酸的重要功能区的变化,进而影响了TDP-43蛋白发挥重要的生理功能,这可能会加快运动神经元死亡的速度,运动神经元快速丢失,延髓肌和呼吸肌萎缩急剧加重,故患者病程短,最后死于延髓麻痹和呼吸衰竭。

本文报告的ALS10大家系的临床表现提示:在有肌跳症状多年的患者,一旦出现一侧肢体肌无力和肌萎缩,病情快速进展,半年内累及呼吸肌和延髓支配的肌肉出现延髓麻痹,要高度重视是否为ALS10,要及时做基因检测明确诊断。对于该家系中3个致病基因携带者,虽然目前尚无肌无力和肌萎缩,但应密切观察,一个患者已进行保护神经元的治疗,现正在密切随访观察中。

目前,对ALS尚无有效的治疗方法,尤其是ALS10类型患者病情进展迅速,患者生存期短,使得对家系其他成员进行致病基因的检测、开展遗传咨询、结合产前基因诊断技术预防携带致病基因的患儿出生显得尤为重要。这对有效地阻止致病基因的传递,预防致病患儿的出生,同时对携带致病基因但尚未发病的家系成员,进行积极的随访,采取积极的治疗来尽可能延迟发病的时间、提高患者生活质量和延长生命时限都具重要意义。

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(编辑 刘清海)

*STAT4*基因rs10181656位点的多态性与不明原因复发性自然流产的相关性

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摘 要：【目的】探讨信号转导与转录激活因子4(*STAT4*)基因在不明原因复发性自然流产(URSA)表达及其表达量与rs10181656位点多态性的关系。【方法】分别采用聚合酶链反应-限制性片段长度多态性法检测332名URSA患者和260名健康对照者rs10181656位点的基因型频率；应用免疫组织化学技术检测其中的86名URSA患者及77名健康对照者蜕膜STAT4基因的蛋白表达量。【结果】rs10181656C/G：URSA组中C/C、C/G及G/G基因型频率分别是36.45%、46.99%及16.57%，而对照组中的3种基因型的频率分别是46.54%、45.38%及8.08%，具有统计学差异($P < 0.05$)，G等位基因增加URSA组发病风险($OR = 1.50$，$P < 0.05$)。STAT4蛋白表达量在URSA组和对照组中的差异具有统计学意义($P < 0.05$)；在URSA组内分为C/C、C/G及G/G基因型的三个亚组STAT4蛋白表达：三个亚组的STAT4蛋白表达差异具有统计学意义($P < 0.05$)，在对照组中亦然($P < 0.05$)，具有G/G基因型样本STAT4蛋白高表达于C/C基因型(P均< 0.05)；相同基因型在URSA组和对照组中STAT4蛋白表达：C/C基因型样本在URSA组和对照组中STAT4蛋白的表达差异无统计学意义($P > 0.05$)，C/G及G/G基因型样本亦然($P > 0.05$)。【结论】rs10181656位点多态性通过影响STAT4蛋白表达，从而与增加URSA发病风险相关。

关键词：复发性自然流产；信号转导与转录激活因子4；基因多态性；蛋白表达
中图分类号：R714.21 **文献标志码**：A **文章编号**：1672-3554(2017)04-0551-05

Association of rs10181656 Polymorphism in Signal Transducer and Activator of Transcription 4 Gene with Susceptibility to Unexplained Recurrent Spontaneous Abortion

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Abstract: 【Objective】To investigate associations between the functional polymorphisms of signal transducer and activator of transcription 4 (*STAT4*) gene and unexplained recurrent spontaneous abortion (URSA) and between STAT4 protein expression and the genotypes of rs10181656 locus. 【Methods】PCR-restriction fragment length polymorphism was used to genotype rs10181656 locus polymorphism in 332 URSA cases and 260 normal controls, in 86 URSA cases and 77 normal controls of which immunohistochemical technique was used to detect STAT4 protein expression. 【Results】The frequencies of rs10181656 C/G were 36.45%, 46.54% in genotype C/C, 46.99%, 45.38% in genotype C/G and 16.57%, 8.08% in genotype G/G between URSA patients and normal controls. They reached statistical difference ($P < 0.05$). The carriers of rs10181656 G allele increased the risk of URSA ($OR = 1.50$, $P < 0.05$). STAT4 protein expression in decidual tissues: ①There was statistical difference in the STAT4 protein expression in decidual tissues between cases and controls ($P < 0.05$). In URSA patients there was statistical difference in the STAT4 protein expression among genotype CC, CG and GG of rs10181656 locus ($P < 0.05$). So was in normal controls ($P < 0.05$). In genotype CC there was no difference in the STAT4 protein expression between cases and controls ($P > 0.05$). Neither was In genotype CG and GG respectively (P all > 0.05). 【Conclusion】Functional polymorphisms of the rs10181656 locus could probably associate with the susceptibility of URSA via STAT4 protein expression increased by genotype G/G in maternal decidual tissue.

