genotype (OR = 2.795, 95% CI = 0.689–11.331, p = 0.029) were all probably associated with the effective response compared to the TT genotype respectively (Table 4). The details were seen in Table 3.

4. Discussion

Methotrexate is widely used in the treatment of many autoimmune diseases, including rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease (IBD). In the past 10 years, there have been preliminary studies that gene SNPs are response to the drug-effectiveness and toxicity of methotrexate in the treatment of disease. Genetic variation in genes involved in methotrexate transmembrane transport and mediation could influence efficacy of the methotrexate. It reported that genetic variation in gene ABCC1, ABCG2, and DHFR are associated with response to methotrexate therapy in psoriasis [11,12], in rheumatoid arthritis, there are also studies reported genetic variation in gene MTHFR, ATIC, SLC19A1, and GGH are significantly associated with efficacy of methotrexate [13–15]. Elena V. Tchetina reported that increased baseline gene expressions of RUNX2, p21 and caspase 3 in the peripheral blood might predict better responses to methotrexate therapy [16]. However, these fragmented genetic loci need to be further validated in clinical, and because of the disease and geographical differences, the findings also need to be validated in multicenter large sample populations. With the development of second-generation sequencing technologies and the reduction of sequencing costs, it is possible to identify genetic variations of all the protein coding regions (exomes) and search for new genes that predict the efficacy of methotrexate quickly and efficiently, providing a new target gene for methotrexate applied to patients for individualized and precise medication.

In this study, we performed all exon sequencing of 22 patients with psoriasis vulgaris treated with methotrexate, and we searched 31,522 variants which might affect the efficacy of methotrexate according the p value ($p < 0.05$). So, according the Fisher. p value, OR and the gene function, we selected 36 significant

Table 2
Quality control of 50 positive SNPs.

