·神经免疫学基础与临床研究·

*IL-2Rβ*基因多态性与重症肌无力关联性研究

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【摘要】 目的 观察重症肌无力患者白细胞介素-2受体β亚单位(*IL-2Rβ*)基因rs228942、rs228941和rs743777位点多态性,探讨其与重症肌无力易感性和严重程度的关联性。**方法** 采用SNPscan™技术对480例重症肌无力患者和487例正常对照者*IL-2Rβ*基因rs228942、rs228941和rs743777位点进行基因分型,根据性别、发病年龄、抗乙酰胆碱受体(AChR)抗体、伴与不伴胸腺瘤、发病后2年最严重临床分型和疾病最严重时Oosterhuis评分将重症肌无力患者分为不同亚组,比较重症肌无力组与对照组、重症肌无力各亚组与对照组、重症肌无力各亚组间等位基因频率,并在共显性、加性和过显性遗传模型下比较基因型频率。**结果** 重症肌无力组*IL-2Rβ*基因rs228942位点*T*等位基因频率高于对照组($\chi^2 = 4.692$, $P = 0.030$, $OR = 1.242$, 95%CI: 1.021 ~ 1.511),基因型频率在加性遗传模型下差异有统计学意义($P = 0.036$, $OR = 1.230$, 95%CI: 1.010 ~ 1.480)。重症肌无力组rs228942和rs228941位点组成的单倍域中*GT*单倍型频率高于对照组($\chi^2 = 4.286$, $P = 0.038$),*GG*单倍型频率低于对照组($\chi^2 = 5.333$, $P = 0.021$)。rs228942位点*T*等位基因频率在15 ~ 50岁亚组($\chi^2 = 7.474$, $P = 0.006$, $OR = 1.380$, 95%CI: 1.095 ~ 1.740)、不伴胸腺瘤亚组($\chi^2 = 4.700$, $P = 0.030$, $OR = 1.261$, 95%CI: 1.022 ~ 1.555)和发病后2年最严重全身型重症肌无力亚组($\chi^2 = 4.715$, $P = 0.030$, $OR = 1.287$, 95%CI: 1.025 ~ 1.617)均高于对照组。多因素前进法Logistic回归分析显示,发病年龄15 ~ 50岁($OR = 9.026$, 95%CI: 4.225 ~ 19.284; $P = 0.000$)和>50岁($OR = 9.956$, 95%CI: 4.475 ~ 22.149; $P = 0.000$)、伴胸腺瘤($OR = 2.578$, 95%CI: 1.393 ~ 4.773; $P = 0.003$)和抗AChR抗体阳性($OR = 1.946$, 95%CI: 1.179 ~ 3.214; $P = 0.009$)均是发病后2年最严重临床分型的独立危险因素,而基因型不是独立危险因素。**结论** *IL-2Rβ*基因rs228942位点可能与重症肌无力易感性相关,但未发现与其严重程度相关;亦未发现rs228941和rs743777位点多态性与重症肌无力易感性和严重程度相关。

【关键词】 白细胞介素类; 多态现象,遗传; 重症肌无力; 基因; 等位基因; 回归分析

Association analysis between *IL-2Rβ* gene polymorphism and myasthenia gravis

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【Abstract】 Objective To explore the association of three single nucleotide polymorphisms (SNPs; rs228942, rs228941 and rs743777) in interleukin-2 receptor β subunit (*IL-2Rβ*) gene with the susceptibility and severity of myasthenia gravis (MG). **Methods** There were 480 MG patients and 487 normal controls enrolled in this study, and their three SNPs (rs228942, rs228941 and rs743777) in *IL-2Rβ* gene were

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evaluated through SNPscan™ technique. Subgroups were classified by gender, age of onset, anti-acetylcholine receptor antibodies (AChR-Ab), thymus status, maximal involvement after 2 years of onset and maximal Oosterhuis score. Frequencies of alleles and genotypes were compared between MG group and control group, between MG subgroups and control group, and among subgroups under codominant, log-additive and overdominant models. **Results** The frequency of *T* allele of rs228942 in MG group was significantly higher than that in control group (χ^2 = 4.692, *P* = 0.030, *OR* = 1.242, 95% CI: 1.021–1.511). There was significant difference in the frequency of rs228942 genotype between MG group and control group under log-additive model (*P* = 0.036, *OR* = 1.230, 95% CI: 1.010–1.480). The rs278942/rs228941 *GT* haplotype frequency in MG group was higher than that in control group (χ^2 = 4.286, *P* = 0.038), while *GG* haplotype frequency in MG group was lower than that in control group (χ^2 = 5.333, *P* = 0.021). The frequencies of *T* allele of rs228942 was significantly higher in MG subgroups of onset age 15–50 years (χ^2 = 7.474, *P* = 0.006, *OR* = 1.380, 95% CI: 1.095–1.740), non-thymoma (χ^2 = 4.700, *P* = 0.030, *OR* = 1.261, 95% CI: 1.022–1.555) and maximal generalized involvement (χ^2 = 4.715, *P* = 0.030, *OR* = 1.287, 95% CI: 1.025–1.617) than those in control group. Multivariate forward Logistic regression analysis found that onset age 15–50 years (*OR* = 9.026, 95% CI: 4.225–19.284; *P* = 0.000), onset age > 50 years (*OR* = 9.956, 95% CI: 4.475–22.149; *P* = 0.000), thymoma (*OR* = 2.578, 95% CI: 1.393–4.773; *P* = 0.003) and positive AChR-Ab (*OR* = 1.946, 95% CI: 1.179–3.214; *P* = 0.009) were the independent risk factors for maximal involvement, while genotypes were not. **Conclusions** The rs228942 polymorphisms of *IL-2Rβ* gene may be associated with the susceptibility of MG, but not with the severity of MG. The rs228941 and rs743777 polymorphisms were not found to be associated with the susceptibility and severity of MG.

