

山东省济宁地区新生儿脂肪酸氧化代谢病筛查及随访分析

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[摘要] **目的:**了解山东省济宁地区脂肪酸氧化代谢病的发病率、基因突变特征,并评估治疗效果。**方法:**采集2014年7月14日—2019年12月31日出生的新生儿血样,用串联质谱法测定血肉碱和酰基肉碱水平,筛查脂肪酸氧化代谢病。提取筛查阳性新生儿外周血DNA,用MassARRAY和高通量测序进行基因突变分析,用桑格-库森法验证。对确诊患儿早期干预治疗并随访。**结果:**从608 818名新生儿中筛查出脂肪酸氧化代谢病患儿42例,总发病率为1/14 496。以原发性肉碱缺乏症(16例,38.10%)和短链酰基辅酶A脱氢酶缺乏症(16例,38.10%)多见,其次为极长链酰基辅酶A脱氢酶缺乏症(6例,14.29%)和中链酰基辅酶A脱氢酶缺乏症(4例,9.53%)。原发性肉碱缺乏症患儿SLC22A5突变以c.1400C>G(p.S467C)和c.51C>G(p.F17L)常见,新发现c.278C>T(p.S93L)、c.1049T>C(p.L350P)、c.572A>G(p.K191R)、c.431T>C(p.L144P)突变。随访期内,肉碱替代治疗10例患儿发育正常;未用肉碱替代治疗6例患儿中5例发育正常,另1例新生儿期出现低血糖,肌酸激酶增高,后期出现智力和语言发育落后。短链酰基辅酶A脱氢酶缺乏症患儿ACADS基因突变以c.1031A>G(p.E344G)和c.164C>T(p.P55L)常见,随访期内发育正常。极长链酰基辅酶A脱氢酶缺乏症患儿ACADVL基因突变以c.1349G>A(p.R450H)常见,新发现c.488T>A(p.L163*)、c.1228G>T(p.D410Y)、c.1276G>A(p.A426T)、c.1522C>T(p.Q508*)、c.1226C>T(p.T409M)突变。3例使用中链脂肪酸奶粉患儿随访期内发育正常;3例合并肉碱降低患儿使用左卡尼汀和中链脂肪酸奶粉治疗,其中1例患儿随访期内发育正常,1例患儿3月龄时急性发病死亡,1例患儿8月龄时曾急性发病,治疗后症状消失,随访期内发育正常。中链酰基辅酶A脱氢酶缺乏症患儿ACADM基因突变以c.449_452del(p.T150Rfs*4)常见,新发现c.718A>G(p.M240V)突变。所有患儿确诊后进行低脂肪饮食并避免饥饿和疲劳,1例患儿补充左卡尼汀,其余3例患儿未使用药物治疗,随访期内发育均正



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常。**结论:**济宁地区脂肪酸氧化代谢病以原发性肉碱缺乏症和短链酰基辅酶A脱氢酶缺乏症常见,存在基因热点突变或新发现的基因突变,通过新生儿筛查早期诊治,患儿预后良好。

[关键词] 脂质代谢缺陷,先天性;基因突变;串联质谱法;新生儿筛查;随访研究

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Screening and follow-up results of fatty acid oxidative metabolism disorders in 608 818 newborns in Jining, Shandong province

