Polymorphisms in drug metabolism genes predict the risk of refractory myasthenia gravis

Qing Zhang[1]^, Huizhen Ge[1], Mengcui Gui[1], Mengge Yang[1], Zhuajin Bi[1], Xue Ma[1], Zhongya Gu[1], Shu Peng[2], Tao Chen[3#], Bitao Bu[1#]

[1]Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [2]Department of Thoracic Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [3]Department of Cardiothoracic and Vascular Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Contributions: (I) Conception and design: B Bu, T Chen; (II) Administrative support: Q Zhang, H Ge; (III) Provision of study materials or patients: Q Zhang, M Gui, M Yang; (IV) Collection and assembly of data: Q Zhang, X Ma, Z Bi; (V) Data analysis and interpretation: Q Zhang, Z Gu, S Peng; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Bitao Bu. Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. Email: bubitao@tjh.tjmu.edu.cn; Tao Chen. Department of Cardiothoracic and Vascular Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. Email: tongji892@sina.com.

Background: Nearly 10% to 20% of myasthenia gravis (MG) patients are refractory to conventional treatment for unclear reasons. The study aimed to explore the relationship between drug metabolism gene polymorphisms and refractory MG.

Methods: One hundred and thirty-one MG patients (33 in the refractory group; 98 in the non-refractory group) admitted to Tongji Hospital were included in this retrospective study. Improved multiplex ligation detection reaction (iMLDR) was used to genotype 13 polymorphisms (NR3C1 rs17209237, rs9324921; FKBP5 rs1360780, rs4713904, rs9296158; HSP90AA1 rs10873531, rs2298877, rs7160651; MDR1 rs1045642, rs1128503, rs2032582; CYP3A4 rs2242480; and CYP3A5 rs776746). We applied multivariable logistic regression to investigate the association between refractory MG and nucleotide polymorphisms. Generalized multifactor dimensionality reduction (GMDR) was used to examine gene-gene interactions.

Results: CC genotype of HSP90AA1 rs7160651 was associated with the increased risk of refractory MG than CT genotype [odds ratio (OR) =0.26; P=0.041] and CT + TT genotype (dominant model, OR =0.24; P=0.022). For CYP3A5 rs776746, AA genotype was associated with refractory MG compared with AG genotype (OR =0.11; P=0.017), GG genotype (OR =0.18; P=0.033), and AG + GG genotype (dominant model, OR =0.16; P=0.020). The frequency of CAT haplotype of HSP90AA1 rs10873531, rs2298877, rs7160651 was less common in refractory patients (OR =0.33; P=0.044). No significant gene-gene interactions were observed.

Conclusions: HSP90AA1 rs7160651 and CYP3A5 rs776746 were significantly associated with refractory MG. Further studies are warranted to confirm the results and investigate the use of polymorphisms for treatment individualization.

Keywords: Refractory myasthenia gravis (MG); single nucleotide polymorphisms (SNPs); CYP3A5; HSP90AA1

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^ ORCID: 0000-0002-5094-2747.

Introduction

Myasthenia gravis (MG) is a rare autoantibody-mediated disease characterized by muscle weakness and fatiguability (1). The majority of MG patients showed marked improvement or remission via the use of pyridostigmine (Pyri), glucocorticoids (GCs), immunosuppressants, and/or thymectomy (2). However, about 10% to 20% of MG patients are classified as refractory due to a suboptimal response or intolerance to conventional treatment (3,4).

It is well recognized that single nucleotide polymorphisms (SNPs) in genes coding for drug-metabolizing enzymes and transporters influence the response to medicines (5). GCs are used as first-line immunosuppressants for MG patients (2). Glucocorticoid receptor (GR; NR3C1, nuclear receptor subfamily 3 group C member 1) polymorphisms contribute to the efficacy of GCs in MG patients (6). FK506 binding protein 5 (FKBP5) represents a target for drugs such as rapamycin and tacrolimus and its genetic variation exerts a role in chemoresistance (7). Allelic variants of heat shock protein 90 Alpha family class A member 1 (HSP90AA1) (8,9) and multidrug resistance protein 1 (MDR1) are reasons for the refractory to pharmaceutical treatment (10,11). In addition, genetic polymorphisms of cytochrome P450 (CYP) 3A4/3A5 influence the blood trough concentration and efficacy of tacrolimus for MG patients (12,13).

Genetic risk factors that determine the patients at high risk for refractory MG remain unknown (14). In this study, we analyzed 13 SNPs in six drug metabolism genes (NR3C1, FKBP5, HSP90AA1, MDR1, CYP3A4, and CYP3A5) to explore their roles in developing refractory MG. We present the following article in accordance with the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2543/rc).

Methods

Study design and participants

The sample size was calculated to be at least 26 for the refractory group [α =0.05; power =0.80; minor allele frequency (MAF) =0.204 (minimum MAF for all candidate SNPs from databases)] using Quanto 1.2.4 (http://biostats.usc.edu/Quanto.html). One hundred and thirty-one MG patients who were admitted to the Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology from October 2013 to July 2020 were eventually included in this retrospective study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participants or their legal guardians gave written informed consents. Ethical approval for this study was obtained from the Ethics Committee of Tongji Hospital (protocol ID: TJ-IRB20191208). Clinical data as well as blood samples were collected at the time of enrollment.

Inclusion criteria: (I) patients of all ages with a diagnosis of MG; (II) followed up for at least 1 year. MG diagnosis was based on typical fluctuating muscle weakness with one or more of the following criteria (15): (I) presence of AChR or MuSK antibodies; (II) a decrement over 10% on repetitive nerve stimulation; (III) Pyri treatment was effective. Patients complicated with malignancies, infections, neurodegenerative diseases, or other autoimmune diseases were excluded.

