·Original Article in English·

Expression of Hoxd 13 Gene in Children with Congenital Anorectal Malformation

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Abstract: Objective To study the Hoxd 13 gene expression in the children with anorectal malformation (CAM) and normal children, and to explore the relationship between Hoxd 13 gene and CAM. **Methods** Hoxd 13 gene mRNA expression was detected by RT-PCR in the rectal pouch in both 16 cases of anorectal malformations and 5 normal children. **Results** The expression level of Hoxd 13 gene mRNA was significantly higher in normal children than that in children with CAM (0.32 ± 0.26 vs 0.73 ± 0.10), ($P < 0.01$). **Conclusions** Hoxd 13 gene expression may be involved in the pathogenesis of anorectal malformation. **[Chin J Contemp Pediatr, 2003, 5(3): 201-204]**

Key words: Anorectal malformation; Hoxd 13 gene; RT-PCR; Gene expression

先天性肛门直肠畸形 Hoxd 13 基因表达的研究

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[**摘 要**] **目的** 检测 Hoxd 13 基因在肛门直肠畸形病人和正常人的直肠末端表达状况,探讨 Hoxd 13 基因与人类先天性肛门直肠畸形发生的关系。**方法** 应用 RT-PCR 方法检测 Hoxd 13 基因在 16 例肛门直肠畸形患儿和 5 例正常儿童直肠末端的表达水平。**结果** Hoxd 13 基因 mRNA 在先天性无肛畸形直肠末端表达水平明显低于正常直肠末端的表达(0.32 ± 0.36 vs 0.73 ± 0.10)。($P < 0.01$)。**结论** Hoxd 13 基因表达可能与人类先天性肛门直肠畸形的发生有关。 [**中国当代儿科杂志,2003, 5(3): 201-204**]

[**关 键 词**] 肛门直肠畸形;Hoxd 13 基因;逆转录多聚酶链反应;基因表达
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Congenital anorectal malformation (CAM) is the most common gastrointestinal disorder, its morbidity is 1/5 000 to 1/1 500. The etiology of CAM still remains unknown. According to studies, epidemiological and animal experiments led to the conclusion that genetic factors played an important role in the occurrence of CAM[1-3]. Up to now, genetic research about CAM is still in the preliminary stage, and most genetic studies about CAM are just limited to chromosomal level and animal experiments. Little is known about the genetics of CAM on the basis of gene in the world. The candidate genes are still unknown, much less about the pathogenic genes[4-6].

Animal experiments showed that Hox genes played an essential role in the development and differentiation of gastrointestinal tracts in vertebrates, of which Hoxd 13 gene was closely related to the development of terminal part of the gastrointestinal tract-anorectum[7-9].

There is no report about whether Hoxd 13 gene is related to CAM or not. In the current study of the expression levels, Hoxd 13 gene in children with CAM or without CAM was determined by reverse transcription polymerase chain reaction (RT-PCR) to explore the relationship of Hoxd 13 gene with human CAM and probe the pathogenic genes of human CAM.

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Materials and methods

Patients and specimens

Among 16 children with CAM, 10 were males and 6 were females, aged from 2 days to 3 years old. Eight patients were low anorectal malformation, 4 children had intermediate anorectal malformations, and 4 children had high anorectal malformations.

During the operation, 0.3 ×0.3 ×0.3 cm^3 to 0.8 ×0.8 ×0.8 cm^3 terminal anorectum tissues were removed, and stored at - 76 ℃. Terminal anorectum tissues 1 cm high above the dentate line of 5 children with acquired anal fistula or rectal trauma were collected as controls and treated with the same method as above.

Total RNA extraction

Tissues from 16 cases of CAM patients and 5 controls were ground after frozen and melted, then 1 ml Trizol solution was added. Total RNA was electrophoresed on 2 % agarose gel and the absorption rate of RNA at A260/A280 was detected by ultraviolet spectrophotometry. The extraction of RNA was stored at - 20 ℃.

Synthesis of the first chain of cDNA by reverse transcription

The reverse transcription kits were obtained from Takara Biotechnology Co., Ltd. Dalian, and the reaction condition was set according to the instruction book. The volume of reaction system was 20 μl. The condition of reaction was at 65 ℃ (1 min), 30 ℃ (5 min), 65 ℃ (30 min), 65 ℃ (15 min), 98 ℃ (5 min), 5 ℃ (5 min).