【Key words】 Interleukins; Polymorphism, genetic; Myasthenia gravis; Genes; Alleles; Regression analysis

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重症肌无力(MG)是主要由抗乙酰胆碱受体(AChR)抗体介导的、细胞免疫依赖的和补体系统参与的神经-肌肉接头(NMJ)处获得性自身免疫性疾病。白细胞介素-2(IL-2)是$CD4^+$T细胞产生的细胞因子,通过与活化T细胞表达的白细胞介素-2受体(IL-2R)结合而发挥生物学作用。IL-2Rβ是IL-2R的3个亚单位之一,对细胞内信号转导具有重要作用[1]。*IL-2Rβ*基因多态性与重症肌无力[2]、支气管哮喘[3]和类风湿性关节炎(RA)[4]等自身免疫性疾病有关。本研究通过观察重症肌无力患者*IL-2Rβ*基因rs228942、rs228941和rs743777位点多态性,探讨其与重症肌无力易感性和严重程度的关联性。

对象与方法

一、研究对象

1. 重症肌无力组(MG组) 共选择青岛大学附属医院和首都医科大学附属北京友谊医院2007年11月–2013年6月治疗和随访的重症肌无力患者480例,均为汉族且无血缘关系,均符合重症肌无力诊断标准[5]:必备条件为典型的骨骼肌无力呈现病态疲劳且症状呈波动性,肌疲劳试验和新斯的明试验均阳性;支持条件为血浆抗AChR抗体阳性和(或)肌电图低频重复神经电刺激(RNS)波幅递减>10%。研究对象需同时满足必备条件和支持条件,每年至少随访2次且于病情加重和调整治疗方案后2~3个月随访。按照性别、发病年龄[6]、血浆抗AChR抗体是否阳性、是否伴胸腺瘤[胸腺CT和(或)病理学检查]、发病后2年最严重临床分型(眼肌型或全身型重症肌无力)和病情最严重时Oosterhuis评分[7]分为相应亚组。有研究显示,约82%重症肌无力患者发病后2年疾病进展至最严重程度[8],因此,本研究对病程≥2年且随访资料完善的患者分析最严重临床分型和病情最严重时Oosterhuis评分。

2. 正常对照组(对照组) 正常对照者为同期在青岛大学附属医院和首都医科大学附属北京友谊医院进行体格检查的健康志愿者共487例,均为汉族且彼此之间或与患者之间无血缘关系,排除自身免疫性疾病和慢性感染性疾病。

本研究获青岛大学附属医院和首都医科大学附属北京友谊医院道德伦理委员会审核批准,所有受试者均知情同意并签署知情同意书。

二、研究方法

1. 血液标本采集 所有受试者均于清晨采集外

周静脉血约4 ml，置乙二胺四乙酸（EDTA）抗凝管中，于离心半径12 cm、转速2500 r/min离心10 min，收集下层细胞用于DNA提取、收集上清液用于血浆抗AChR抗体检测，于-80 ℃保存备用。

2. DNA提取和基因分型 （1）DNA提取：取0.50 ml保存备用的外周血细胞，采用全血基因组DNA试剂盒（美国BioChain公司）提取DNA。（2）基因分型：采用上海天昊生物科技有限公司设计的SNPscan™技术进行基因分型[9]。以连接酶连接反应的高特异性实现对单核苷酸多态性（SNP）位点等位基因的识别，通过在连接探针末端引入不同长度的非特异性序列和连接酶加接反应获得相应位点对应的不同长度连接产物，采用荧光标记的通用引物（上海天昊生物科技有限公司设计，上游引物序列：5'-ACACGACCGGTAACGCTTAGA-3'，下游引物序列：5'-ATTAAATCACCCGCTCTAGGGAAG-3'）对连接产物进行聚合酶链反应（PCR），荧光毛细管电泳（ABI3730XL型，美国Applied Biosystems公司）对扩增产物进行分离，并对电泳图谱进行分析以获得单核苷酸多态性位点的基因型。（3）质量控制：采用随机数字表法选取4%的样本进行双盲质量控制，其结果与原分型结果一致。

3. 血浆抗乙酰胆碱受体抗体检测 采用双抗夹心竞争抑制酶联免疫吸附试验（ELISA）检测血浆抗AChR抗体水平，检测用试剂盒购自英国RSR公司，抗体水平采用抑制率（%）表示，以81例正常对照者均值+2倍标准差（SD）为阳性临界值，抑制率>33.75%为抗体阳性。