Refractory MG was defined as at least one of the following during the follow-up (16): (I) patients respond insufficiently to maximal safe doses of GCs and at least one immunosuppressive drug at an adequate dose and duration; (II) inability to reduce immunosuppressive therapy without clinical relapse or require repeated rescue therapy such as intravenous immunoglobulin (IVIG) or plasma exchange (PLEX); (III) intolerable adverse effects from immunosuppressive therapy; (IV) comorbid conditions that restrict the use of conventional therapies; (V) frequent myasthenic crises even while on therapy.

The severity of the disease was assessed by Myasthenia Gravis Foundation of America (MGFA) clinical classification (17) and quantified MG (QMG) scores (18). Clinical status of the patients was determined by the MGFA post-intervention status (PIS) (17).

SNP selection and genotyping

Gene information was available from the National Center for Biotechnology Information (NCBI) dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP). We investigated the impact of 13 polymorphisms in six genes potentially associated with pharmacogenetics of immunologic agents, including NR3C1 rs17209237, rs9324921; FKBP5 rs1360780, rs4713904, rs9296158; HSP90AA1 rs10873531, rs2298877, rs7160651; MDR1 rs1045642, rs1128503, rs2032582; CYP3A4 rs2242480; and CYP3A5 rs776746. DNA from blood was extracted from peripheral blood using Gentra Puregene Blood Kit (Qiagen). SNP genotyping was performed by the improved multiplex ligation detection reaction (iMLDR) technique (Genesky Biotechnologies Inc., Shanghai, China) as

Table 1 SNPs information

SNPs	Gene	Chr	Chr position	HGVS names	SNP property	Functional change	P value for HWE	MAF	
								Database[#]	Detected in this study
rs17209237	NR3C1	5	142657212	NM_000176.2:c.*4242T>C	3'-Flanking	–	0.347	0.204	0.176
rs9324921	NR3C1	5	142767740	NM_000176.2:c.1184+11481G>T	intron2	–	0.899	0.266	0.267
rs1360780	FKBP5	6	35607571	NM_004117.3:c.106-2636A>G	intron2	–	0.805	0.286	0.263
rs4713904	FKBP5	6	35625147	NM_004117.3:c.-19-14527G>A	intron1	–	1.000	0.324	0.271
rs9296158	FKBP5	6	35567082	NM_004117.3:c.509-1901T>C	intron5	–	0.249	0.344	0.321
rs10873531	HSP90AA1	14	102568296	NM_001017963.2:c.282C>T	synon_exon2	p.=(Thr94Thr)	0.378	0.258	0.271
rs2298877	HSP90AA1	14	102548224	NM_001017963.2:c.2456-66A>G	intron11	–	0.331	0.235	0.240
rs7160651	HSP90AA1	14	102564159	NM_001017963.2:c.366+4053C>T	intron2	–	0.222	0.226	0.218
rs1045642	MDR1	7	87138645	NM_000927.4:c.3435T>C	synon_exon27	p.=(Ile1145Ile)	0.902	0.383	0.370
rs1128503	MDR1	7	87179601	NM_000927.4:c.1236T>C	synon_exon13	p.=(Gly412Gly)	0.471	0.374	0.389
rs2032582	MDR1	7	87160618	NM_000927.4:c.2677T>G	nonsynon_exon22	p.Ser893Ala	0.233	0.434	0.355
				NM_000927.4:c.2677T>A		p.Ser893Thr			
rs2242480	CYP3A4	7	99361466	NM_017460.5:c.1026+12G>A	intron10	–	0.280	0.321	0.290
rs776746	CYP3A5	7	99270539	NM_000777.5:c.219-237A>G	intron3	–	0.191	0.287	0.244

[#], MAF in the Asian population from dbSNP databases. SNPs, single nucleotide polymorphisms; Chr, chromosome; HGVS, Human Genome Variation Society; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

previously described (19). Primer sequences for multiplex polymerase chain reaction (PCR). are provided in Table S1. Negative controls and duplicate samples were used to monitor genotyping quality. Genotypes were conducted using GeneMapper 4.0 (Applied Biosystems, USA).

Statistical analysis

Continuous variables were expressed as median (interquartile range) and categorical variables were described as frequency (percentages). Comparisons between groups were analyzed using Mann-Whitney U-test or Chi-squared test as appropriate. All genotypes were examined for Hardy-Weinberg equilibrium (HWE). Multivariable logistic regression was performed to determine the genetic risk factors associated with the incidence of refractory MG after adjusting for age, sex, MGFA classification, QMG scores, medicine treatment, and history of thymectomy. We used receiver operating characteristics curves with area under the curve (AUC) values to evaluate the logistic regression model.

No correction for multiple testing was applied due to the exploratory character of this study. Two-sided statistical significance was set at P value <0.05. Data were analyzed by GraphPad Prism 8.01 (GraphPad Prism, San Diego, CA, USA) and SPSS 24 (IBM, Armonk, NY, USA). Linkage disequilibrium (LD) blocks were constructed with Haploview software (http://www.broad.mit.edu/mpg/haploview) and haplotype-based association analyses were performed by SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). LD analysis was assessed by D' and r^2 values as described elsewhere (20). Gene-gene interactions were analyzed using generalized multifactor dimensionality reduction (GMDR) software (21).

Results

SNPs information

All the polymorphisms were in HWE (P>0.05). The 13 SNPs included one in the 3' flanking sequence, four in exons, and eight in introns. The genotype information for all candidate SNPs is summarized in *Table 1*.