Amplification of Hoxd 13 gene by PCR

The upstream of the primer of Hoxd 13 gene was 5'- TGCTCCTCTTCTGCCGTTGT - 3' and its downstream sequence was 5'- CCTGTGGCTGGTCCTTGGT - 3'. The primer was provided by Beijing Augct Biotechnology Co., Ltd. The product of amplification was 487 bp. The condition was at 94 ℃ 3 min, 94 ℃ 30 s, 55 ℃ 45 s, 72 ℃ 90 s, 72 ℃ 5 min, 30 cycles. The volume of reaction system was 25 μl.

β-actin was used as internal control. Its primer was provided by Shanghai Biotechnology Co., Ltd. The upstream sequence was 5'- GTG GGG CGC CCC AGG CAC CA - 3' and the downstream was 5' - CTC CTT ATT GTC ACG CAC GAT TTC - 3'. The final product was 690 bp fragment. The condition of proliferation was at 94 ℃ 3 min, 94 ℃ 40 s, 60 ℃ 40 s, 72 ℃ 1 min, 72 ℃ 5 min, 20 cycles.

Analysis of the products of Hoxd 13 gene

The products of Hoxd 13 gene and β-actin were determined by Kodak 1D gel imaging analytic system. Expression level of mRNA was estimated by the brightness of products bands. The relative content of the products was determined by the ratio of mRNA density of Hoxd 13 gene to the density of β-actin.

Statistical analysis

All data were expressed in the form of $\bar{x} \pm s$. Statistical analysis was performed using Student's t test.

Results

Concentration of total RNA

Total RNA at A260/A280 detected by ultraviolet spectrophotometry was between 1.7 and 1.9, which implicated that the concentration of total RNA was fairly good.

The expression of Hoxd 13 mRNA

The bands representing mRNA of Hoxd 13 gene was obscure and even disappeared in CAM patients, yet was very bright in normal intestinal tissues. But there were no differences among the bands of β-actin. See Figure 1. The mRNA expression level of Hoxd 13 gene in terminal rectums of CAM was significantly lower than that in normal rectum (0.32 ±0.26 vs 0.73 ±0.10) ($P < 0.01$).

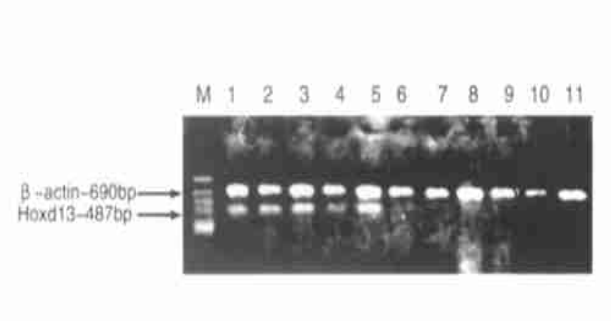

Figure 1 The electrophoretic analysis of Hoxd 13 gene mRNA in the terminal part of the rectum
1 - 5 control group, 6 - 11 CAM group

Discussion

The etiology of CAM remains unknown. It is known that the pathogenesis and pathological changes of CAM are very complex, so it is often associated with other malformations, or appears as a part of syndromes. The spectrum of CAM is fairly wide with various phenotypes. The genetic pathways of CAM are poorly understood and more than one gene is involved in its pathogenesis. Therefore, it is very difficult to investigate the pathogenic genes of CAM[4].

Animal experiments led to the conclusion that Hox gene played a key role in the development of gastrointestinal tracts in vertebrates embryos[7-9]. Hox gene in human beings and mice can be divided into four clusters: Hoxa, Hoxb, Hoxc and Hoxd. If classified on the basis of homology, these different genes can be divided into 13 groups. Hox genes are expressed in a collinear fashion and temporo-spatial sequence. In one cluster, the upstream genes express early and are differentiated into the cephalic end, while the downstream genes express later and are differentiated into the tail end. For example, in the development of gastrointestinal tract of chicken embryos, Hoxd 9 expressed before Hoxd 10, then Hox 11 and Hox 12 expressed successively. Hoxd 13 expressed latest. On the fourth day of chicken embryos, Hoxd 9 expressed in the mesoderm of small intestine and cecum, Hoxd 10 expressed in the cecal mesoderm. Hoxd 11 expressed in the mesoderm of large intestine and the cloaca, whereas Hoxd 13 expressed only in the cloaca mesoderm. Moreover, every gene expression region is demarcated clearly. The expression of Hox gene may be used as clues of morphological boundary[8]. So it can be concluded that Hoxd 13 gene is closely related to the development of the tail ends of vertebrates, such as terminal gastrointestinal tract and urogenital system. Recently Kondo[10] reported that anorectum muscule was maldeveloped in Hoxd 13 - knock - out mice. Warot[11] pointed that anorectum developed abnormally in Hoxa $13^{+/-}$/ Hoxd $13^{+/-}$ mutation mice in the research of complex mutation of Hoxa 13 and Hoxd 13 genes in mice. Furthermore, transgenic mice with other types of complex mutations of Hoxa 13 and Hoxd 13 genes showed malformations of gonad, vagina, urethra, position of anal orifice and so on. All of these experiments suggested that Hoxd 13 gene may be very important in the development of terminal part of gastrointestinal tract and urogenital system, whose abnormalities would result in CAM.