Number	SNP ID	Gene	Chr	MAF	Location	Genotype	n	Genotyping rate	p for H–W
1	rs216195	SMG6	17	0.4014	missense_variant	TT/GT/GG	12/51/37	99%	0.346398562
2	rs2290610	IL5RA	3	0.4281	missense_variant	TT/CT/CC	31/49/20	99%	0.856440726
3	rs4689254	ZBTB49	4	0.1508	missense_variant	GG/AG/AA	37/54/7	97%	**0.040151884**
4	rs2499953	MMP26	11	0.1667	missense_variant	AA/AG/GG	59/37/5	100%	0.771325839
5	rs1130409	APEX1	14	0.3756	missense_variant	TT/GT/GG	34/45/20	98%	0.518747634
6	rs2240432	INPP5J	22	0.3784	missense_variant	GG/AG/AA	22/46/32	99%	0.417197593
7	rs2285421	UPK1A	19	0.3413	missense_variant	TT/CT/CC	20/45/35	100%	0.427871704
8	rs818817	SLC22A14	3	0.1637	missense_variant	AA/AG/GG	5/37/59	100%	0.771325839
9	rs2301354	KRT36	17	0.4844	missense_variant	GG/AG/AA	54/33/13	99%	**0.043125755**
10	rs2235592	EMILIN3	20	0.2831	missense_variant	CC/CT/TT	19/49/30	98%	0.899483105
11	rs2071588	KRT81	12	0.2788	missense_variant	CC/GC/GG	31/61/6	97%	**0.000819461**
12	rs2306359	BNIP2	15	0.1779	missense_variant	TT/GT/GG	50/46/3	99%	**0.046941295**
13	rs2017252	ZNF273	7	0.4559	missense_variant	AA/CA/CC	29/47/21	96%	0.878522401
14	rs17138089	CRCP	7	0.0978	missense_variant	AA/AG/GG	47/40/13	99%	0.365712296
15	rs1050301	IMMT	2	0.3293	missense_variant	GG/AG/AA	28/45/27	99%	0.269315996
16	rs1051340	TRIP11	14	0.2714	missense_variant	CC/CT/TT	49/41/10	99%	0.775985656
17	rs1048197	SMPD2	6	0.1100	missense_variant	CC/CT/TT	60/36/4	99%	0.60524231
18	rs4871827	DEPTOR	8	0.2995	missense_variant	GG/AG/AA	17/43/41	100%	0.357755973
19	rs2272094	CRTAM	11	0.4497	missense_variant	AA/AG/GG	35/46/19	99%	0.508012836
20	rs7927048	ADAMTS8	11	0.4692	missense_variant	CC/GC/GG	12/55/20	**86%**	**0.013361838**
21	rs30489	TCF7	5	0.1534	missense_variant	GG/AG/AA	44/40/17	100%	0.187020572
22	rs1529151	ECT2L	6	0.2762	missense_variant	GG/AG/AA	49/45/6	99%	0.341632942
23	rs299290	HMMR	5	0.3127	missense_variant	TT/CT/CC	27/51/21	98%	0.810300859
24	rs3749172	GPR35	2	0.4840	missense_variant	AA/CA/CC	10/44/46	99%	0.991599255
25	rs34639489	ERV3-1	7	0.4714	missense_variant	CC/CT/TT	34/40/26	99%	0.061598824
26	rs3742264	CPB2	13	0.3073	missense_variant	CC/CT/TT	63/35/3	100%	0.531971058
27	rs1051266	SLC19A1	21	0.4886	missense_variant	TT/CT/CC	18/57/24	98%	0.145176604
28	rs10987746	ENG	9	0.4796	intron_variant	CC/CT/TT	55/34/12	100%	0.057062208
29	rs11161732	COL24A1	1	0.3702	missense_variant	GG/GA/AA	30/51/20	100%	0.919542442
30	rs11702425	COL18A1	21	0.3199	synonymous_codon	CC/CT/TT	7/35/59	100%	0.589706042
31	rs32209	FBN2	5	0.2328	missense_variant	TT/CT/CC	13/47/40	99%	0.749802984
32	rs12681874	GGH	8	0.2386	intron_variant	CC/CT/TT	37/45/14	95%	0.876015576
33	rs12995526	ATIC	2	0.4683	intron_variant	CC/CT/TT	57/38/6	100%	0.893929767
34	rs156697	GSTO2	10	0.4407	missense_variant	AA/AG/GG	54/41/6	100%	0.599607729
35	rs10030708	TMPRSS11F	4	0.3289	missense_variant	CC/CT/TT	26/44/29	98%	0.319581416
36	rs1476413	MTHFR	1	0.2512	intron_variant	TT/CT/CC	6/33/62	100%	0.499046565
37	rs248709	CTXN3	5	0.3466	missense_variant	AA/AT/TT	45/27/29	100%	**7.61602E-06**
38	rs17731538	ABCG2	4	0.096	intron_variant	GG/GA	13/89	100%	0.486938014
39	rs1136001	NTAN1	16	0.3433	missense_variant	GG/GT/TT	43/41/13	96%	0.453652995
40	rs2838956	LOC107987304	21	0.4545	missense_variant	AA/AG/GG	23/57/18	97%	0.120347858
41	rs228104	SLC37A1	21	0.1801	missense_variant	GG/AG/AA	18/54/29	100%	0.465493521
42	rs35592	ABCC1	16	0.3704	intron_variant	CC/TT	12/80	92%	**8.66765E-22**
43	rs3821353	ATIC	2	0.2454	intron_variant	GG/GT/TT	27/31/42	99%	**0.000158118**
44	rs753778	SLC45A4	8	0.3754	missense_variant	GG/AG/AA	57/36/7	100%	0.22674307
45	rs3744786	KRT32	17	0.3255	missense_variant	TT/CT/CC	52/35/14	100%	0.058798105
46	rs10489990	CD207	2	0.2843	missense_variant	GG/AG/AA	58/36/6	99%	0.920742285
47	rs7499	COL18A1	21	0.4673	intron_variant	AA/AG/GG	25/56/20	100%	0.304152649
48	rs7563206	ATIC	2	0.4018	intron_variant	CC/CT/TT	57/36/7	99%	0.714570206
49	rs7986131	LMO7	13	0.3131	missense_variant	TT/CT/CC	21/49/31	100%	0.90450551
50	rs843358	EIF2B5	3	0.3900	missense_variant	AA/AG/GG	19/53/26	97%	0.443663049