Demographic and disease characteristics

Clinical characteristics are shown in *Table 2*. The median

Table 2 Baseline characteristics in refractory and non-refractory patients

Variables	Non-refractory (n=98)	Refractory (n=33)	P value
Current age, years, median (25% to 75%)	23.55 (10.82–47.03)	29.84 (13.80–52.77)	0.395
Sex, females, n (%)	67 (68.4)	19 (57.6)	0.259
Age at onset, years, median (25% to 75%)	14.00 (3.42–40.75)	24.00 (4.50–47.75)	0.395
MGFA classification, n (%)			
I–II	77 (78.6)	19 (57.6)	0.012*
III–IV	19 (19.4)	9 (27.3)	
V	2 (2.0)	5 (15.2)	
Antibodies, n (%)			
AChR-Ab (+)	87 (88.8)	31 (93.9)	0.602
Seronegative	11 (11.2)	2 (6.1)	
QMG scores, years, median (25% to 75%)	3.00 (2.00–8.00)	5.50 (4.00–20.00)	<0.001*
Medicine treatment, n (%)			
Pyri	25 (25.5)	4 (12.1)	0.025*
Pyri + Pred	48 (49.0)	12 (36.4)	
Pyri + Pred + TAC	11 (11.2)	9 (27.3)	
Pyri + Pred + MMF	2 (2.0)	1 (3.0)	
Pyri + Pred + AZA	1 (1.0)	0 (0.0)	
IVIG/PLEX	2 (2.0)	5 (15.2)	
History of thymectomy, n (%)	16 (16.3)	11 (33.3)	0.037*
Thymus histology, n (%)			
Hyperplasia	4 (4.1)	1 (3.0)	0.080
Thymoma	8 (8.2)	10 (30.3)	
Thymolipoma	3 (3.1)	0 (0.0)	
Normal	1 (1.0)	0 (0.0)	
Follow-up, years, median (25% to 75%)	3.32 (1.38–7.40)	1.98 (1.56–6.13)	0.280
MGFA-PIS at last visit, n (%)			
CSR + PR	11 (11.2)	0 (0.0)	<0.001*
MM	51 (52.0)	1 (3.0)	
Improvement	27 (27.6)	10 (30.3)	
Unchanged	2 (2.0)	18 (54.5)	
Worse	3 (3.1)	2 (6.1)	
Exacerbation	1 (1.0)	0 (0.0)	
Death	3 (3.1)	2 (6.1)	

*, P value <0.05. MGFA, Myasthenia Gravis Foundation of America; AChR-Ab, acetylcholine receptor antibody; QMG, quantified myasthenia gravis; Pyri, pyridostigmine; Pred, prednisone; TAC, tacrolimus; MMF, mycophenolate mofetil; AZA, azathioprine; IVIG, intravenous immunoglobulin; PLEX, plasma exchange; PIS, post-intervention status; CSR, complete stable remission; PR, pharmacologic remission; MM, minimal manifestations.

Table 3 The distribution of SNPs in refractory and non-refractory patients

SNPs	Genotype (total =131)	Non-refractory (n=98), n (%)	Refractory (n=33), n (%)	χ^2	P value[a]	Logistic regression	
						OR (95% CIs)	P value[b]
HSP90AA1 (rs7160651)	CC	56 (57.1%)	27 (81.8%)	7.016	0.030*	Reference	
	CT	34 (34.7%)	5 (15.2%)			0.26 (0.07–0.94)	0.041*
	TT	8 (8.2%)	1 (3.0%)			0.19 (0.02–2.28)	0.188
	CT + TT	42 (42.9%)	6 (18.2%)	6.475	0.011*	0.24 (0.07–0.82)	0.022*
	C-allele	146 (74.5%)	59 (89.4%)	6.443	0.011*	Reference	
	T-allele	50 (25.5%)	7 (10.6)			0.31 (0.11–0.84)	0.022*
CYP3A5 (rs776746)	AA	5 (5.1%)	6 (18.2%)	5.211	0.074	Reference	
	AG	34 (34.7%)	8 (24.2%)			0.11 (0.02–0.68)	0.017*
	GG	59 (60.2%)	19 (57.6%)			0.18 (0.04–0.87)	0.033*
	AG + GG	93 (94.9%)	27 (81.8%)	3.922	0.048*	0.16 (0.03–0.75)	0.020*
	A-allele	44 (22.4%)	20 (30.3%)	1.650	0.199	Reference	
	G-allele	152 (77.6%)	46 (69.7%)			0.58 (0.27–1.24)	0.157

The allele frequency was calculated by dividing the number of alleles by twice the number of cases. [a], P value for genotype frequencies using χ^2 test; [b], P value from multivariable logistic regression analysis after adjusting for age, sex, MGFA classification, QMG scores, medicine treatment, and history of thymectomy; *, P value <0.05. SNPs, single nucleotide polymorphisms; OR, odds ratio; CIs, confidence intervals; MGFA, Myasthenia Gravis Foundation of America; QMG, quantified myasthenia gravis.

age of the patients in the study (86 females and 45 males) was 24.50 (11.76 to 47.14) years and the median follow-up duration was 2.53 (1.47–7.20) years. Patients were divided into refractory group (n=33, 25.2%) and non-refractory group (n=98, 74.8%) based on their treatment responses throughout the follow-up period. Refractory patients were more severely ill at enrollment, with higher MGFA classification (P=0.012), QMG scores (P<0.001). More patients in the refractory group received aggressive medicine treatment (P=0.025) and underwent thymectomy (P=0.037) compared with patients in the non-refractory group.

The distribution of SNPs in the MG patients

Results of multivariable logistic regression are presented in *Table 3*. CC genotype of the HSP90AA1 rs7160651 was associated with the increased risk of refractory MG than CT genotype [odds ratio (OR) =0.26; 95% confidence interval (CI): 0.07–0.94; P=0.041] and CT + TT genotype (dominant model, OR =0.24; 95% CI: 0.07–0.82; P=0.022). T-allele of HSP90AA1 rs7160651 frequency was less susceptible to refractory MG compared with C-allele (OR =0.31; 95% CI: 0.11–0.84; P=0.022). For CYP3A5 rs776746, AA genotype was associated with refractory MG compared with AG genotype (OR =0.11; 95% CI: 0.02–0.68; P=0.017), GG genotype (OR =0.18; 95% CI: 0.04–0.87; P=0.033), and AG + GG genotype (dominant model, OR =0.16; 95% CI: 0.03–0.75; P=0.020). There were no statistical differences in the other SNPs (Table S2).