It was known that Hoxd 13 mutation could cause congenital synpolydactyly, but it was never reported that it was related to CAM in human beings.

In this current study, Hoxd 13 gene expression level was determined in human rectal terminus by RT - PCR, and Hoxd 13 level in CAM patients was found significantly lower than that in children without CAM, which indicated that Hoxd 13 gene was likely to be related to CAM. In normal individuals, intestinal epithelium and muscular tissues above dentate line originate from endoderm and mesoderm, and intestinal epithelium below dentate line is from exoderm. The normal anorectum is the result of coordinate development of these three structures. Hoxd 13 gene expression level in hindgut can not only be used as the marker of morphological boundary of hindgut, but also induce the proliferation and differentiation of interstitial cells and epitheliocytes in hindgut[8,9]. Hence, we conjecture that during embryonic stage primitive rectum is connected to urogenital sinus in cloaca. And during the differentiation of cloaca, because of the abnormal expression of Hoxd 13 gene, the boundaries between different parts of cloaca are unclear. For instance, if the boundary between primitive rectum and urogenital sinus is unclear during this stage, interstitial cells and epitheliocytes will develop abnormally and result in CAM.

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·病例报告·

甘露醇致幼儿严重皮疹 1 例

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[中图分类号] R758.25 [文献标识码] E

患儿,男,2 岁,因高热 2 d,嗜睡 1 d、抽搐 3 次入院。入院前 3 h 出现四肢抽搐、两眼凝视、口吐白沫、神志不清、持续 4～5 min 停止,呈间断发作 3 次,呕吐胃内容物 1 次,非喷射状,无腹泻。既往无药物、食物过敏史。查体:T 39 ℃,P 142 次/min,R 35 次/min,体重 11 kg。发育正常,营养中等,嗜睡状。皮肤粘膜无黄染、皮疹及出血点,球结膜无水肿,双侧瞳孔等大正圆,对光反射正常,颈抗(+),双肺无异常,心律齐、心脏各瓣膜区无杂音。腰椎穿刺脑脊液滴速 56 滴/min,无色透明,潘氏试验(+),镜检 WBC 46 × 10^6/L,N 0.85,L 0.15,糖 5.12 mmol/L、氯化物 118 mmol/L。初步诊断:乙型脑炎。给予对症、抗病毒、中药等治疗,病情无好转,出现昏迷、间断呼吸暂停、频繁抽搐,加用甘露醇静脉滴注,约半小时后,全身皮肤出现大小不等的充血性皮疹,部分呈风团状,眼睑、口唇肿胀。即刻停用甘露醇,给予抗组织胺和皮质类固醇药物治疗,皮疹在 4 h 后逐渐消失,不留痕迹。次日再用甘露醇时,上述皮疹复发,躯干、四肢受压部位出现水疱,在其他药物不变的情况下停用甘露醇,换用甘油和速尿治疗,并予抗过敏和局部处理,皮疹逐渐好转,未再出现。

甘露醇为不显药理作用的低分子有机化合物,其脱水作用机理主要是静脉注射其高渗溶液后,血浆渗透压迅速升高,将组织中水分吸收回血浆,产生脱水作用,从而降低颅内压,有报道[1]每 8 g 甘露醇带出水分 100 ml,脱水降颅压作用可靠确实,是临床常用药物。甘露醇致严重皮疹的报道少见[2],本例应用甘露醇 2 次均出现同样的皮疹,不用甘露醇改用其他药物后未再出现,且抗过敏治疗有效,说明为患儿对甘露醇过敏所致。

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