Key words: recurrent spontaneous abortion; signal transducer and activator of transcription 4; polymorphism; expression
[J SUN Yat-sen Univ(Med Sci), 2017, 38(4): 551-555]

收稿日期：2017-03-02
基金项目：广东省自然科学基金(S2011010003522)
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复发性自然流产(recurrent spontaneous abortion, RSA)是指和同一性伴侣连续发生≥2次的自然流产,其病因复杂,大概存在一半患者的病因在现有的医技水平上不能明确诊断,进一步导致在临床上不能得到有效的治疗。这样原因不明确的RSA称之为原因不明复发性自然流产(unexplained recurrent spontaneous abortion, URSA)。我们前期发研究发现,信号转导与转录激活因子4(signal transducer and activator of transcription 4, *STAT4*)基因是URSA患者的易感基因,其中rs10181656 G等位基因显著增加URSA的发病风险[1]。目前研究显示*STAT4*基因在URSA蜕膜中的蛋白表达明显高于健康女性[2],而rs10181656基因型与STAT4蛋白表达在URSA中关系尚未见报道。本文从基因多态性对蛋白表达的影响方面出发,来探讨URSA的易感基因*STAT4*增加发病风险可能机理,为URSA临床治疗提供可能的参考思路。

1 材料与方法

1.1 实验对象与分组

1.1.1 URSA组 URSA病人332例,从2012年12月到2015年12月,于中山大学干细胞中心进行免疫治疗非妊娠病人246例,年龄21~37岁,平均29.6岁;中山大学附属第一医院东院门诊人流间的稽留流产病人86例,年龄22~36岁,平均28.9岁。满足:经历自然流产≥2次,孕周≤10周;父母染色体无明显异常,女方生殖系统无解剖异常,生殖道分泌物无感染性疾病,性激素检查正常,抗心磷脂抗体等临床常规检查的抗体阴性,男方精液常规无异常。

1.1.2 对照组 健康女性260名,来自同期在中山大学附属第一医院东院的体检者183名,年龄28~40岁,平均31.5岁;主动人工流产者77名,年龄23~37岁,平均27.6岁,胚胎发育正常,孕期≤10周。满足:最少一次正常孕育史,无不良妊娠史;无免疫性及代谢内分泌疾病。

1.2 方 法

1.2.1 DNA提取 经实验对象知情同意,并经中山大学附属第一医院医学伦理委员会批复,于中山大学干细胞中心进行免疫治疗非妊娠病人246例和中山大学附属第一医院东院的体检者183例,采取3 mL外周血;中山大学附属第一医院东院门诊人流间的稽留流产病人86例和主动人工流产者77例,收集100 mg蜕膜。用3 mL血或30 mg蜕膜按照DNA试剂盒实验步骤提取基因组DNA,-20 ℃保存备用。

1.2.2 PCR-RFLP法检测基因型 利用Primer premier 5.0对rs10181656位点设计一对引物,正向引物:5′-ACTGTGATAGATAACTAGCTGGAAT-3′,反向引物:5′-AAAGCAGGGAACAGGAAGAT-3′。每管PCR配兑体系:样本DNA5 μL、上下游特异扩增引物各2 μL、Takara Ex Tap Mix 15 μL、加入双蒸水配制到30 μL;反应条件:先95℃预变性5 min 1次,接着94 ℃变性30 s,56 ℃退火30 s,72 ℃延伸1 min,共35个循环,72℃延伸7 min。取10 μL PCR产物加入1 μL限制性内切酶(*Dde*I)和2 μL缓冲液(Fermentas),加注射灭菌水配成30 μL的酶切反应液,在37 ℃恒温箱反应12 h。取10 μL酶切后的反应液行电泳,随机挑出10%的样本重复上述过程,所得实验图像和上次结果完全一致。

1.2.3 SP法检测蛋白表达 每个标本剩余的约70 mg蜕膜组织,生理盐水清洗除杂,固定、脱水、包埋及切片备用。选择兔抗人STAT4抗体(1∶125,abcam),DAB及SP试剂盒(北京中杉金桥生物技术有限公司),按照内附实验步骤的说明操作实验。以细胞浆内出现棕色或棕黄色颗粒为阳性,一张切片选取5个高倍视野(×400),利用Image-Pro Plus7.0(Media Cybernetics)软件分析实验图像。随机选取5个高倍视野,取其平均值来代表该样本的蛋白表达水平。