SNP: single nucleotide polymorphism; Chr: chromosome; MAF: minor allele frequency; H–W: Hardy–Weinberg equilibrium; missense variant: a type of non-synonymous; bold in Genotyping rate mean the result is unqualified; bold in p for H–W mean the result is not consistent with Hardy–Weinberg equilibrium.

statistically significant SNPs and other 14 SNPs that have been reported in articles associated with the response of methotrexate to verify with MassARRAY method in other 100 methotrexate treated psoriasis patients.

After the verification result came out, we firstly used the single factor binary logistic regression method to analyze these 50 SNPs separately and got a crude P value. Then we adjusted the baseline of age, BMI and PASI and sex of onset of psoriasis with logistic regression analysis and got another adjusted P value. Among these 50 SNPs, there are 6 SNPs ($p < 0.05$). However these differences became non-significant ($P > 0.001$) with Bonferroni correction ($n = 50$). Although these differences are not statistically significant, the variation of these SNPs is different between psoriasis patients responded and non-responded to MTX treatment, which implied that these SNPs still has some meaning to some extent. So we still try to make further statistical analysis based on this results. We excluded 2 SNPs that did not meet gene dose dependence and conducted a multivariate binary logistic regression analysis using the other 4 SNPs together with factors such as the baseline of age, BMI and PASI and sex of onset of psoriasis as independent variables. Finally we got 3 SNPs ($P < 0.05$), they are respectively rs216195 ($T > G$), rs1050301 ($G > A$), rs2285421($T > C$). We conclude that the 3 SNPs variation might associate with the response of methotrexate and will provide reference meanings for further studies based on larger samples.

rs216195$T > G$ is a missense variant in SMG6 gene on chromosome 17 resulting in a change of amino acid lysine to glutamine, SMG6 is broadly expressed in all human tissues. It has dual functions in telomere maintenance and RNA surveillance pathways. SMG6 is an NMD factor. Nonsense-mediated mRNA decay (NMD) controls gene expression by eliminating mRNAs with premature or aberrant translation termination. Degradation of NMD substrates is initiated by the central NMD factor UPF1, which recruits the endonuclease SMG6 and the deadenylation-promoting

Table 3
Binary logistic regression analysis of 6 SNPs.

Gene	rs number	Genotype frequencies	responder	non-responder	P	Crude OR(95%)[a]	P	Adj Adjust OR(95%)[b]
			N = 63	N = 37				
SMG6	rs216195	TT,n(%)	3(4.8)	9(25)		1		1
		GT,n(%)	35(55.6)	16(44.4)	**0.01**	6.562[1.564-27.54]	**0.024**	5.989[1.269-28.262]
		GG,n(%)	25(39.7)	11(30.6)	**0.011**	6.818[1.542-30.152]	**0.019**	6.88[1.365-34.67]
UPK1A	rs2285421	TT,n(%)	9(14.3)	11(29.7)		1		1
		TC,n(%)	31(49.2)	14(37.8)	0.072	2.706[0.916-7.999]	**0.017**	4.632[1.318-16.272]
		CC,n(%)	23(36.5)	12(32.4)	0.138	2.343[0.761-7.208]	0.097	2.875[0.827-9.995]
IMMT	rs1050301	GG,n(%)	19(30.6)	9(24.3)		1		1
		GA,n(%)	32(51.6)	12(32.4)	0.658	1.263[0.449-3.552]	0.886	1.084[0.361-3.256]
		AA,n(%)	11(17.7)	16(43.2)	**0.046**	0.326[0.108-0.982]	**0.042**	0.294[0.09-0.958]
TMPRSS11F	rs10030708	CC,n(%)	19(31.1)	6(16.2)		1		1
		CT,n(%)	27(44.3)	17(45.9)	0.293	0.553[0.183-1.668]	0.259	0.51[0.158-1.642]
		TT,n(%)	15(24.6)	14(37.8)	**0.041**	0.295[0.091-0.951]	**0.025**	0.232[0.064-0.835]
LOC107987304	rs2838956	AA,n(%)	18(30)	5(13.5)		1		1
		AG,n(%)	31(51.7)	25(67.6)	0.063	0.344[0.112-1.058]	**0.041**	0.289[0.088-0.95]
		GG,n(%)	11(18.3)	7(18.9)	0.236	0.437[0.111-1.72]	0.293	0.458[0.107-1.967]
KRT32	rs3744786	TT,n(%)	27(42.9)	24(64.9)		1		1
		TC,n(%)	26(41.3)	9(24.3)	**0.048**	2.568[1.007-6.55]	**0.04**	2.835[1.049-7.667]
		CC,n(%)	10(15.9)	4(10.8)	0.223	2.222[0.616-8.019]	0.205	2.427[0.615-9.575]