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[Abstract] **Objective:** To investigate the incidence and gene mutation characteristics of fatty acid oxidative metabolism disorders in Jining area of Shandong province, and to evaluate the therapeutic effect. **Methods:** Blood samples of newborns were collected in Jining of Shandong province between July 14, 2014 and December 31, 2019. Tandem mass spectrometry was used to determine the levels of carnitine and acylcarnitine in the blood to screen for fatty acid oxidative metabolism disorder. For newborns with positive screening result, blood DNA was analyzed by MassARRAY and high-throughput sequencing, then verified by Sanger sequencing. The diagnosed children were given early intervention and treatment, and followed up. **Results:** Forty-two children with fatty acid oxidative metabolism disorders were screened out of 608 818 newborns, with an incidence rate of 1/14 496. Primary carnitine deficiency (16 cases, 38.10%) and short-chain acyl-CoA dehydrogenase deficiency (16 cases, 38.10%) were the most common, followed by very long-chain acyl-CoA dehydrogenase deficiency (6 cases, 14.29%), medium-chain acyl-CoA dehydrogenase deficiency (4 cases, 9.53%). In children with primary carnitine deficiency, c.1400C>G (p.S467C) and c.51C>G (p.F17L) were the most common in *SLC22A5* mutations; and c.278C>T (p.S93L), c.1049T >C (p.L350P), c.572A>G (p.K191R), c.431T>C (p.L144P) were newly discovered mutations. Ten children with carnitine replacement therapy showed normal development during the follow-up. In 6 children without carnitine replacement treatment, hypoglycemia developed during the neonatal period in 1 case, in whom the creatine kinase was increased, and the intellectual and language development delayed in the later period; the other 5 children developed normally during the follow-up period. The *ACADS* gene mutations c.1031A>G (p.E344G) and c.164C>T (p.P55L) were common in children with short-chain acyl-CoA dehydrogenase deficiency, and the children developed normally during the follow-up. In

children with very long-chain acyl-CoA dehydrogenase deficiency, the c.1349G>A (p.R450H) was common in *ACADVL* gene mutations; and c.488T>A (p.L163*), c.1228G>T (p.D410Y), c.1276G>A (p.A426T), c.1522C>T (p.Q508*), c.1226C>T (p.T409M) were newly discovered mutations. Three children treated with milk powder rich in medium-chain fatty acids had normal development during the follow-up. The other 3 cases with combined carnitine reduction were treated with levocarnitine and milk powder enriched of medium-chain fatty acids, 1 case developed normally during the follow-up, 1 case died of acute illness at the age of 3 months, and 1 case had acute illness and recovered after treatment, and developed normally during the follow-up. c.449_452del (p.T150Rfs*4) was the most common *ACADM* gene mutation in children with medium-chain acyl-CoA dehydrogenase deficiency, and c.718A>G (p.M240V) was a newly discovered mutation. All children received low-fat diet, and hunger and fatigue were avoided; 1 child was supplemented with L-carnitine, and the other 3 children were not treated with drugs, and all of them developed normal during the follow-up. **Conclusions:** Primary carnitine deficiency and short-chain acyl-CoA dehydrogenase deficiency are the most common fatty acid oxidative metabolism disorders in Jining area. There are gene hotspot mutations and new discovered gene mutations in patients. Patients with early diagnosis and treatment through neonatal screening have a good prognosis.

[**Key words**] Lipid metabolism, inborn errors; Gene mutation; Tandem mass spectrometry; Neonatal screening; Follow-up studies

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[**缩略语**] 游离肉碱(free carnitine, C0);乙酰肉碱(acetylcarnitine, C2);丙酰肉碱(propionylcarnitine, C3);丁酰肉碱(butyrylcarnitine, C4);辛酰肉碱(octanoylcarnitine, C8);癸酰肉碱(decanyl carnitine, C10);肉豆蔻烯酰肉碱(tetradecenoylcarnitine, C14:1);棕榈酰肉碱(palmitoylcarnitine, C16);聚合酶链反应(polymerase chain reaction, PCR)

脂肪酸氧化代谢病是一种常见的新生儿遗传代谢病,是由于脂肪酸进入线粒体进行 β 氧化代谢途径中的酶或转运蛋白功能缺陷,导致脂肪酸 β 氧化代谢发生障碍所引起的一组疾病;属于常染色体隐性遗传病,发病时多累及肝脏、心肌和骨骼肌,导致患儿运动发育落后、肌无力、肝大、低血糖、酸中毒甚至猝死。串联质谱法用于新生儿筛查可以实现脂肪酸氧化代谢病的早期诊断和及时治疗。本文回顾性分析了山东省济宁市2014—2019年新生儿遗传代谢病串联质谱法筛查资料,分析脂肪酸氧化代谢病筛查确诊患儿的基因突变特征和治疗效果,探讨脂肪酸氧化代谢病患儿的基因型与临床表型、治疗效果的关系,为脂肪酸氧化代谢病的精准诊断和早期干预