4. 统计分析方法 采用SPSS 17.0统计软件进行数据处理与分析。计数资料以相对数构成比（%）或率（%）表示，MG组及其亚组与对照组以及MG亚组间等位基因频率的比较采用χ^2检验。采用SNPstats在线软件（http://bioinfo.iconcologia.net/snpstats/start.htm）行Hardy-Weinberg平衡检验，并在共显性、加性和过显性（存在杂合子优势时）遗传模型下对MG组及其亚组与对照组以及MG亚组间的基因型频率进行比较。当MG亚组基因型频率差异有统计学意义（$P \leqslant 0.05$）时，采用多因素前进法Logistic回归分析对作为亚组分类依据的临床特征和基因型是否为重症肌无力的危险因素进行筛选。采用SHEsis在线软件（http://analysis.bio-x.cn/SHEsisMain.htm）进行连锁不平衡分析和构建单倍型，并对MG组与对照组的单倍型频率进行比较。以$P \leqslant 0.05$为差异具有统计学意义。

结 果

一、一般资料

共计480例重症肌无力患者，男性189例，女性291例；发病年龄1～86岁、中位值40（8，72）岁，其中，发病年龄<15岁71例、15～50岁253例、>50岁156例；血浆抗AChR抗体阳性338例，抗AChR抗体阴性124例；伴胸腺瘤107例，不伴胸腺瘤367例；根据发病后2年最严重临床分型，眼肌型重症肌无力151例，全身型重症肌无力274例；根据发病后2年疾病最严重时Oosterhuis评分，轻度重症肌无力（评分0～2分）216例，重度重症肌无力（评分3～5分）154例。共计487例正常对照者，男性249例，女性238例；年龄14～78岁，中位年龄45（21，69）岁。

二、基因型分析

1. 基因分型 MG组*IL-2Rβ*基因rs228942（*G/T*）、rs228941（*C/G*）和rs743777（*A/G*）位点在NCBI dbSNP数据库（https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/）所提供的中国汉族人群的少数等位基因频率（MAF）分别为0.257、0.374和0.107，与对照组MAF值（0.276，0.403和0.111）相近（表1）。MG组*IL-2Rβ*基因rs228942、rs228941和rs743777位点的成功分型率分别为98.75%、97.50%和97.92%，对照组为99.18%、100%和99.79%。对照组3个位点基因型分布均符合Hardy-Weinberg平衡定律（$P=0.069$，0.400，1.000；表1），表明*IL-2Rβ*基因rs228942、rs228941和rs743777位点处于平衡状态，具有群体代表性。

2. MG组与对照组等位基因和基因型频率的比较 MG组患者*IL-2Rβ*基因rs228942位点*T*等位基因频率（0.322）高于对照组（0.276）且差异有统计学意义（$P=0.030$，表1）；MG组与对照组患者*IL-2Rβ*基因rs228942位点基因型频率在加性遗传模型下差异亦有统计学意义（$P=0.036$），而在共显性遗传模型下差异无统计学意义（$P>0.05$，表2）。两组患者*IL-2Rβ*基因rs228941和rs743777位点等位基因和基因型频率差异均无统计学意义（$P>0.05$，表2）。

3. 连锁不平衡和单倍型分析 *IL-2Rβ*基因rs228942和rs228941位点的连锁不平衡参数为$D'=0.990$，$r^2=0.619$，提示两位点连锁程度较高（图1），故rs228942和rs228941位点可以构建1个连锁不平衡的单倍域。由表3可见，MG组患者*TG*单倍型频

表1 MG组与对照组受试者*IL-2Rβ*基因rs228942、rs228941和rs743777位点遗传学特征和等位基因频率的比较

Table 1. Genetic characteristics of rs228942, rs228941 and rs743777 in *IL-2Rβ* gene and comparison of their allele frequencies between MG group and control group

SNP	Location and function	Risk allele	MAF*		χ^2 value	*P* value	*OR*	*OR* 95%CI
			MG (N = 480)	Control (N = 487)				
rs228942	Chromosome 22 missense	*T*	0.322	0.276	4.692	0.030	1.242	1.021–1.511
rs228941	Chromosome 22 3′UTR	*G*	0.409	0.403	0.064	0.800	1.024	0.853–1.229
rs743777	Chromosome 22 5′near region	*G*	0.119	0.111	0.303	0.582	1.082	0.817–1.433

*According to NCBI dbSNP, the MAF of Han people in China was 0.257, 0.374 and 0.107, which was similar to those in control group of this study (0.276, 0.403 and 0.111), indicating the representativeness of controls. The genotypes of three SNPs in control group were consistent with Hardy-Weinberg equilibrium: *P* = 0.069, 0.400, 1.000. SNP, single nucleotide polymorphism, 单核苷酸多态性; MAF, minor allele frequency, 少数等位基因频率; MG, myasthenia gravis, 重症肌无力; UTR, untranslated region, 非翻译区

表2 MG组与对照组受试者*IL-2Rβ*基因rs228942、rs228941和rs743777位点基因型频率的比较[例(%)]

Table 2. Comparison of genotpye frequencies of rs228942, rs228941 and rs743777 in *IL-2Rβ* gene between MG group and control group [case (%)]