[a] Without adjusted for the baseline of age, BMI and PASI and sex. Bold and italics in p value mean the significant result;
[b] adjusted for the baseline of age, BMI and PASI and sex. Bold and italics in p value mean the significant result.

Table 4
Association of 3 positive SNPs with response to methotrexate via logistic regression analysis.

Gene	SNPs	Genotypes/Alleles	PASI<50%	PASI ≥ 75%	Adjusted OR[95%CI][a]	P
			n = 37	n = 63		
SMG6	rs216195	TT,n(%)	9(25)	3(4.8)	1	
		GT,n(%)	16(44.4)	35(55.6)	5.574[1.035-30.032]	0.046
		GG,n(%)	11(30.6)	25(39.7)	6.147[1.114-33.922]	0.037
IMMT	rs1050301	GG,n(%)	9(24.3)	19(30.6)	1	
		GA,n(%)	12(32.4)	32(51.6)	0.809[0.224-2.926]	0.747
		AA,n(%)	16(43.2)	11(17.7)	0.246[0.061-0.990]	0.048
UPK1A	rs2285421	TT,n(%)	11(29.7)	9(14.3)	1	
		TC,n(%)	14(37.8)	31(49.2)	4.420[1.175-16.630]	0.028
		CC,n(%)	12(32.4)	23(36.5)	2.795[0.689-11.331]	0.150

[a] adjusted for the baseline of age, BMI and PASI and sex. Bold and italics in p value mean the significant result.

SMG5/7 complex [17–21]. It has been reported that overexpression or knockdown of SMG6 in human cells causes the shortening or loss of telomeres and cell cycle arrest. These studies highlight the important function of Smg6 in various cellular process [22]. In addition, there are several reports that Smg6 regulates embryonic stem cell differentiation through NMD function [23,24]. As we all know, the pathological features of psoriasis are excessive proliferation and abnormal differentiation of keratinocytes. Therefore, we speculate that SMG6 is likely to participate in the development of psoriasis, and the mechanism deserves to study.

rs2285421T > C is a missense variant in UPK1A gene on chromosome19 resulting in a change of amino acid methionine to threonine. The protein encoded by this gene is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility [25]. UPK1A is a specific marker of mammalian urothelium. It has been reported that inhibition of UPK1A can suppress proliferation and enhance apoptosis of bladder transitional cell carcinoma cells [26]. And Carlsson H et al have reported that UPK1A upregulated in the inflammatory skin disorder such as psoriasis but the mechanism is unknown [27]. But there is no evidence that it directly affects the methotrexate transport and metabolic processes. We speculated that UPK1A may play a role in the development of psoriasis and influence the efficacy of methotrexate.