提供参考。

1 对象与方法

1.1 对象

筛查对象为山东省济宁地区2014年7月14日—2019年12月31日出生的新生儿共608 818名,其中男性335 558名,女性273 260名,男女比例为1.23:1;正常足月儿596 249名,早产儿4023名,过期产儿8546名;正常体重儿596 215名,低出生体重儿3972名,巨大儿8631名。健康新生儿出生后3~7 d充分哺乳后采血,早产儿和低出生体重儿采血时间不超过出生后20 d。本研究中的标本采集和检测经监护人知情同意,且研究方案通过济宁市妇幼保健计划生育服务中心伦理委员

会批准。

1.2 仪器及试剂

S&S903采血滤纸为英国Whatman公司产品; AQCUTY TQ-D三重四极杆串联质谱仪为美国Waters公司产品; MassARRAY Analyzer 4核酸质谱仪和RS-1000芯片点样机为美国Sequen公司; Illumina HiSeq 2500测序平台为美国Illumina公司产品; ABI 3500XL基因分析仪为美国Applied Biosystems公司产品。

NeoBase新生儿非衍生生化法筛查试剂盒为芬兰PerkinElmer公司产品; DNA提取试剂盒为德国Qiagen公司产品; 核酸质谱分析MassARRAY试剂盒为美国Agena公司产品; 高通量测序Invitrogen Qubit dsDNA检测试剂盒为美国Invitrogen公司产品; Covaris LE220试剂盒为美国Covaris公司产品; Illumina DNA标准和引物预混试剂盒为美国Kapa Biosystems公司产品; 桑格-库森法PCR试剂盒为日本TaKaRa公司产品; PCR产物纯化试剂盒为德国Macherey-Nagel公司产品; BigDye Terminator 3.1循环测序试剂盒为美国Applied Biosystems公司产品。

1.3 串联质谱法筛查脂肪酸氧化代谢病

采集新生儿足跟末梢血滴于采血滤纸上, 采用非衍生串联质谱法测定干血斑C0和酰基肉碱含量。串联质谱法检测的C0和酰基肉碱正常参考区间采用百分位数法, 并通过模型分析确定各自疾病的特异性筛查指标。其中, 原发性肉碱缺乏症为C0降低(正常参考值10~55 $\mu\text{mol/L}$), 可伴多种酰基肉碱降低判定筛查阳性; 短链酰基辅酶A脱氢酶缺乏症为C4升高(正常参考值0.07~0.45 $\mu\text{mol/L}$), 可伴C4/C3比值(正常参考值0.05~0.42)升高判定筛查阳性; 极长链酰基辅酶A脱氢酶缺乏症为C14:1升高(正常参考值0.02~0.22 $\mu\text{mol/L}$), 可伴C14:1/C2比值(正常参考值0~0.01)或C14:1/C16比值(正常参考值0~0.09)升高判定筛查阳性; 中链酰基辅酶A脱氢酶缺乏症为C8升高(正常参考值0.01~0.12 $\mu\text{mol/L}$), 可伴C8/C2比值(正常参考值0~0.01)或C8/C10比值(正常参考值0.40~1.33)升高判定筛查阳性^[1-2]。