SNP	Genotype	Genotype frequency		Codominant model			Log-additive model		
		MG (N = 480)	Control (N = 487)	*P* value	*OR*	*OR* 95%CI	*P* value	*OR*	*OR* 95%CI
rs228942		474△	483△						
	G/G	222 (46.84)	261 (54.04)	0.082	1.000		0.036	1.230	1.010–1.480
	G/T	199 (41.98)	177 (36.65)		1.320	1.010–1.730			
	T/T	53 (11.18)	45 (9.32)		1.380	0.900–2.140			
rs228941		468△	487△						
	C/C	166 (35.47)	178 (36.55)	0.940	1.000		0.800	1.020	0.850–1.220
	C/G	221 (47.22)	225 (46.20)		1.050	0.790–1.400			
	G/G	81 (17.31)	84 (17.25)		1.030	0.710–1.500			
rs743777		470△	486△						
	A/A	365 (77.66)	384 (79.01)	0.860	1.000		0.580	1.080	0.820–1.430
	A/G	98 (20.85)	96 (19.75)		1.070	0.780–1.470			
	G/G	7 (1.49)	6 (1.23)		1.230	0.410–3.690			

△subjects with relevant data and successful genotyping, 有相应资料且分型成功病例数。SNP, single nucleotide polymorphism, 单核苷酸多态性; MG, myasthenia gravis, 重症肌无力

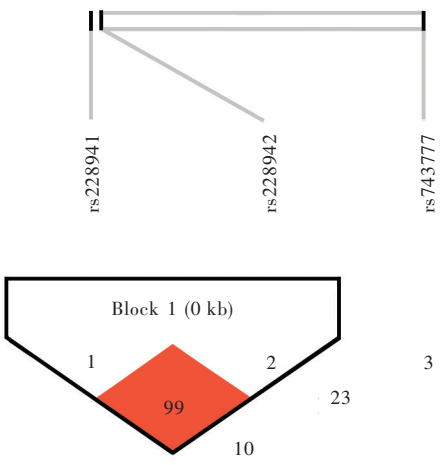

表3 *IL-2Rβ*基因rs228942和rs228941位点构建的单倍型频率

Table 3. Frequencies of rs228942/rs228941 haplotypes

SNP		MG (N = 480)	Control (N = 487)	χ^2 value	*P* value	*OR*	*OR* 95%CI
rs228942	rs228941						
G	*C*	0.589	0.601	0.230	0.631	0.956	0.796–1.149
T	*G*	0.319	0.276	4.286	0.038	1.231	1.011–1.500
G	*G*	0.089	0.122	5.333	0.021	0.706	0.525–0.950
T	*C*	0.004	0.000	–	–	–	–

–, no statistic value because of few number, 数量极少而无法计算。SNP, single nucleotide polymorphism, 单核苷酸多态性; MG, myasthenia gravis, 重症肌无力

图1 连锁不平衡和单倍型分析可见*IL-2Rβ*基因3个单核苷酸多态性位点构建的单倍域，其中，rs228942和rs228941位点的连锁不平衡参数为D' = 0.990，r^2 = 0.619，提示2个位点连锁程度较高

Figure 1 Linkage disequilibrium and haplotype analysis showed D' = 0.990, r^2 = 0.619 in rs228942/rs228941, indicating high linkage of these 2 loci.

表4 相同性别的MG组与对照组受试者*IL-2Rβ*基因rs228942、rs228941和rs743777位点等位基因和基因型频率的比较[例(%)]

Table 4. Comparison of frequencies of alleles and genotypes between MG and control groups of the same gender [case (%)]

SNP	Male				Female			
	MG (N = 189)	Control (N = 249)	χ^2 value	*P* value	MG (N = 291)	Control (N = 238)	χ^2 value	*P* value
rs228942	185△	247△			289△	236△		
GG	92 (49.73)	136 (55.06)		0.520* and 0.260#	130 (44.98)	125 (52.97)		0.190* and 0.100#
GT	73 (39.46)	89 (36.03)			126 (43.60)	88 (37.29)		
TT	20 (10.81)	22 (8.91)			33 (11.42)	23 (9.75)		
T	0.305	0.269	1.359	0.244	0.332	0.284	2.829	0.093
rs228941	182△	249△			286△	238△		
CC	74 (40.66)	88 (35.34)		0.430* and 0.190#	92 (32.17)	90 (37.82)		0.360* and 0.160#
CG	81 (44.51)	115 (46.18)			140 (48.95)	110 (46.22)		
GG	27 (14.84)	46 (18.47)			54 (18.88)	38 (15.97)		
G	0.371	0.416	1.762	0.184	0.434	0.391	1.962	0.161
rs743777	184△	249△			286△	237△		
AA	145 (78.80)	193 (77.51)		0.560* and 0.590#	220 (76.92)	191 (80.59)		0.370* and 0.220#
AG	38 (20.65)	52 (20.88)			60 (20.98)	44 (18.57)		
GG	1 (0.54)	4 (1.61)			6 (2.10)	2 (0.84)		
G	0.109	0.120	0.288	0.592	0.126	0.101	1.546	0.214

△subjects with relevant data and successful genotyping, 有相应资料且分型成功病例数; *codominant model, 共显性遗传模型; #log-additive model, 加性遗传模型。SNP, single nucleotide polymorphism, 单核苷酸多态性; MG, myasthenia gravis, 重症肌无力

率(0.319)高于对照组(0.276, *P* = 0.038), *GG*单倍型频率(0.089)低于对照组(0.122, *P* = 0.021), 且差异有统计学意义。

