

证 的 研 究

HLA-DQA1 等位基因与急性脑梗塞多种
中医体质类型的相关性研究沈乃莹¹ 闫 征² 金宇安¹ 韩 静¹ 何培英² 王申五²

摘要 对急性脑梗塞多种中医体质进行 HLA-DQA1 等位基因分型,分析体质类型的遗传易感性及体质与“证”和治疗的密切关系。采用 PCR-SSP 技术对观察组急性脑梗塞阴虚质、阳虚质、气虚质、痰湿质、血瘀质共 103 例及正常质对照组 99 例进行 HLA-DQA1 等位基因的基因分型。结果表明,HLA-DQA1 * 0501 基因型在阴虚质明显高于正常质对照组 ($P < 0.01$),HLA-DQA1 * 0301 基因型在气虚质、痰湿质、血瘀质均明显高于正常质对照组 ($P < 0.01$)。提示急性脑梗塞患者,HLA-DQA1 * 0501 基因与阴虚质相关联;HLA-DQA1 * 0301 基因与气虚质、痰湿质及血瘀质相关联;从分子生物学水平阐明了体质与“证”,体质与“同病异治”的遗传学背景。

主题词 脑梗塞/遗传学 @ 中医体质类型

急性脑梗塞属“中风”范畴,其发病率高,复发率高,致残率高,严重危害着人类的健康。脑梗塞多种中医体质类型与人类白细胞抗原(Human Leukocyte Antigen, HLA)基因相关性研究经中国医学科学院信息研究所检索,目前国内尚未见文献报道,脑血管疾病是一种多基因遗传性疾病,其产生具有强烈的遗传易感性^[1]。我们对 103 例具有颈动脉粥样硬化病史的急性脑梗塞患者进行中医体质分型,并采用聚合酶链反应-序列特异性引物(Polymerase Chain Reaction, PCR-SSP)技术对多种中医体质进行 HLA-DQA1 等位基因的 DNA 分型,从而进一步探讨中医体质类型的遗传易感性,体质与“证”的密切关系,体质与“同病异治、异病同治”的密切关系及其遗传学背景。

1 临床资料

观察组按照 1996 年全国脑血管病学术会议修订的诊断标准^[2],并经 CT 或 MRI 证实。随机选择了黄河以北的北方汉族人中具有颈动脉粥样硬化病史(多普勒超声 DUS 确诊)的急性脑梗塞患者 103 例,全部病例均系 1998 年 10 月~2000 年 5 月在我院首次发

病 1 周内住院或留观的急性脑梗塞患者,男 61 例,女 42 例,年龄 41~86 岁,平均 58.6 ± 7.9 岁。梗塞直径 0.6~7.6cm,103 例中有脑梗塞阳性家族史 31 例,阴性家族史 72 例;伴发原发性高血压病 71 例,高脂血症 52 例,糖尿病 20 例,冠心病 11 例。并对观察组 103 例患者按照匡调元、王琦的分型标准进行中医体质分型^[3],其中阴虚质 19 例,阳虚质 4 例,气虚质 32 例,痰湿质 23 例,血瘀质 25 例。对照组 99 例,男 56 例,女 43 例。年龄 39~72 岁,平均 57.6 ± 7.2 岁,均为体检健康者,其父母为原籍黄河以北的北方汉族,家族一、二级亲属中无一人患有原发性高血压、心脑血管病或糖尿病,99 例对照组按匡、王二氏体质分型标准确定为正常质^[3]。

2 观察方法

2.1 DNA 提取:取外周静脉血 5ml,加 2%EDTA 1/10 体积(500 μ l)抗凝。采用改良酚/氯仿抽提法提取 DNA^[4]。

2.2 PCR-SSP:采用 Qlerup^[5]PCR-SSP 分型方法,并用第 11 届 HLA 国际会议标准寡核苷酸探针,分别对 12 对特异性引物(北京大学医学部附属人民医院中心实验室合成)进行鉴定,符合率 100%。PCR 扩增体系 15 μ l 内含 5pmmol 特异性引物,内参照引物 0.5pmmol, dNTP 各 50 μ mol/L, Taq 酶 0.5u, 10 $\times$ PCR 缓冲液 1.5 μ l。预变性:94 $^{\circ}$ C 60 秒;变性:94 $^{\circ}$ C 30 秒;退火:58 $^{\circ}$ C 30 秒;延伸:72 $^{\circ}$ C 45 秒;后延伸 72 $^{\circ}$ C 300 秒;共 35 个循环。2%琼脂糖电泳鉴定并拍照(图略)。

2.3 统计学处理:基因频率采用基因计数法计算;组间等位基因频率的比较采用卡方检验。

3 结果

附表示,观察组阴虚质 HLA-DQA1 * 0501 基因频率,两组 DQA1 位点等位基因的基因频率比较:气虚质、痰湿质、血瘀质 HLA-DQA1 * 0301 基因频率明显高于正常质对照组 ($P < 0.01$)。附表示,正常质对照组 HLA-DQA1 * 0103 基因是高分布频率基因,而气虚质、痰湿质、血瘀质组此基因分布频率却明显降低 ($P < 0.01$)。