1.4 核酸质谱法、高通量测序、桑格-库森法检测基因突变

筛查阳性新生儿召回复查筛查指标仍为阳性者, 采集其外周血, 提取DNA。一部分患儿先用核

酸质谱法检测已知基因突变, 若未发现基因突变, 再用高通量测序检测; 另一部分患儿直接用高通量测序检测基因突变。阳性结果用桑格-库森法验证。

1.4.1 核酸质谱法 采用核酸质谱分析系统检测脂肪酸氧化代谢病4种常见基因(*ACADS*、*ACADM*、*ACADVL*和*SLC22A5*)的突变情况。按照试剂盒说明, 应用多重PCR扩增目标靶序列, 然后使用虾碱性磷酸酶移除PCR反应残留的脱氧核苷三磷酸, 使用延伸引物和测定特异性复合物终止子核苷酸混合物, 将单碱基延伸至突变位点, 在干扰离子与树脂结合后将产物转移到芯片上。使用核酸质谱仪从飞行时间质谱图上检索质谱图, 用SpectroTYPER软件进行基因分型。

1.4.2 高通量测序 用紫外分光光度计检测DNA的浓度和纯度, 并将DNA片段剪切至150~200 bp。通过多重PCR将剪切的DNA用于靶区域的文库制备, 用DNA标准和引物预混试剂盒进行定量, HiSeq 2500平台测序后, 与人类基因组参考序列进行比对, 使用GATK软件对突变进行命名, 用ANNOVAR软件对突变进行分类。参照美国医学遗传学和基因组学学会联合分子病理协会提出的“序列变异解读标准和指南”分析突变的致病性^[3]。

1.4.3 桑格-库森法 使用待测位点的特异性引物, 按照试剂盒操作流程进行PCR, PCR产物纯化后, 用ABI 3500XL平台测序。测序结果采用SeqMan软件进行序列比对分析。根据脂肪酸氧化代谢病的基因检测结果, 与PubMed数据库、dbSNP数据库、千人基因组(1000 Genomes)数据库、人类基因突变数据库等收录的基因突变信息比较, 确定脂肪酸氧化代谢病的基因热点突变或新发现的基因突变。

1.5 诊断标准

满足下面其中一项即诊断为脂肪酸氧化代谢病: ①串联质谱法筛查特征性指标阳性, 且检测到相应的基因复合杂合突变、纯合突变; ②如果检测到杂合突变, 特征性指标随访持续明显异常, 或者出现相应的临床症状^[4]。

1.6 治疗和随访

对于脂肪酸氧化代谢病确诊病例, 嘱其避免饥饿和剧烈运动。原发性肉碱缺乏症患者使用肉碱替代治疗, 按照50~100 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ 剂量补充

左卡尼汀,维持血C0正常或接近正常水平;短链酰基辅酶A脱氢酶缺乏症和中链酰基辅酶A脱氢酶缺乏症患者若出现血C0降低可适当补充左卡尼汀;极长链酰基辅酶A脱氢酶缺乏症患者以高糖低脂饮食治疗为主,尤其要控制长链脂肪酸摄入,使用富含中链脂肪酸的奶粉,C0降低的患儿补充左卡尼汀。急性期治疗主要为补充足量葡萄糖、纠正酸中毒、电解质紊乱和对症治疗。

患儿病情稳定后2~3个月随访复查一次,随访内容主要包括体格检查、智力发育、血肉碱水平、肝功能、心肌酶谱、心电图及心脏彩超检查等,随访时间截至2020年12月31日。

1.7 统计学方法

采用SPSS 20.0软件进行统计分析,正态分布的计量资料用均数±标准差表示,非正态分布的计量资料用中位数(范围)表示;计数资料采用例数(百分率)表示。

2 结果

确诊4种脂肪酸氧化代谢病42例,发病率为1/14 496。其中原发性肉碱缺乏症16例(38.10%),发病率为1/38 051;短链酰基辅酶A脱氢酶缺乏症16例(38.10%),发病率为1/38 051;极长链酰基辅酶A脱氢酶缺乏症6例(14.29%),发病率为1/101 470;中链酰基辅酶A脱氢酶缺乏症4例(9.53%),发病率为1/152 205。