Analysis of LD haplotypes

Two haplotype blocks were identified: Block 1 contained FKBP5 rs1360780 and rs4713904 (17 kb) and Block 2 contained HSP90AA1 rs7160651 and rs10873531 (4 kb). The results of LD analysis revealed strong linkages between FKBP5 rs1360780 and rs4713904 (D' =0.96, r^2=0.88) as well as HSP90AA1 rs10873531 and rs2298877 (D' =1.00, r^2=0.85). *Figure 1* illustrates LD among SNPs.

Considering LD of genes, haplotype analyses were further performed (*Table 4*). Haplotype analysis at rs10873531, rs2298877, rs7160651 of HSP90AA1 gene manifested that the refractory group had a lower CAT haplotype frequency (OR =0.33; 95% CI: 0.11–0.97; P=0.044).

Discrimination of the predictive model

AUC values were calculated from the logistic regression model including clinical covariates together with genetic variants. As shown in *Figure 2*, CYP3A5 rs776746 and

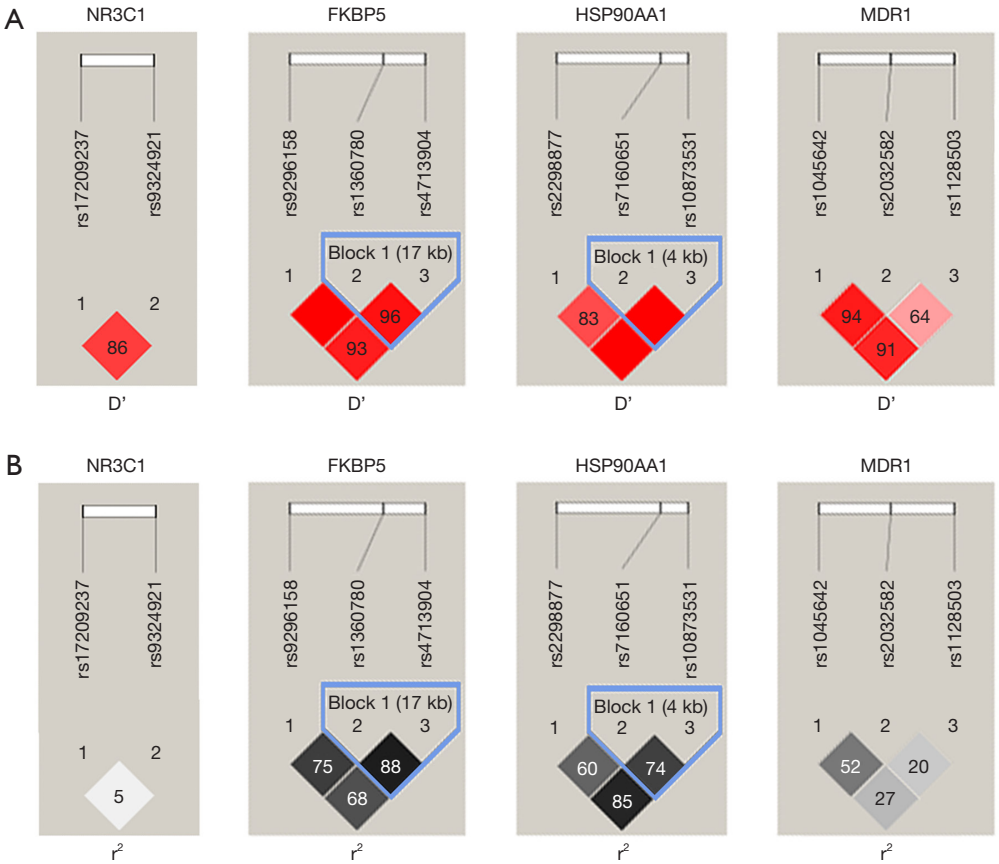

Figure 1 Gene haplotypes generated by Haploview. (A) LD plot showing D' values within squares. The D' value is displayed in gradients from red (strong linkage) to white (weak linkage). (B) LD plot showing r^2 values within squares. The r^2 value is displayed in gradients from black (strong linkage) to white (weak linkage). Alleles in strong LD are highlighted in one blue block. LD, linkage disequilibrium.

HSP90AA1 rs7160651 had good predictive accuracy (AUC >0.800). Among these models, the allele comparison model of HSP90AA1 rs7160651 achieves the best prediction performance with the highest AUC value of 0.852 (*Figure 2E*).

Gene-gene interactions

GMDR analysis showed that the single-locus model (rs7160651) was regarded as the optimal model based on the testing balanced accuracy, cross-validation consistency, and significant P values. Therefore, our results yielded no gene-related interactions for all possible one- to thirteen-locus models (*Table 5*).

Discussion

In the current study, we have evaluated 13 SNPs for the treatment response in 131 patients with MG. After adjusting for possible confounding factors by logistic regression, genetic polymorphisms of CYP3A5 rs776746 and HSP90AA1 rs7160651 showed statistically significant associations with the risk of refractory MG. No significant SNP pairs were found in the gene-gene interaction analyses.