rs1050301G > A is a missense variant in IMMT gene on chromosome 2 resulting in a change of amino acid proline to serine. IMMT gene encodes MICOS complex subunit Mic60 protein (also known as mitofilin), is a protein of the inner mitochondrial membrane and a key component of the mitochondrial contact site and cristae junction organizing system (MICOS). Mic60 is critical for maintaining mitochondrial membrane structure and function [28,29]. It has reported that Mic60 knockdown compromises mitochondrial transcription and OXPHOS activities. And precise regulation of mtDNA transcription and oxidative phosphorylation (OXPHOS) is crucial for human health [30]. Mitofilin has also been reported that can participate in protein input along with other mitochondrial proteins [31,32] Besides, it has been reported that mitofilin distinctly functions in cristae remodeling and controls cytochrome c release during apoptosis [33]. In a word, mitofilin involved in transcription, protein import, metabolism and apoptosis. Psoriasis is a hyperplastic and metabolic disorder, and methotrexate requires transporter. Although there is currently no report on the association between mitofilin and psoriasis, a lot of evidence suggests that it will play a role in psoriasis.

Simultaneously, we selected 14 SNPs previously reported associated with response to methotrexate therapy such as ABCC1 (rs35592), ABCG2 (rs13120400, rs17731538), SLC19A1 (rs1051266, rs11702425, rs7499, rs2838956), ATIC (rs3821353, rs12995526, rs7563206), DHFR(rs1232027), MTHFR(rs1476413), GGH (rs12681874), FPGS(rs10987746) to verify. Unfortunately, none of the SNPs were found to be associated with the efficacy of methotrexate in the treatment of psoriasis in our case series. We speculate on the one hand because the results of this study are limited by the number of samples, on the other hand, due to characteristics of disease and geographical differences, the

reliability of these previously reported gene locus is still worth further study to demonstrate.

In conclusion, rs216195T > G, rs1050301G > A, rs2285421T > C may influence the efficacy of methotrexate in Chinese patients with psoriasis vulgaris. There is accumulating evidence regarding the functional effects of these genes, however, the mechanisms is not clearly understood. We believe that in future, these variants will be proved to be useful as markers for targeting therapies with additional work and they will be widely used in clinical as a precision medical treatment that is more accurate, more effective and more personalized treatment. Besides, it will reduce unnecessary expenditure for patients and be prioritized by the patients. Although the deficiencies of this study are the small sample size, we are now expanding the sample size to further verify these SNP sites.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant number 81573049, grant number 81773329, grant number 81620108024, grant number 2017JJ2378. We sincerely thank the participating patients and the volunteers for their generous and enthusiastic support of this study. We are also very grateful to Shanghai Huashan Hospital for providing partly of the samples.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2018.06.009.