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附表 两组 DQA1 位点等位基因的基因频率比较

等位基因	对照组		观察组		
	正常质 (99 例)	阴虚质 (19 例)	气虚质 (32 例)	痰湿质 (23 例)	血瘀质 (25 例)
DQA1 * 0101	6.5657	7.8974	7.8125	6.5217	8.0000
DQA1 * 0102	10.6061	5.2632	12.5000	13.0435	12.0000
DQA1 * 0103	18.6867	13.1579	4.6875*	4.3478*	4.0000*
DQA1 * 0104	1.5152	0.0000	1.5625	2.1739	4.0000
DQA1 * 0201	5.0505	2.6316	6.2500	10.8696	12.0000
DQA1 * 0301	22.2222	23.6842	37.5000*	41.3043*	38.0000*
DQA1 * 0302	13.6364	7.8947	6.2500	6.5217	6.0000
DQA1 * 0401	1.0101	2.6316	1.5625	0.0000	0.0000
DQA1 * 0501	19.6970	36.8421*	20.3135	13.0434	14.0000
DQA1 * 0601	1.0101	0.0000	1.5625	2.1739	2.0000

* 与对照组比较, $P < 0.01$

4 讨论

HLA 基因是由一组紧密连锁的复等位基因位点组成,位于第 6 号染色体短臂上。以往的实验证明,HLA 血清学表型相同,DNA 核苷酸序列不一定相同,故不能用传统的血清学分型方法鉴定^[6]。近年随着 HLA 分型技术的发展,将 HLA-Ⅱ 类基因主要分为 DR、DQ 及 DP 位点,研究表明,HLA-Ⅱ 类基因编码的Ⅱ类抗原主要存在于抗原提呈细胞,在抗原提呈细胞参与的免疫过程中具有重要意义,其自身极大的等位基因多态性可以作为遗传学标记^[7],以基因本身分析,更易揭示疾病的本质。现代分子生物学理论和技术把脑梗塞的中医体质分型的研究带入了分子遗传学领域。匡调元指出:“体质是人体及人群中的个体在遗传的基础上,在环境、习惯的影响下,在其生长、发育及衰老的过程中形成的机能、结构与代谢上相对稳定的特殊状态^[8]”。随着医疗实践的不断丰富和发展,许多古代医家依稀感觉到,体质的遗传是有其物质基础的,如《类经》中指出:“夫人器质,既禀于有生之初,则具一定之数。”这里所说人的“器质”在受生之时已有“定数”说的是体质的遗传,它是一种附于气血之内,宰乎气血之先的“元气”来完成的,虽然我们不能简单地将此“元气”与现代遗传学的染色体相类比,但说明中医在数百年前就对遗传的物质性有了一个初步的认识,体质及体质的遗传性在临床工作中占有重要地位,其原因在于:(1)中医治病论疾非常重视个体差异。(2)中医的辨证就其实质而言与辨体密不可分。体质与发病学的联系是体质与“证”的联系,突出反应“证”随体质而转移。“证”是体质特征的反应,而体质是产生各种不同“证”的物质基础。所谓“同病异证、异病同证”是以体质学说为主要依据的。(3)中医治疗学与体质因素密不可分,最重要表现在“治疗求本”的原则上。这里所谓“本”从

某种意义上可指患者的体质,治则是建立在“证”的基础上,而“证”又是以体质为机转,因此不同的体质所表现的证候不同,治疗方法也就随之而异,这种以体质为“本”来确定治疗的方法,在具体临床应用上就体现出“同病异治”和“异病同治”两个方面。

据此,我们采用 PCR-SSP 技术对急性脑梗塞多种中医体质(阴虚质、阳虚质、气虚质、痰湿质、血瘀质)进行 HLA-DQA1 等位基因分型,发现 HLA-DQA1 * 0501 基因型在阴虚质明显高于正常质对照组($P < 0.01$),两组显著性差异提示 HLA-DQA1 * 0501 基因与阴虚质相关联;HLA-DQA1 * 0301 基因型在气虚质、痰湿质、血瘀质均明显高于正常质对照组(P 均 < 0.01),这种显著差异提示 HLA-DQA1 * 0301 基因同与气虚质、痰湿质、血瘀质相关联,而中医认为气虚水湿受障、血流不畅而致痰湿、血瘀,因而我们的研究为气虚质、痰湿质、血瘀质具有内在联系的中医理论找到了可能的遗传学依据。在基因分型后还发现 HLA-DQA1 * 0103 基因在气虚质、痰湿质、血瘀质的分布频率均远低于正常质对照组(P 均 < 0.01),这种显著的连锁不平衡差异提示 HLA-DQA1 * 0103 基因可能是一个保护性基因,在这三种体质中 HLA-DQA1 * 0301 基因分布频率高,而 HLA-DQA1 * 0103 基因分布频率低的现象,提示 HLA 的发病基因强于保护基因,可能是导致气虚质、痰湿质及血瘀质发生脑梗塞的一个因素;阳虚质仅 4 例,其中 3 例均与 HLA-DQA1 * 0301 基因相关,但例数少,未做统计处理。