HSP90AA1 is efficiently expressed under pathological conditions, such as tumors, infection, and autoimmune disorders (9,22). A previous study has found that HSP90AA1 is closely associated with resistance to cancer therapy by inhibiting apoptosis and inducing autophagy (23). But the relationship between autophagy and therapy resistance in MG remains unclear and needs further investigation. In the present study, rs7160651 in HSP90AA1 was significantly associated with the response to immunosuppressive therapy under a dominant genetic model, which was consistent with the previous report (8). Furthermore, we found that HSP90AA1 CAT haplotype and T-allele were associated

Table 4 Haplotype analysis between refractory and non-refractory patients

Haplotype	Frequencies n (%)		χ^2	P value[a]	Logistic regression	
	Non-refractory (n=196)	Refractory (n=66)			OR (95% CIs)	P value[b]
NR3C1 (rs17209237, rs9324921)						
TT	54 (27.6%)	14 (21.2%)	1.032	0.310	0.61 (0.27–1.34)	0.217
TG	108 (55.1%)	40 (60.6%)	0.609	0.435	1.77 (0.87–3.59)	0.115
CT	2 (1.0%)	0 (0.0%)	0.000	1.000	–	–
CG	32 (16.3%)	12 (18.2%)	0.122	0.727	0.84 (0.32–2.23)	0.726
KBP5 (rs1360780, rs4713904, rs9296158)						
GGC	2 (1.0%)	1 (1.5%)	0.000	0.987	0.19 (0.01–2.87)	0.232
GAT	11 (5.6%)	3 (4.5%)	0.000	0.987	0.64 (0.14–2.83)	0.554
GAC	129 (65.8%)	46 (69.7%)	0.335	0.563	1.35 (0.64–2.83)	0.428
AGT	53 (27.0%)	14 (21.2%)	0.881	0.348	0.93 (0.40–2.14)	0.861
AAT	1 (0.5%)	1 (1.5%)	0.000	1.000	0.16 (0.01–20.76)	0.464
GGT	0 (0.0%)	1 (1.5%)	0.328	0.567	–	–
HSP90AA1 (rs10873531, rs2298877, rs7160651)						
TGC	138 (70.4%)	53 (80.3%)	2.447	0.118	1.65 (0.73–3.74)	0.226
CGT	7 (3.6%)	1 (1.5%)	0.182	0.670	0.37 (0.04–3.43)	0.379
CAT	43 (21.9%)	6 (9.1%)	5.360	0.021	0.33 (0.11–0.97)	0.044*
CAC	8 (4.1%)	6 (9.1%)	1.559	0.118	3.60 (0.98–13.26)	0.054
MDR1 (rs1045642, rs1128503, rs2032582)						
TTT	69 (35.2%)	25 (37.9%)	0.154	0.695	1.31 (0.65–2.61)	0.449
TCG	1 (0.5%)	0 (0.0%)	0.000	1.000	–	–
TCA	2 (1.0%)	0 (0.0%)	0.000	0.995	–	–
CTT	6 (3.1%)	2 (3.0%)	0.000	1.000	2.54 (0.31–2.23)	0.382
CTG	44 (22.4%)	19 (28.8%)	1.086	0.297	1.27 (0.59–2.74)	0.549
CTA	3 (1.5%)	1 (1.5%)	0.000	1.000	–	–
CCG	44 (22.4%)	10 (15.2%)	1.067	0.205	0.42 (0.16–1.08)	0.072
CCA	27 (13.8%)	9 (13.6%)	0.001	0.977	1.28 (0.50–3.27)	0.600

[a], P value for genotype frequencies using χ^2 test; [b], P value from multivariable logistic regression analysis after adjusting for age, sex, MGFA classification, QMG scores, medicine treatment, and history of thymectomy; *, P value <0.05. OR, odds ratio; CIs, confidence intervals; MGFA, Myasthenia Gravis Foundation of America; QMG, quantified myasthenia gravis.

with a lower risk of refractory MG. The protein encoded by the HSP90AA1 gene is the cytoplasmic/nuclear form of heat shock protein 90 (HSP90) (24). Ouyang *et al.* demonstrated that the increased presence of HSP90 in the nucleus could hinder DNA-binding and transcriptional activity of GR, which might lead to steroid resistance in patients with idiopathic nephrotic syndrome (25). However, the functional significance of the polymorphisms in HSP90AA1 is not fully understood, thus how HSP90AA1 genetic polymorphisms influence the treatment response of MG remains to be determined.

CYP3A5 protein is polymorphically expressed in the liver and intestines and impacts the drug disposition (26,27). GCs (28) and tacrolimus (29) are the most frequently used