References

[1] W.H. Boehncke, M.P. Schon, Psoriasis, Lancet (Lond., Engl.) 386 (2015) 942–983.
[2] C.E. Griffiths, J.N. Barker, Pathogenesis and clinical features of psoriasis, Lancet (Lond., Engl.) 370 (2007) 263–271.
[3] A.M. Bowcock, Genetics of psoriasis and autoimmunity, Annu. Rev. Genom. Hum. G. 6 (2005) 93–122.
[4] Y. Deng, C. Chang, Q. Lu, The inflammatory response in psoriasis: a comprehensive review, Clin. Rev. Allergy Immunol. 50 (2016) 377–389.
[5] W.B. Kim, D. Jerome, J. Yeung, Diagnosis and management of psoriasis, Can. Fam. Physician. 63 (2017) 278–285.
[6] G. Murdaca, S. Negrini, O. Magnani, E. Penza, M. Pellecchio, F. Puppo, Impact of pharmacogenomics upon the therapeutic response to etanercept in psoriasis and psoriatic arthritis expert, Opin. Drug Saf. 16 (2017) 1173–1179.
[7] R. Nishikawa, H. Nagai, T. Bito, T. Ikeda, T. Horikawa, A. Adachi, T. Matsubara, C. Nishigori, Genetic prediction of the effectiveness of biologics for psoriasis treatment, J. Dermatol. 43 (2016) 1273–1277.
[8] J.J. Wu, A. Guerin, M. Sundaram, K. Dea, M. Cloutier, P. Mulani, Cardiovascular event risk assessment in psoriasis patients treated with tumor necrosis factor-alpha inhibitors versus methotrexate, J. Am. Acad. Dermatol. 76 (2017) 81–90.
[9] W.P. Gulliver, H.M. Young, H. Bachelez, S. Randell, S. Gulliver, N. Al-Mutairi, Psoriasis patients treated with biologics and methotrexate have a reduced rate of myocardial infarction: a collaborative analysis using International cohorts, J. Cutan. Med. Surg. 20 (2016) 550–554.
[10] Z.S. Yang, N.N. Lin, L. Li, Y. Li, The effect of TNF inhibitors on cardiovascular events in psoriasis and psoriatic arthritis: an updated meta-analysis, Clin. Rev. Allergy Immunol. 51 (2016) 240–247.
[11] R.B. Warren, R.L. Smith, E. Campalani, S. Eyre, C.H. Smith, J.N. Barker, J. Worthington, C.E. Griffiths, Genetic variation in efflux transporters influences outcome to methotrexate therapy in patients with psoriasis, Invest. Dermatol. 128 (2008) 1925–1929.
[12] V. Chandran, F. Siannis, P. Rahman, F.J. Pellett, V.T. Farewell, D.D. Gladman, Folate pathway enzyme gene polymorphisms and the efficacy and toxicity of methotrexate in psoriatic arthritis, J. Rheumatol. 37 (2010) 1508–1512.
[13] S.A. Owen, S.L. Hider, P. Martin, I.N. Bruce, A. Barton, W. Thomson, Genetic polymorphisms in key methotrexate pathway genes are associated with response to treatment in rheumatoid arthritis patients, Pharmacogenomics J. 13 (2013) 227–234.
[14] Y.C. Lee, J. Cui, K.H. Costenbader, N.A. Shadick, M.E. Weinblatt, E.W. Karlson, Investigation of candidate polymorphisms and disease activity in rheumatoid arthritis patients on methotrexate, Rheumatology (Oxf.) 48 (2009) 613–617.
[15] J.A. Wessels, J.K. de Vries-Bouwstra, B.T. Heijmans, P.E. Slagboom, Y.P. Goekoop-Ruiterman, C.F. Allaart, P.J. Kerstens, D. van Zeben, F.C. Breedveld, B.A. Dijkmans, T.W. Huizinga, H.J. Guchelaar, Efficacy and toxicity of methotrexate in early rheumatoid arthritis are associated with single-nucleotide polymorphisms in genes coding for folate pathway enzymes, Arthritis Rheum. 54 (2006) 1087–1895.
[16] E.V. Tchetina, N.V. Demidova, G.A. Markova, E.A. Taskina, S.I. Glukhova, D.E. Karateev, Increased baseline RUNX2, caspase 3 and p21 gene expressions in the peripheral blood of disease-modifying anti-rheumatic drug-naive rheumatoid arthritis patients are associated with improved clinical response to methotrexate therapy, Int. J. Rheum. Dis. 20 (2017) 1468–1480.
[17] F. Ottens, V. Boehm, C.R. Sibley, J. Ule, N.H. Gehring, Transcript-specific characteristics determine the contribution of endo- and exonucleolytic decay pathways during the degradation of nonsense-mediated decay substrates, RNA 23 (2017) 1224–1236.