4. 重症肌无力各亚组与对照组等位基因和基因型频率的比较 相同性别的MG组与对照组受试者*IL-2Rβ*基因rs228942、rs228941和rs743777位点等位基因和基因型频率差异均无统计学意义(*P* > 0.05, 表4)。rs228942位点*T*等位基因频率在15～50岁亚组(*P* = 0.006)、不伴胸腺瘤亚组(*P* = 0.030)和发病后2年最严重全身型重症肌无力亚组(*P* = 0.030)均高于对照组且差异有统计学意义。基因型频率比较, 15～50岁亚组在共显性(*P* = 0.012)、加性(*P* = 0.008)和过显性(*P* = 0.014)遗传模型下, 不伴胸腺瘤亚组在加性遗传模型下(*P* = 0.035), 发病后2年最严重全身型重症肌无力亚组在加性遗传模型下(*P* = 0.037)与对照组差异均有统计学意义(表5)。将发病后2年最严重临床分型(眼肌型或全身型重症肌无力)作为因变量, rs228942位点基因型(共显性、加性或过显性遗传模型)、性别(男性或女性)、年龄(< 15岁、15～50岁或> 50岁)、胸腺瘤(伴或不伴)和抗AChR抗体(阳性或阴性)作为自变量, 行多因素前进法Logistic回归分析, 结果显示, 发病年龄15～50岁(*OR* = 9.026, 95% CI: 4.225～19.284; *P* = 0.000)和> 50岁(*OR* = 9.956, 95% CI: 4.475～22.149; *P* = 0.000)、伴胸腺瘤(*OR* = 2.578, 95% CI: 1.393～4.773; *P* = 0.003)和抗AChR抗体阳性(*OR* = 1.946, 95% CI: 1.179～3.214; *P* = 0.009)是发病后2年最严重临床分型的独立危险因素, 但基因型不是独立危险因素(表6)。

5. 重症肌无力各亚组间等位基因和基因型频率的比较 *IL-2Rβ*基因rs228941位点*G*等位基因频率在15～50岁亚组高于> 50岁亚组(*P* = 0.049), 两亚组在加性遗传模型下基因型频率差异亦有统计学意义(*P* = 0.049)。rs743777位点*G*等位基因频率在Oosterhuis评分3～5分亚组高于0～2分亚组(*P* = 0.013), 两亚组在共显性(*P* = 0.028)和加性(*P* = 0.015)遗传模型下基因型频率差异亦有统计学意义; 将发病后2年疾病最严重时Oosterhuis评分(3～5分或0～2分)作为因变量, rs743777位点基因型(共显性或加性遗传模型)、性别(男性或女性)、年龄(< 15岁、15～50岁或> 50岁)、胸腺瘤(伴或不伴)和抗AChR抗体(阳性或阴性)作为自变量, 行多因素前进法Logistic回归分析, 结果显示, 发病年龄15～50岁(*OR* = 18.703, 95% CI: 4.370～80.047; *P* =

表5 MG亚组与对照组受试者*IL-2Rβ*基因rs228942、rs228941和rs743777位点等位基因和基因型的比较[例(%)]

Table 5. Comparison of frequencies of alleles and genotypes between MG subgroups and control group [case (%)]

SNP	Control (N = 487)	Onset age (year)			AChR-Ab		Thymoma		Maximal involvement		Oosterhuis (score)	
		< 15 (N = 71)	15–50 (N = 253)	> 50 (N = 156)	Positive (N = 338)	Negative (N = 124)	Positive (N = 107)	Negative (N = 367)	OMG (N = 151)	GMG (N = 274)	0–2 (N = 216)	3–5 (N = 154)
rs228942	483△	70△	252△	152△	333△	124△	105△	363△	151△	270△	216△	152△
GG	261 (54.04)	37 (52.86)	107 (42.46)	78 (51.32)	161 (48.35)	52 (41.94)	52 (49.52)	167 (46.00)	70 (46.36)	125 (46.30)	97 (44.91)	78 (51.32)
GT	177 (36.65)	23 (32.86)	116 (46.03)	60 (39.47)	132 (39.64)	60 (48.39)	40 (38.10)	156 (42.98)	71 (47.02)	112 (41.48)	99 (45.83)	59 (38.82)
TT	45 (9.32)	10 (14.29)	29 (11.51)	14 (9.21)	40 (12.01)	12 (9.68)	13 (12.38)	40 (11.02)	10 (6.62)	33 (12.22)	20 (9.26)	15 (9.87)
T	0.276	0.307	0.345[a]	0.289	0.318	0.339	0.314	0.325[b]	0.301	0.330[c]	0.322	0.293
rs228941	487△	70△	248△	150△	328△	122△	104△	358△	148△	266△	212△	150△
CC	178 (36.55)	27 (38.57)	78 (31.45)	61 (40.67)	120 (36.59)	39 (31.97)	43 (41.35)	122 (34.08)	48 (32.43)	97 (36.47)	69 (32.55)	58 (38.67)
CG	225 (46.20)	30 (42.86)	123 (49.60)	68 (45.33)	148 (45.12)	65 (53.28)	42 (40.38)	174 (48.60)	81 (54.73)	119 (44.74)	112 (52.83)	62 (41.33)
GG	84 (17.25)	13 (18.57)	47 (18.95)	21 (14.00)	60 (18.29)	18 (14.75)	19 (18.27)	62 (17.32)	19 (12.84)	50 (18.80)	31 (14.62)	30 (20.00)
G	0.403	0.400	0.438[d]	0.367	0.409	0.414	0.385	0.416	0.402	0.412	0.410	0.407
rs743777	486△	68△	250△	152△	330△	123△	105△	359△	148△	269△	213△	152△
AA	384 (79.01)	55 (80.88)	193 (77.20)	117 (76.97)	257 (77.88)	95 (77.24)	76 (72.38)	286 (79.67)	119 (80.40)	206 (76.58)	175 (82.16)	111 (73.03)
AG	96 (19.75)	13 (19.12)	53 (21.20)	32 (21.05)	68 (20.61)	27 (21.95)	26 (24.76)	69 (19.22)	28 (18.92)	57 (21.19)	37 (17.37)	36 (23.68)
GG	6 (1.23)	0 (0.00)	4 (1.60)	3 (1.97)	5 (1.52)	1 (0.81)	3 (2.86)	4 (1.11)	1 (0.68)	6 (2.23)	1 (0.47)	5 (3.29)
G	0.111	0.096	0.122	0.125	0.118	0.118	0.152	0.107	0.101	0.128	0.092	0.151[e]