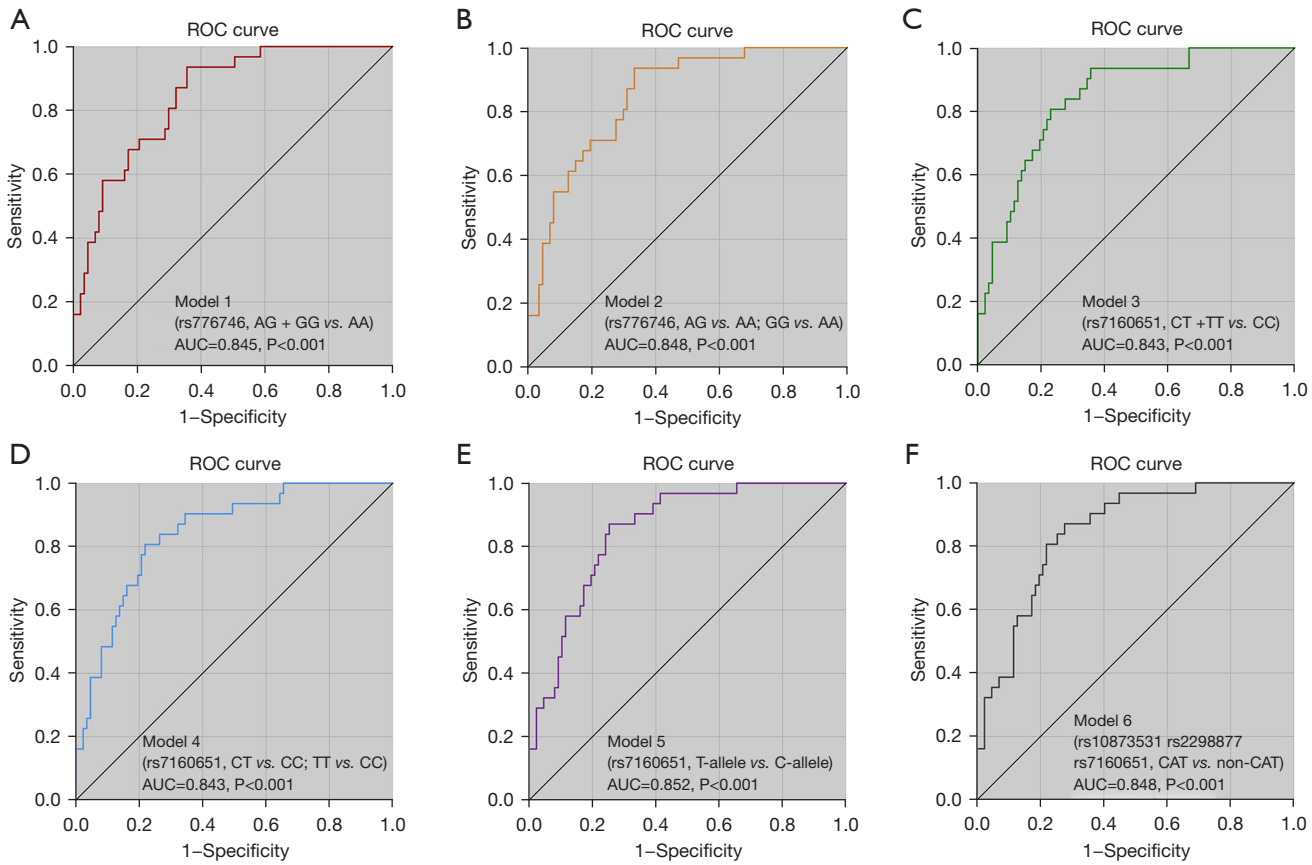

Figure 2 ROC curves for the model proposed to predict MG patients with refractory status. (A,B) Dominant model and genotype model of CYP3A5 rs776746. (C-E) Dominant model, genotype model, and allele comparison model of HSP90AA 1 rs7160651. (F) Haplotype model of HSP90AA 1 rs10873531, rs2298877, rs7160651. ROC, receiver operating characteristic; AUC, area under the curve; MG, myasthenia gravis.

pharmacological agents for our cohort, which are both metabolized by CYP3A5 enzyme. The A to G substitution at rs776746 polymorphism is responsible for aberrantly spliced transcript and low protein expression of CYP3A5 in many populations (30). Consistent with the previous study (29), our results demonstrated an association between the dominant model of CYP3A5 rs776746 polymorphism and poor response to therapy. Therefore, we speculate that rs776746 variation significantly affects drug disposal and may play a predictive role in the refractory status of MG.

Non-genetic factors related to refractory MG were used as covariates to correct the disease model. A recent study showed that patients with drug-refractory MG were more frequently younger at onset, females, and thymectomized (14). However, age at onset and sex were not significantly different between refractory and non-refractory MG in our current study. Childhood-onset MG mostly occurs in women with a high rate of spontaneous remission, which accounts for 10–20% of all MG patients in western countries, but more than 50% in Asians (31,32). Differences in the age distribution between Asian and Western populations could be one possible reason for the different clinical characteristics across studies. We demonstrated that the patients in the refractory group were more often treated with thymectomy but did not achieve symptom remission. The ineffectiveness of thymectomy can be attributed to circulating plasma cells that are often long-lived and can secrete antibodies in the absence of T cells (33).

MG is a multifactorial disease involving complex gene-gene and gene-environment interactions (34-36). No gene-gene interactions were identified among possible SNP combinations in GMDR analysis, but we cannot exclude the possibility of other multiple interactions that influence the therapeutic effects of MG. Several investigations suggested

Table 5 The gene-gene interaction models obtained by GMDR

Interacting SNPs	Testing balanced accuracy	CVC	P value
rs7160651	0.626	10/10	0.011*
rs2032582*rs7160651	0.502	5/10	0.828
rs2032582*rs9296158*rs7160651	0.590	5/10	0.055
rs2032582*rs17209237*rs9296158*rs7160651	0.466	4/10	0.623
rs2032582*rs2242480*rs17209237*rs9296158*rs10873531	–	5/10	0.377
rs1128503*rs2032582*rs2242480*rs17209237*rs9296158*rs7160651	–	5/10	0.172
rs1128503*rs2032582* rs776746*rs2242480* rs9324921* rs4713904*rs10873531	–	5/10	0.828
rs1045642*rs1128503*rs2032582*rs776746*rs2242480*rs17209237*rs9324921* rs9296158	–	2/10	0.999
rs1045642*rs1128503*rs2032582*rs776746*rs2242480*rs17209237*rs9324921*rs9296158* rs10873531	–	4/10	0.989
rs1045642*rs1128503*rs2032582*rs776746*rs2242480*rs17209237*rs9324921*rs1360780*rs9296158*rs10873531	–	10/10	0.945
rs1045642*rs1128503*rs2032582*rs776746*rs2242480*rs17209237*rs9324921*rs1360780*rs4713904*rs9296158*rs10873531	–	9/10	0.945
rs1045642*rs1128503*rs2032582*rs776746*rs2242480*rs17209237*rs9324921*rs1360780*rs4713904*rs9296158*rs10873531*rs2298877	–	10/10	0.945
rs1045642*rs1128503*rs2032582*rs776746*rs2242480*rs17209237*rs9324921*rs1360780*rs4713904*rs9296158*rs10873531*rs2298877*rs7160651	–	10/10	0.945

P value was calculated with covariate adjustment for age, sex, MGFA classification, QMG scores, medicine treatment, and history of thymectomy. *, P value <0.05. GMDR, generalized multifactor dimensionality reduction; SNPs, single nucleotide polymorphisms; CVC, cross validation consistency; MGFA, Myasthenia Gravis Foundation of America; QMG, quantified myasthenia gravis.

that MDR1, NR3C1, and FKBP5 polymorphisms may confer resistance to immunosuppressive therapy (37-39). The present study did not find any association between their genotypes and refractory MG. Further work targeting more diverse genotypes will be necessary.