[18] S. Nasif, L. Contu, O. Muhlemann, Beyond quality control: the role of nonsense-mediated mRNA decay (NMD) in regulating gene expression, Semin. Cell. Dev. Biol. 75 (2018) 78–87.
[19] P. Nicholson, C. Josi, H. Kurosawa, A. Yamashita, O. Muhlemann, A novel phosphorylation-independent interaction between SMG6 and UPF1 is essential for human NMD, Nucleic Acids Res. 42 (2014) 9217–9235.
[20] M. Colombo, E.D. Karousis, J. Bourquin, R. Bruggmann, O. Muhlemann, Transcriptome-wide identification of NMD-targeted human mRNAs reveals extensive redundancy between SMG6- and SMG7-mediated degradation pathways, RNA 23 (2017) 189–201.
[21] S.A. Schmidt, P.L. Foley, D.H. Jeong, L.A. Rymarquis, F. Doyle, S.A. Tenenbaum, J. G. Belasco, P.J. Green, Identification of SMG6 cleavage sites and a preferred RNA cleavage motif by global analysis of endogenous NMD targets in human cells, Nucleic Acids Res. 43 (2015) 309–323.
[22] C.M. Azzalin, J. Lingner, The human RNA surveillance factor UPF1 is required for S phase progression and genome stability, Curr. Biol. 16 (2006) 433–439.
[23] T. Li, Y. Shi, P. Wang, L.M. Guachalla, B. Sun, T. Joerss, Y.S. Chen, M. Groth, A. Krueger, M. Platzer, Y.G. Yang, K.L. Rudolph, Z.Q. Wang, Smg6/Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay, EMBO J. 34 (2015) 1630–1647.
[24] C.H. Lou, E.Y. Shum, M.F. Wilkinson, RNA degradation drives stem cell differentiation, EMBO J. 34 (2015) 1606–1608.
[25] J. Olsburgh, P. Harnden, R. Weeks, B. Smith, A. Joyce, G. Hall, R. Poulsom, P. Selby, J. Southgate, Uroplakin gene expression in normal human tissues and locally advanced bladder cancer, J. Pathol. 199 (2003) 41–49.
[26] H. Zhu, Y. Tang, X. Zhang, X. Jiang, Y. Wang, Y. Gan, J. Yang, Downregulation of UPK1A suppresses proliferation and enhances apoptosis of bladder transitional cell carcinoma cells, Med. Oncol. 32 (2015) 84.
[27] H. Carlsson, S. Petersson, C. Enerback, Cluster analysis of S100 gene expression and genes correlating to psoriasin (S100A7) expression at different stages of breast cancer development, Int. J. Oncol. 27 (2005) 1473–1481.
[28] C. Ding, Z. Wu, L. Huang, Y. Wang, J. Xue, S. Chen, Z. Deng, L. Wang, Z. Song, S. Chen, Mitofilin and CHCHD6 physically interact with Sam50 to sustain cristae structure, Sci., Rep. 5 (2015) 16064.
[29] H. Li, Y. Ruan, K. Zhang, F. Jian, C. Hu, L. Miao, L. Gong, L. Sun, X. Zhang, S. Chen, H. Chen, D. Liu, Z. Song, Mic60/Mitofilin determines MICOS assembly essential for mitochondrial dynamics and mtDNA nucleoid organization, Cell. Death Differ. 23 (2016) 380–392.
[30] R.F. Yang, L.H. Sun, R. Zhang, Y. Zhang, Y.X. Luo, W. Zheng, Z.Q. Zhang, H.Z. Chen, D.P. Liu, Suppression of Mic60 compromises mitochondrial transcription and oxidative phosphorylation, Sci. Rep. 5 (2015) 7990.
[31] J. Xie, M.F. Marusich, P. Souda, J. Whitelegge, R.A. Capaldi, The mitochondrial inner membrane protein mitofilin exists as a complex with SAM50, metaxins 1 and 2, coiled-coil-helix coiled-coil-helix domain-containing protein 3 and 6 and DnaJC11, FEBS Lett. 581 (2007) 3545–3549.
[32] T.K. von der Malsburg, J.M. Muller, M. Bohnert, S. Oeljeklaus, P. Kwiatkowska, T. Becker, A. Loniewska-Lwowska, S. Wiese, S. Rao, D. Milenkovic, D.P. Hutu, R.M. Zerbes, A. Schulze-Specking, H.E. Meyer, J.C. Martinou, S. Rospert, P. Rehling, C. Meisinger, M. Veenhuis, B. Warscheid, I.J. van der Klei, N. Pfanner, A. Chacinska, M. van der Laan, Dual role of mitofilin in mitochondrial membrane organization and protein biogenesis, Dev. Cell. 21 (2011) 694–707.
[33] R.F. Yang, G.W. Zhao, S.T. Liang, Y. Zhang, L.H. Sun, H.Z. Chen, D.P. Liu, Mitofilin regulates cytochrome c release during apoptosis by controlling mitochondrial cristae remodeling, Biochem. Biophys. Res. Commun. 428 (2012) 38–93.