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ABSTRACTS FROM ORIGINAL ARTICLES

Clinical and Experimental Study on Sputum—Removing Action of Tan Jing Nebulization Liquid

Peng Zhaolin, Peng Wenjuan

103 children of respiratory tract infection were treated with ultrasonic aerosol inhalation of Tan Jing Nebulization Liquid in order to study mechanisms of sputum—removing action in pharyngeal portion. Clinical results indicated that Tang Jing Nebulization Liquid in sputum—removing action and reduction of sputum sound was superior to the control group ($P < 0.01$). Results in the experiment of animal showed that the drug promoted obviously ciliary motion in air passage as compared with the control group ($P < 0.05$ or 0.01). Electron microscopy indicated that chain of sputum shortened, loosened and dispersed.

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Key words: respiratory tract infection / TCM therapy, Chinese herb steam inhalation, @ Tan Jing Nebulization Liquid

(Original article on page 216)

Comparative Analysis of Therapeutic Effects in Removing Stone in 584 Cases of Cholelithiasis

Chen Weimin, Pang Xiaoxia, Zhu Weihua

581 cases of cholelithiasis were assigned to Western medicine group, Chinese herb group and Combined treatment group. The Western medicine group received oral administration of Trepibutone, Ai Hou Dan Tong Tablet; The Chinese herb group were orally administrated self—formulated prescription Si Jin Pai Shi Decoction and Gan Dan Jie Shi Tablet; The combined treatment group were orally administrated Si Jin Pai Shi Decoction and received treatment with Tui An Yun Jing Instrument. Results showed that the therapeutic effect in the combined treatment group was the best, the all—stone ejection rate, total lithecbole rate and the total effective rate being 16.84%, 49.27%, 84.82%, respectively; in the Chinese herb group was next, the three indexes being 11.29%, 37.10% and 67.7%, respectively; in the Western medicine group was the worst, the three indexes being 2.44%, 7.32% and 13.90%, respectively. There were significantly differences in the all—stone ejection rate ($P < 0.05$), total lithecbole rate ($P < 0.01$) and the total effective rate ($P < 0.01$) among the three groups. It is suggested that the combined treatment is a better method for clinical treatment of middle and small stone with transverse diameter less than 1.0cm at present.

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Key words: cholelithiasis/Chinese herb therapy

(Original article on page 218)

Approach to Correlativity of Treatment of Amenia Based on Difference of Syndromes with Endometrial Thickness—Clinical Observation of 84 Cases

Han Xiaofang, Wang Pujing

It was found in clinical observation of 84 cases of functional secondary amenia that the patient with endometrial thickness of less than 0.5 cm manifested most as deficiency syndrome and the patient with endometrial thickness of more than 0.5 cm manifested most as excess syndrome. Based on the therapeutic principle "illness of deficiency type should be treated by tonifying method and excess syndrome should be treated with the method of purgation and reduction", a good therapeutic effect was attained. It is indicated that treatment of amenia based on differentiation of syndromes and the endometrial thickness have close correlativity.

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Key words: amenia/ Chinese herb therapy, endometrium, medical action, treatment based on differentiation of symptoms and signs

(Original article on page 220)

Study on Correlativity of HLA—DQA1 Allelic Gene with Varied TCM Constitution Types in Acute Cerebral Infarction

Shen Naiying, Yan Zheng, Jin Yu'an, et al

Varied TCM constitutions in the patient of acute cerebral infarction were classified by using HLA—DQA1 allelic gene to analyze hereditary susceptibility of constitution types and relation among constitution, syndrome and treatment. PCR—SSP technique was used for classification of Yin—deficiency, Yang—deficiency, Qi—deficiency, phlegm—dampness and blood stasis constitution in 103 cases of acute cerebral infarction and HLA—DQA1 allelic gene was used for gene classification in 99 cases of normal constitution. Results indicated that HLA—DQA1 * 0501 gene type in Yin—deficiency constitution was significant higher than that of normal constitution ($P < 0.01$); HLA—DQA1 * 0301 gene type in Qi—deficiency, phlegm—dampness and blood stasis constitution all was higher than normal constitution ($P < 0.01$). It is suggested that HLA—DQA1 * 0501 gene is correlated with Yin—deficiency in the patient of acute cerebral infarction. Thus, genetical background of relation of constitution with syndrome, and constitution with "treating the same disease with different methods" are explained from view of point of molecular biology.

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Key words: cerebral infarction/ genetics

(Original article on page 237)