Limitations exist for this study. Firstly, the present study is a single-center retrospective study with small-sample data. Secondly, the relationship between genetic polymorphisms and the effects of different drugs or different drug combinations used by the participants is unclear. Thirdly, the current study spreads over a large age range. Finally, we did not correct for multiple testing as the present study is exploratory.

Conclusions

This article is the first to explore the association between polymorphisms of drug-metabolizing genes and refractory MG. The results suggested that variants of CYP3A5 rs776746 and HSP90AA1 rs7160651 were clinical risk factors for the refractory status of MG. Drugs that are not metabolized by CYP3A5 and HSP90AA1 might be an option for refractory MG with rs776746 AA genotype and rs7160651 CC genotype, respectively. Further studies are required to confirm these findings and their clinical applications.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethical Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (protocol ID: TJ-IRB20191208). Informed consents were obtained from all individual participants or their legal guardians.

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Supplementary

Table S1 Primers used in the PCR to amplify the coding region

SNPs	Primer sequences (from 5′ to 3′)		Fragment size
rs17209237	F: CTGCCCGACCTTTCTATTCTATGTG	R: CCTGGTGCCAAAGACCTGAAGA	267
rs9324921	F: CCTCTAACCCTTCATTTACAAACATTGG	R: GCCCAGGTTATCTTCCCCAGAT	219
rs1360780	F: TGAGGACAGCCTGCAAAGTCTC	R: TTAATATCTCTTGTGCCAGCAGTAGCA	283
rs4713904	F: GAGATCACAAGTCCAGAATGGGTCT	R: CAGTATTCCCGGCTGAAGATGG	174
rs9296158	F: TTCTGTTATACTCATTCCATGCCCAATA	R: GCCTGGGCTAGGGGTAATTCAA	224
rs10873531	F: TGCAGATCCTTGTAGAGGTGTTGC	R: CCCAAGTGTTTCTCTGGCATCTG	154
rs2298877	F: GCGTGATGTGTCGTCATCTCCT	R: CCTGCTTGCTGCTTGGAGGTAT	284
rs7160651	F: CTGCCTGGTAGGGGAGCTGATAG	R: GCAGAAGCTGACAGGACCAGGTT	213
rs1045642	F: CAGAGAGGCTGCCACATGCT	R: CAGGAGCCCATCCTGTTTGACT	188
rs1128503	F: GCTCTTCCCACAGCCACTGTTT	R: TGTGTCTGTGAATTGCCTTGAAGTTT	125
rs2032582	F: TGAAGACAATGGCCTGAAAACTGA	R: TGTTGTCTGGACAAGCACTGAAAGA	296
rs2242480	F: CTTCTGCCAGTAGCAACCATTTG	R: ACTGCAGGAGGAAATTGATGCAG	218
rs776746	F: CCAGGAAGCCAGACTTTGATCATT	R: TGCCCTTGCAGCATTTAGTCCT	391

PCR, polymerase chain reaction; SNPs, single nucleotide polymorphisms.

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Table S2 The distribution of SNPs in refractory and non-refractory patients