△subjects with relevant data and successful genotyping，有相应资料且分型成功病例数；[a]The frequency of *T* allele of rs228942 was significantly higher in onset age 15–50 years subgroup than control group (χ^2 = 7.474, *P* = 0.006, *OR* = 1.380, 95% CI: 1.095–1.740); [b]The frequency of *T* allele of rs228942 was significantly higher in MG thymoma (–) subgroup than control group (χ^2 = 4.700, *P* = 0.030, *OR* = 1.261, 95% CI: 1.022–1.555); [c]The frequency of *T* allele of rs228942 was significantly higher in GMG subgroup than control group (χ^2 = 4.715, *P* = 0.030, *OR* = 1.287, 95% CI: 1.025–1.617); [d]The frequency of *G* allele of rs228941 was significantly higher in onset age 15–50 years subgroup than onset age > 50 years subgroup (*P* = 0.049, *OR* = 1.343, 95% CI: 1.001–1.803); [e]The frequency of *G* allele of rs743777 was significantly higher in Oosterhuis 3–5 score subgroup than 0–2 score subgroup (*P* = 0.013, *OR* = 1.769, 95%CI: 1.123–2.788)。SNP，single nucleotide polymorphism，单核苷酸多态性；AChR-Ab，anti-acetylcholine receptor antibodies，抗乙酰胆碱受体抗体；OMG，ocular myasthenia gravis，眼肌型重症肌无力；GMG，generalized myasthenia gravis，全身型重症肌无力

表6 *IL-2Rβ*基因rs228942位点在发病后2年最严重疾病分型(眼肌型或全身型重症肌无力)亚组的Logistic回归分析

Table 6. Logistic regression analysis of rs228942 in maximal involvement (OMG/GMG) subgroups

Variable	*b*	*SE*	Wald χ^2	*P* value	*OR*	*OR* 95%CI
Onset age (score)						
15–50	2.200	0.387	32.262	0.000	9.026	4.225–19.284
> 50	2.298	0.408	31.733	0.000	9.956	4.475–22.149
Thymoma (+)	0.947	0.314	9.087	0.003	2.578	1.393– 4.773
AChR-Ab (+)	0.666	0.256	6.776	0.009	1.946	1.179– 3.214
Constant	-2.019	0.395	26.181	0.000		

+，positive，阳性。AChR - Ab，anti - acetylcholine receptor antibodies，抗乙酰胆碱受体抗体

表7 *IL-2Rβ*基因rs743777位点在发病后2年疾病最严重时Oosterhuis评分(3～5分或0～2分)亚组的Logistic回归分析

Table 7. Logistic regression analysis of rs743777 in maximal severity (Oosterhuis 3–5/0–2) subgroups

Variable	*b*	*SE*	Wald χ^2	*P* value	*OR*	*OR* 95%CI
Onset age (score)						
15–50	2.929	0.742	15.586	0.000	18.703	4.370–80.047
> 50	2.939	0.753	15.217	0.000	18.900	4.316–82.759
Thymoma (+)	0.608	0.271	5.043	0.025	1.838	1.080– 3.125
AChR-Ab (+)	1.062	0.303	12.246	0.000	2.892	1.596– 5.243
Constant	-3.989	0.763	27.316	0.000		

+，positive，阳性。AChR - Ab，anti - acetylcholine receptor antibodies，抗乙酰胆碱受体抗体

0.000）和 > 50 岁（*OR* = 18.900，95% CI：4.316～82.759；*P* = 0.000）、伴胸腺瘤（*OR* = 1.838，95% CI：1.080～3.125；*P* = 0.025）和抗AChR抗体阳性（*OR* = 2.892，95%CI：1.596～5.243；*P* = 0.000）是发病后2年疾病最严重时Oosterhuis评分的独立危险因素，但基因型不是独立危险因素（表7）。

讨　论

IL-2主要由$CD4^+$T细胞受抗原刺激后产生，通过与其特异性受体IL-2R相结合，在维持机体免疫

平衡中发挥重要作用。IL-2R有3个亚单位，即α亚单位（CD25）、β亚单位（IL-2Rβ，CD122）和γ亚单位（CD132）。单个亚单位与IL-2亲和力较低，而3个亚单位构成的三聚体型IL-2R与IL-2有较高的亲和力并能够更好地完成信号转导[1]。其中，β亚单位相对分子质量约70×10^3，*IL-2Rβ*基因定位于染色体22q11.2-q12；而α和γ亚单位不能单独与IL-2结合，唯3个亚单位聚合方可与IL-2形成较高的亲和力。