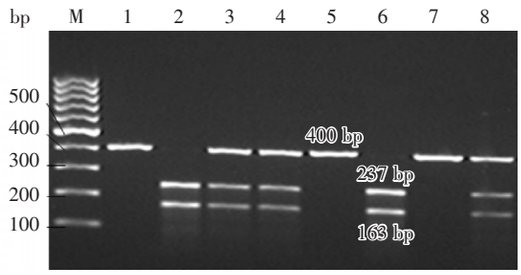
1.3 统计学分析

采用Excel软件计数基因型及等位基因频率,利用SPSS18.0软件对基因型和等位基因频率等定性资料差异比较行χ^2检验、非条件Logistic回归分析,对STAT4蛋白表达量等独立样本定量资料的差异比较,多组行单因素方差分析,两组行t检验,设定$\alpha = 0.05$为检验水准。

2 结 果

2.1 基因型判定

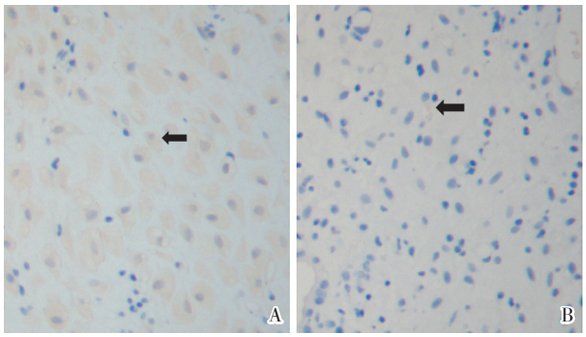
PCR扩增目的产物片段长度400 bp(图1),完全没有被*Dde*I内切的是CC基因型,只有部分被酶切为400、237及163 bp片段的是C/G,彻底被酶切为237和163 bp片段是G/G。



Panel is the band pattern for rs10181656. Lane 1, 5, 7 indicated C/C genotype; Lane 2 and 6 indicated G/G genotype; Lane 3, 4, 8 indicated C/G genotype. M represented 100 bp DNA ladder.

图1　rs10181656位点电泳结果图

Fig.1　Typical band patterns of PCR-RFLP products in rs10181656 locus



Immunohistochemical positive signal of STAT4 protein expression is brown and located in the cytoplasm, which is indicated by the arrow. Fig A and Fig B were both magnified 400 times.

图2　*STAT4*基因蛋白在URSA组和对照组蜕膜组织中免疫组化结果

Fig.2　The protein expression of STAT4 in decidual tissue between the URSA group and control group

2.2　基因频率分布

经χ^2检验及非条件Logistic回归统计分析，rs10181656位点基因型和等位基因频率在两组中的差异具有统计学意义（表1）。

2.3　蛋白表达

STAT4蛋白免疫组化阳性表达信号为棕色，位于胞质，如箭头所示（图2）。

2.4　*STAT4*基因表达水平与基因型的亚组统计分析

按基因型的不同分为C/C、C/G及G/G基因型亚组，再分析不同亚组之间*STAT4*基因表达水平的情况，*STAT4*在URSA组和对照组中的蛋白表达水平各自是154.7 ± 13.4和149.9 ± 11.5，差异达到统计学意义（$P = 0.015$），STAT4在URSA组中的表达水平显著高于对照组；每一个亚组STAT4蛋白总体表达水平在URSA组和对照组之间无差异（$P > 0.05$）；单因素方差分析，三个亚组STAT4蛋白表达水平在URSA组内存在显著差异，在对照组内亦然，携带G/G基因型样本的STAT4蛋白表达量高于C/C基因型（表2）。

表1　*STAT4*基因rs10181656位点的基因型及等位基因的频率及统计分析

Table 1　Genotype and allele frequencies of rs10181656 locus in *STAT4* gene in the URSA group and control group　［*n*(%)］

Site	Genotype Allele	URSA(*N*=332)	Control(*N*=260)	χ^2	*P*	OR(95%CI)
rs10181656	C/C	121(36.45)	121(46.54)	11.9	0.003[1)]	1
	C/G	156(46.99)	118(45.38)	2.485	0.115[2)]	0.76(0.53,1.07)
	G/G	55(16.57)	21(8.08)	11.71	0.001[2)]	2.62(1.49,4.60)
	C/C	121(36.45)	121(46.54)			
	C/G+G/G	211(63.55)	139(53.46)	6.146	0.013[2)]	0.66(0.47,0.92)
	C/G	156(46.99)	118(45.38)			
	C/C+G/G	176(53.01)	142(54.62)	0.151	0.698[2)]	1.07(0.77,1.48)
	G/G	55(16.57)	21(8.08)			
	C/C+C/G	277(83.43)	239(91.92)	9.391	0.002[2)]	2.26(1.33,3.85)
	C	398(59.94)	360(69.23)			
	G	266(40.06)	160(30.77)	10.929	0.001[3)]	1.50(1.18,1.92)

URSA(332): 332 cases included 246 cases of 3 mL peripheral blood and 86 cases of missed abortion decidual; Control(260): 260 cases included 183 cases of 3 mL peripheral blood and 77 cases of induced abortion decidual. 1) *P* was determined by Pearson's chi-square test for 3×2 contingency tables, $P \leq 0.05$ was considered statistically significant; 2), 3) *P* was determined by Pearson's chi-square test for 2×2 contingency tables, $P \leq 0.017$ or $P \leq 0.05$ are considered statistically significant, respectively.