2.1 16例原发性肉碱缺乏症患者检测和随访结果

16例原发性肉碱缺乏症患者中,男性8例,女性8例;早产儿1例,足月儿15例;确诊时均无临床症状,确诊时间为45(21~57)日龄。检出SLC22A5基因突变11种,其中纯合突变3例(18.75%),复合杂合突变11例(68.75%),杂合突变2例(12.50%)。突变频率较高的位点为c. 1400C>G(p. S467C)(14/30,46.67%)和c. 51C>G(p. F17L)(6/30,20.0%),突变频率较低的位点包括c. 95A>G(p. N32S)、c. 428C>T(p. P143L)、c. 246C>T(p. R82R)、c. 505C>T(p. R169W)、c. 278C>T(p. S93L)、c. 1049T>C(p. L350P)、c. 572A>G(p. K191R)、c. 431T>C(p. L144P)和c. 695C>T(p. T232M)。新发现c. 278C>T(p. S93L)、c. 1049T>C(p. L350P)、c. 572A>G(p. K191R)、c. 431T>C(p. L144P)突变位点。

16例患儿串联质谱法筛查C0浓度为5.90(1.85~8.91) $\mu\text{mol/L}$,均低于正常范围。治疗随访35(10~69)个月,其中9例患儿长期补充左卡尼汀,血C0浓度为(11.54±2.44)~(32.68±3.36) $\mu\text{mol/L}$,均在正常范围,智力和语言发育正常;1例患儿补充左卡尼汀4年后自行停药,血C0(9.69±1.19) $\mu\text{mol/L}$,体格和智力发育正常。6例未补充左卡尼汀患儿中5例患儿随访期内血C0浓度为(4.32±0.62)~(7.63±1.33) $\mu\text{mol/L}$,仍低于正常范围,但体格和智力发育正常;1例患儿新生儿期出现低血糖,肌酸激酶增高(362 U/L),随访C0为(4.32±0.62) $\mu\text{mol/L}$,随后出现智力和语言发育落后。

结果提示,原发性肉碱缺乏症为济宁地区较为常见的脂肪酸氧化代谢病之一,c. 1400C>G(p. S467C)和c. 51C>G(p. F17L)为热点突变,早期使用肉碱替代治疗患儿预后较好。

2.2 16例短链酰基辅酶A脱氢酶缺乏症患者检测和随访结果

短链酰基辅酶A脱氢酶缺乏症患者中,男性8例,女性8例,均为足月儿,确诊时体格检查未见异常,无临床症状,确诊时间为43(29~57)日龄。检出ACADS基因突变7种,其中纯合突变3例(18.75%),复合杂合突变10例(62.50%),杂合突变3例(18.75%)。突变频率较高的位点为c. 1031A>G(p. E344G)(12/29,41.38%)和c. 164C>T(p. P55L)(9/29,31.03%),突变频率较低的位点包括c. 989G>A(p. R330H)、c. 682_683del(p. E228Rfs*16)、c. 1054G>A(p. A352T)、c. 1130C>T(p. P337L)和c. 682G>A(p. E228K)。

16例患儿串联质谱法初筛C4浓度为1.58(0.65~2.73) $\mu\text{mol/L}$,C4/C3比值为1.40(0.81~4.13)。治疗随访中位时间43(13~73)个月,C4浓度为(0.60±0.02)~(2.23±0.13) $\mu\text{mol/L}$,C4/C3比值为(0.69±0.02)~(3.29±0.39),体格和智力发育均正常。

结果提示,短链酰基辅酶A脱氢酶缺乏症为济宁地区较为常见的脂肪酸氧化代谢病之一,c. 1031A>G(p. E344G)和c. 164C>T(p. P55L)为热点突变,早期饮食干预后患儿预后较好。