$CD4^+$T细胞是机体有效特异性免疫应答的关键。重症肌无力患者辅助性T细胞（Th）和调节性T细胞（Treg）活性失衡是重要发病机制之一[10]。Fattorossi等[11]研究显示，未经治疗的重症肌无力患者外周血Treg细胞计数显著低于正常对照者，予以泼尼松联合硫唑嘌呤治疗后，Treg细胞计数显著增加或恢复正常。而IL-2/IL-2R信号转导通路对Treg细胞的分化、增殖和功能维持具有重要作用[12-13]，并在1型糖尿病和多发性硬化（MS）等自身免疫性疾病的发病过程中影响免疫调节从而增加疾病的易感性[14-15]。Pál等[2]发现，*IL-2Rβ*基因功能调控区rs743777位点多态性与重症肌无力易感性相关。

本研究在NCBI dbSNP数据库提供的中国汉族人群数据中选取*IL-2Rβ*基因rs228942、rs228941和rs743777位点，其中，rs228942位点位于*IL-2Rβ*基因第10外显子，属于编码区错义突变，使第391位氨基酸由天冬氨酸突变为谷氨酸；rs228941位点位于3'端非翻译区（3'UTR），转录非翻译区可以影响信使RNA（mRNA）的剪切和稳定性，进而影响IL-2Rβ功能；rs743777位点位于5'端启动子调控区，其多态性与重症肌无力[2]和类风湿性关节炎[4]有关。两组受试者3个位点的分型成功率均超过98.50%，且对照组3个位点的基因型分布符合Hardy-Weinberg平衡定律，其基因型频率与NCBI dbSNP数据库提供的中国汉族人群数据相近，表明*IL-2Rβ*基因rs228942、rs228941和rs743777位点基因型频率处于平衡状态，具有群体代表性。

本研究结果显示，MG组与对照组*IL-2Rβ*基因rs228942位点*T*等位基因和基因型频率差异均有统计学意义，且含该位点的单倍型（*TG*和*GG*单倍型）差异亦有统计学意义；亚组分析显示，15～50岁亚组、不伴胸腺瘤亚组和发病后2年全身型重症肌无力亚组与对照组比较，该位点*T*等位基因和基因型频率差异均有统计学意义，而发病后2年疾病最严重时Oosterhuis评分（3～5分或0～2分）亚组与对照组差异无统计学意义；进一步行多因素前进法Logistic回归分析显示，基因型并非上述存在频率差异的重症肌无力各亚组的独立危险因素。rs228942位点位于外显子区域，属编码区错义突变，该突变可能引起IL-2Rβ改变，影响IL-2R信号转导，从而增加重症肌无力的易感性，但不影响其严重程度。

rs228941位点*G*等位基因和基因型频率仅在发病年龄亚组中差异有统计学意义，但接近临界值，且年龄不是结局事件。rs743777位点位于5'端启动子调控区，既往研究显示，该位点基因多态性与重症肌无力有关[2]。但本研究rs743777位点*G*等位基因和基因型频率仅在发病后2年疾病最严重时Oosterhuis评分（3～5分或0～2分）亚组中差异有统计学意义，进一步行多因素前进法Logistic回归分析，基因型并非疾病最严重时Oosterhuis评分的独立危险因素。而且，这2个位点等位基因和基因型频率在MG组与对照组无差异，个别亚组存在差异可能是抽样误差所致，故不认为rs743777位点与重症肌无力易感性和严重程度有关。

综上所述，*IL-2Rβ*基因rs228942位点可能与重症肌无力易感性相关，但未发现与其严重程度相关；亦未发现rs228941和rs743777位点多态性与重症肌无力易感性和严重程度相关。

参 考 文 献

[1] Liao W, Lin JX, Leonard WJ. Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. Immunity, 2013, 38:13-25.

[2] Pál Z, Antal P, Millinghoffer A, Hullám G, Pálóczi K, Tóth S, Gabius HJ, Molnár MJ, Falus A, Buzás EI. A novel galectin-1 and interleukin 2 receptor β haplotype is associated with autoimmune myasthenia gravis. J Neuroimmunol, 2010, 229(1/2): 107-111.

[3] Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson WOZ; GABRIEL Consortium. A large - scale, consortium - based genomewide association study of asthma. N Engl J Med, 2010, 363:1211-1221.

[4] Ruyssen-Witrand A, Lukas C, Nigon D, Dawidowicz K, Morel J, Sibilia J, Jamard B, Cambon-Thomsen A, Cantagrel A, Dieudé P, Constantin A. Association of IL-2RA and IL-2RB genes with erosive status in early rheumatoid arthritis patients (ESPOIR and RMP cohorts). Joint Bone Spine, 2014, 81:228-234.