表2 两组对象STAT4基因三种基因型的表达水平

Table 2 STAT4 gene expression in different Genotype in two groups [$\bar{x} \pm s(n)$]

Group	N	Expression volume			F	P
		C/C	C/G	G/G		
URSA	86	151.7 ± 12.8(32)	154.2 ± 14.4(39)	162.5 ± 9.1(15)	3.558	0.033
Control	77	147.5 ± 9.9(34)	150.4 ± 12.6(37)	160.6 ± 11.5(6)	3.590	0.032

3 讨 论

妊娠是一种半同种异体移植过程,成功妊娠依赖于母方对父方抗原恰当的免疫耐受。而免疫耐受一旦打破,就可能导致不良妊娠结局,如自然流产等[3]。在正常妊娠中表现出免疫相关细胞特殊状态,来维持母方的免疫耐受,如Th1/Th2细胞再平衡呈现出妊娠特有的"Th2现象"[4],免疫调节细胞Foxp3+Treg在母体蜕膜组织中升高[5],而在URSA患者蜕膜中与母-胎排斥反应相关的Th17数量明显增加[6]。研究显示,STAT4能够促进辅助性T细胞向Th1细胞分化[7]及刺激Th17细胞的活化[8],抑制Foxp3+Treg细胞的免疫抑制功能[9]。本研究显示STAT4基因在URSA蜕膜中的蛋白表达明显高于对照组($P = 0.015$),由此推测增高的STAT4通过JAK-STAT信号通路改变Th1/Th2细胞平衡、Th17活化及Foxp3+Treg功能,打破了正常妊娠的免疫耐受,导致URSA发病。

近年关于*STAT4*基因rs10181656位点多态性与免疫性疾病相关性的文献不断出现。研究发现,*STAT4*基因rs10181656位点G等位基因明显增加系统性红斑狼疮(systemic lupus erythematosus,SLE)[10]、银屑病性关节炎[11]等免疫性疾病的发病风险。本研究显示,URSA患者rs10181656GG基因型及G等位基因频率显著高于对照组;与CC基因型比较,携带G等位基因和GG基因型患者发生自然流产的风险明显增加。

Sigurdsson等[12]研究发现在SLE患者中携带rs10181656 G危险等位基因的患者的成骨细胞中出现了*STAT4*基因过度表达的现象,而我们统计分析发现,在URSA组内,把rs10181656位点的CC、CG和GG三种基因型分为三个不同的亚组,在三个亚组中,分析三种基因型之间对应的蜕膜组织中*STAT4*基因的蛋白表达量,存在明显的差异,携带GG基因型亚组的*STAT4*基因蛋白表达量高于携带CC基因型亚组的样本,在健康女性的对照组中亦出现相同的情况,与Sigurdsson在SLE患者中的研究相比较在不同的实验对象中得到相似的结论。我们推测由于G等位基因频率在URSA组的升高和携带G等位基因样本的STAT4高表达,所以*STAT4*基因蛋白表达量在URSA组与对照组相比较时展现出总体显著升高,升高的*STAT4*蛋白通过JAK-STAT信号通路,导致URSA中免疫相关细胞失常,破坏了母体对胎儿携带的父性抗原的免疫耐受而引发自然流产。这也许就是rs10181656 C/G多态性影响URSA易感性的机理。

我们进一步的分析发现,携带GG基因型样本的蛋白表达水平在URSA组中表现出稍高于对照组,但未达到统计学意义,而携带CC、CG基因型样本也出现了类似的结果,由此我们推测,G等位基因的频率和与G等位基因正相关的*STAT4*蛋白表达量,导致*STAT4*基因在URSA组的蜕膜组织的高表达中起了主要作用,但是可能还有未发现的机制协同参与*STAT4*基因表达,所以在本实验中表现出基因型相同,但蛋白表达在两组中存在微小差异现象,此种现象的具体机理还有待于进一步的探讨。

此次研究初次阐述了*STAT4*基因rs10181656位点的G危险等位基因使*STAT4*基因在URSA患者中的蛋白表达明显增加,增加的STAT4蛋白通过信号通路,发挥了级联放大效应 打破了母胎免疫耐受引起URSA发病。

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（编辑　余　菁）

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（编辑　刘清海）