SNPs	Genotype (total =131)	Non-refractory (n=98), n (%)	Refractory (n=33), n (%)	χ^2	P value[a]	Logistic regression	
						OR (95% CIs)	P value[b]
NR3C1 (rs17209237)	TT	68 (69.4%)	23 (69.7%)	0.249	0.883	Reference	
	TC	26 (26.5%)	8 (24.2%)			0.95 (0.31–2.93)	0.925
	CC	4 (4.1%)	2 (6.1%)			0.04 (0.00–56.95)	0.375
	TC + CC	30 (30.6%)	10 (30.3%)	0.001	0.973	0.82 (0.27–2.48)	0.727
	T-allele	162 (82.7%)	54 (81.8%)	0.024	0.877	Reference	
	C-allele	34 (17.3%)	12 (18.2)			0.74 (0.28–1.94)	0.534
NR3C1 (rs9324921)	GG	50 (51.0%)	21 (63.6%)	1.602	0.449	Reference	
	GT	40 (40.8%)	10 (30.3%)			0.47 (0.17–1.36)	0.165
	TT	8 (8.2%)	2 (6.1%)			0.35 (0.04–3.31)	0.358
	GT + TT	48 (49.0%)	12 (36.4%)	1.583	0.208	0.46 (0.17–1.25)	0.127
	G-allele	140 (71.4%)	52 (78.8%)	1.366	0.243	Reference	
	T-allele	56 (28.6%)	14 (21.2%)			0.56 (0.25–1.24)	0.150
FKBP5 (rs1360780)	AA	8 (8.2%)	2 (6.1%)	0.601	0.740	Reference	
	AG	38 (38.8%)	11 (33.3%)			1.74 (0.14–21.90)	0.670
	GG	52 (53.1%)	20 (60.6%)			1.80 (0.15–21.08)	0.640
	AG + GG	90 (91.8%)	31 (93.9%)	0.000	0.988	1.78 (0.16–20.35)	0.644
	A-allele	54 (27.6%)	15 (22.7%)	0.592	0.442	Reference	
	G-allele	142 (72.4%)	51 (77.3%)			0.87 (0.38–1.98)	0.734
FKBP5 (rs4713904)	GG	8 (8.2%)	1 (3.0%)	1.193	0.551	Reference	
	GA	39 (39.8%)	14 (42.4%)			1.80 (0.15–22.07)	0.644
	AA	51 (52.0%)	18 (54.5%)			1.89 (0.16–22.07)	0.612
	GA + AA	90 (91.8%)	32 (97.0%)	0.373	0.542	1.86 (0.16–23.64)	0.618
	G-allele	55 (28.1%)	16 (24.2%)	0.364	0.546	Reference	
	A-allele	141 (71.9%)	50 (75.8%)			1.16 (0.50–2.65)	0.734
FKBP5 (rs9296158)	TT	8 (8.2%)	2 (6.1%)	0.501	0.778	Reference	
	TC	49 (50.0%)	15 (45.5%)			1.63 (0.13–19.96)	0.702
	CC	41 (41.8%)	16 (48.5%)			1.88 (0.16–21.95)	0.615
	TC + CC	90 (91.8%)	31 (93.9%)	0.000	0.988	1.78 (0.16–20.35)	0.644
	T-allele	65 (33.2%)	19 (28.8%)	0.434	0.510	Reference	
	C-allele	131 (66.8%)	47 (71.2%)			1.19 (0.56–2.51)	0.615
HSP90AA1 (rs10873531)	CC	10 (10.2%)	2 (6.1%)	2.526	0.283	Reference	
	CT	38 (38.8%)	9 (27.3%)			1.19 (0.18–7.80)	0.856
	TT	50 (51.0%)	22 (66.7%)			2.20 (0.36–13.36)	0.394
	CT + TT	88 (89.8%)	31 (93.9%)	0.133	0.715	1.70 (0.30–9.56)	0.546
	C-allele	58 (29.6%)	13 (19.7%)	2.447	0.118	Reference	
	T- allele	138 (70.4%)	53 (80.3%)			1.65 (0.73–3.74)	0.226
HSP90AA1 (rs2298877)	AA	8 (8.2%)	2 (6.1%)	1.946	0.378	Reference	
	AG	35 (35.7%)	8 (24.2%)			0.81 (0.11–5.86)	0.833
	GG	55 (56.1%)	23 (69.7%)			1.44 (0.23–9.21)	0.770
	AG + GG	90 (91.8%)	31 (93.9%)	0.000	0.988	1.18 (0.19–7.24)	0.857
	A-allele	51 (26.0%)	12 (18.2%)	1.661	0.197	Reference	
	G-allele	145 (74.0%)	54 (81.8%)			1.45 (0.62–3.34)	0.390
MDR1 (rs1045642)	TT	14 (14.3%)	3 (9.1%)	1.715	0.424	Reference	
	TC	44 (44.9%)	19 (57.6%)			1.43 (0.30–6.79)	0.656
	CC	40 (40.8%)	11 (33.3%)			0.85 (0.17–4.31)	0.841
	TC + CC	84 (85.7%)	30 (90.9%)	0.220	0.639	1.14 (0.26–5.05)	0.865
	T-allele	72 (36.7%)	25 (37.9%)	0.028	0.868	Reference	
	C-allele	124 (63.3%)	41 (62.1%)			0.83 (0.42–1.66)	0.598
MDR1 (rs1128503)	TT	36 (36.7%)	16 (48.5%)	1.976	0.372	Reference	
	TC	50 (51.0%)	15 (45.5%)			0.64 (0.23–1.78)	0.392
	CC	12 (12.2%)	2 (6.1%)			0.31 (0.05–1.82)	0.196
	TC + CC	62 (63.2%)	17 (51.5%)	1.424	0.233	0.55 (0.21–1.45)	0.229
	T-allele	122 (62.2%)	47 (71.2%)	1.734	0.188	Reference	
	C-allele	74 (37.8%)	19 (28.8%)			0.60 (0.29–1.23)	0.162
MDR1 (rs2032582)	TT	13 (13.3%)	3 (9.1%)	3.856	0.570	Reference	
	TG	37 (37.8%)	14 (42.4%)			1.30 (0.26–6.60)	0.754
	GG	17 (17.3%)	6 (18.2%)			0.55 (0.08–3.91)	0.550
	TA	12 (12.2%)	7 (21.2%)			2.16 (0.33–13.96)	0.421
	AA	1 (1.0%)	0 (0.0%)			–	–
	GA	18 (18.4%)	3 (9.1%)			0.63 (0.09–4.43)	0.641
	TG + GG	54 (55.1%)	20 (66.7%)	0.139	0.710	0.99 (0.21–4.69)	0.994
	TA + AA	13 (13.3%)	7 (23.3%)	0.500	0479	2.85 (0.29–11.65)	0.514
	T-allele	75 (38.3%)	27 (40.9%)	0.155	0.926	Reference	
	G-allele	89 (45.4%)	29 (43.9%)			0.66 (0.31–1.38)	0.265
	A-allele	32 (16.3%)	10 (15.2%)			0.84 (0.31–2.25)	0.728
CYP3A4 (rs2242480)	GG	62 (63.3%)	23 (69.7%)	1.734	0.420	Reference	
	GA	31 (31.6%)	7 (21.2%)			0.90 (0.33–2.50)	0.846
	AA	5 (5.1%%)	3 (9.1%)			2.31 (0.39–13.74)	0.359
	GA + AA	36 (36.7%)	10 (30.3%)	0.448	0.503	1.09 (0.43–2.78)	0.861
	G-allele	155 (79.1%)	53 (80.3%)	0.045	0.832	Reference	
	A-allele	41 (20.9%)	13 (19.7%)			1.08 (0.48–2.42)	0.848

The allele frequency was calculated by dividing the number of alleles by twice the number of cases. [a], P value for genotype frequencies using χ^2 test; [b], P value from multivariable logistic regression analysis after adjusting for age, sex, MGFA classification, QMG scores, medicine treatment, and history of thymectomy. SNPs, single nucleotide polymorphisms; OR, odds ratio; CIs, confidence intervals; MGFA, Myasthenia Gravis Foundation of America; QMG, quantified myasthenia gravis.

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