[5] Han JL, Li HF, Xie YC, Sun L, Wang ZX, Xu X, Wang SX, Wang QQ, Gao X, Yao RY, Zhou XB. Association between vitamin D receptor gene Tru9I polymorphism and myasthenia gravis. Zhonghua Yi Xue Za Zhi, 2012, 92:2028-2033.[韩继兰, 李海峰, 谢琰臣, 孙亮, 王梓炫, 徐昕, 王淑霞, 王芹芹, 高翔, 姚如永, 周晓彬. 维生素D受体基因Tru9I位点多态性与重症肌无力的相关性. 中华医学杂志, 2012, 92:2028-2033.]

[6] Aarli JA. Myasthenia gravis in the elderly: is it different? Ann

NY Acad Sci, 2008, 1132:238-243.
[7] Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. Muscle Nerve, 2008, 37:141-149.
[8] Skeie GO, Pandey JP, Aarli JA, Gilhus NE. TNFA and TNFB polymorphisms in myasthenia gravis. Arch Neurol, 1999, 56:457-461.
[9] Song M, Ding XJ, Yue YX, Gao Z, Zhang X, Gao X, Gu CK, Xie YC, Hao HJ, Li HF. Association analysis between B cell activating factor gene polymorphisms and myasthenia gravis. Zhonghua Shen Jing Ke Za Zhi, 2015, 48:585-590.[宋敏, 丁晓君, 岳耀先, 高哲, 张栩, 高翔, 谷传凯, 谢琰臣, 郝洪军, 李海峰. B细胞活化因子基因多态性与重症肌无力的相关性研究. 中华神经科杂志, 2015, 48:585-590.]
[10] Mu L, Sun B, Kong Q, Wang J, Wang G, Zhang S, Wang D, Liu Y, Liu Y, An H, Li H. Disequilibrium of T helper type 1, 2 and 17 cells and regulatory T cells during the development of experimental autoimmune myasthenia gravis. Immunology, 2009, 128(1 Suppl):826-836.
[11] Fattorossi A, Battaglia A, Buzzonetti A, Ciaraffa F, Scambia G, Evoli A. Circulating and thymic CD4 CD25 T regulatory cells in myasthenia gravis: effect of immunosuppressive treatment. Immunology, 2005, 116:134-141.
[12] Zeiser R, Negrin RS. Interleukin-2 receptor downstream events in regulatory T cells: implications for the choice of immunosuppressive drug therapy. Cell Cycle, 2008, 7:458-462.
[13] Bayer AL, Yu A, Malek TR. Function of the IL-2R for thymic and peripheral CD4 + CD25 + Foxp3 + T regulatory cells. J Immunol, 2007, 178:4062-4071.
[14] Chistiakov DA, Voronova NV, Chistiakov PA. The crucial role of IL-2/IL-2RA-mediated immune regulation in the pathogenesis of type 1 diabetes, an evidence coming from genetic and animal model studies. Immunol Lett, 2008, 118:1-5.
[15] Rouse M, Nagarkatti M, Nagarkatti PS. The role of IL-2 in the activation and expansion of regulatory T - cells and the development of experimental autoimmune encephalaomyelitis. Immunobiology, 2013, 218:674-682.

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Systems Biology of Alzheimer´s Disease published

Systems Biology of Alzheimer´s Disease (ISBN: 978-1-4939-2626-8, eBook ISBN: 978-1-4939-2627-5) will be published by Humana Press in 2016. The editors of this book are Juan I. Castrillo and Stephen G. Oliver, Department of Biochemistry & Cambridge Systems Biology Centre, University of Cambridge.

Alzheimer´s disease (AD) and many other neurodegenerative disorders are multifactorial in nature, involving a combination of genomic, epigenomic, network dynamic and environmental factors. A proper investigation requires new integrative Systems Biology approaches, at both the experimental and computational level. The interplay of disease mechanisms and homeostatic networks will underlie the time of onset and rate of progression of the disease.

This book addresses such an integrated approach to AD. It aims to present Systems Biology, including both experimental and computational approaches, as a new strategy for the study of AD and other multifactorial diseases, with the hope that the results will translate into more effective diagnosis and treatment, as well as improved public health policies.

Written for the highly successful Methods in Molecular Biology series, practical and cutting - edge *Systems Biology of Alzheimer´s Disease* is intended for post-graduate students, post-doctoral researchers and experts in different fields with an interest in comprehensive Systems Biology strategies applicable to AD and other complex multifactorial diseases (including other neurodegenerative diseases and cancers).

The price of eBook is 103.52€, and hardcover is 124.99€. Visit link.springer.com for more information.

Epilepsy Towards the Next Decade published

Epilepsy Towards the Next Decade (ISBN: 978-3-319-12282-3, eBook ISBN: 978-3-319-12283-0) was published by Springer International Publishing in 2015. The editor of this book is Pasquale Striano, Pediatric Neurology and Muscular Diseases Unit, University of Genoa.

This volume is a comprehensive collection of the most recent knowledge on the biological bases of various kinds of epilepsies and modern clinical approaches to their treatment. Epilepsy affects about 0.5%-1% of the world´s population and the main goal of its treatment is to eliminate seizures without creating side effects. Despite numerous advances in the treatment of epilepsy and the approval of several new antiepileptic drugs, about 30% of patients continue to experience recurrent seizures which are medically, physically, and/or socially disabling. The editor of this volume hopes that by bridging the gap between the fundamental biology of epilepsy and its clinical implications he might spur further research and treatment options.

The price of eBook is 118.99€, and hardcover is 139.99€. Visit link.springer.com for more information.