2.3 6例极长链酰基辅酶A脱氢酶缺乏症患者检测和随访结果

6例极长链酰基辅酶A脱氢酶缺乏症患者均

为男性、足月儿,确诊时体格检查未见异常,无临床症状,确诊时间为44(35~50)日龄。检出ACADVL基因突变9种,突变频率较高的位点为c. 1349G>A(p. R450H)(3/11, 27. 27%),突变频率较低的位点为c. 1280G>A(p. W427*)、c. 1328T>C(p. M443T)、c. 996dup(p. A333Cf*26)、c. 488T>A(p. L163*)、c. 1228G>T(p. D410Y)、c. 1276G>A(p. A426T)、c. 1522C>T(p. Q508*)和c. 1226C>T(p. T409M)。新发现c. 488T>A(p. L163*)、c. 1228G>T(p. D410Y)、c. 1276G>A(p. A426T)、c. 1522C>T(p. Q508*)、c. 1226C>T(p. T409M)突变位点。

6例患儿串联质谱法初筛C14:1浓度为1. 63(0. 67~4. 65) $\mu\text{mol/L}$,C14:1/C2和C14:1/C16比值分别为0. 22(0. 04~0. 67)和0. 46(0. 31~1. 00),均高于正常范围。治疗随访中位时间20(3~49)个月,3例C0正常患儿采用中链脂肪酸奶粉治疗,C14:1、C14:1/C2和C14:1/C16比值分别为(0. 20 \pm 0. 01)~(2. 61 \pm 0. 32) $\mu\text{mol/L}$ 、(0. 01 \pm 0. 01)~(0. 32 \pm 0. 06)和(0. 07 \pm 0. 01)~(0. 91 \pm 0. 10),均高于正常范围,体格和智力发育正常;3例合并C0降低的患儿使用左卡尼汀和中链脂肪酸奶粉治疗,其中1例c. 488T>A(p. L163*)杂合突变患儿3月龄时因肺炎急性发病死亡;1例患儿8月龄时急性发病,出现心力衰竭、心源性肺水肿、肥厚性心肌病,急性期增加服用地高辛、贝那普利及利尿药物治疗后症状缓解;1例患儿随访期内无急性发病,体格和智力发育正常。

结果提示,极长链酰基辅酶A脱氢酶缺乏症在济宁地区发病率较低,c. 1349G>A(p. R450H)为热点突变,临床危害较重,治疗期间仍有部分病例死亡或急性发病。

2. 4 4例中链酰基辅酶A脱氢酶缺乏症患儿检测和随访结果

4例中链酰基辅酶A脱氢酶缺乏症患儿中男性2例,女性2例,均为足月儿,确诊时体格检查正常,无临床症状,确诊时间为(51 \pm 6)日龄。检出ACADM基因突变4种,突变频率较高的位点为c. 449_452del(p. T150Rfs*4)(3/6, 50. 0%),突变频率较低的位点包括c. 718A>G(p. M240V)、c. 928G>A(p. G310R)和c. 157C>T(p. R53C),新发现c. 718A>G(p. M240V)突变位点。4例患儿C8浓度为4. 56(0. 62~11. 29) $\mu\text{mol/L}$,C8/C2和C8/

C10比值分别为0. 45(0. 04~0. 67)和9. 97(2. 69~16. 71),均高于正常范围。确诊后所有患儿采用低脂肪饮食,避免饥饿和疲劳,随访中位时间36(13~54)个月,1例合并C0降低的患儿服用左卡尼汀治疗,其余3例患儿未用药物治疗,随访C8、C8/C2、C8/C10分别为(0. 59 \pm 0. 08)~(2. 11 \pm 0. 23) $\mu\text{mol/L}$ 、(0. 04 \pm 0. 01)~(0. 65 \pm 0. 13)、(2. 82 \pm 0. 35)~(11. 32 \pm 2. 50),随访期内患儿体格和智力发育均正常。结果提示,中链酰基辅酶A脱氢酶缺乏症在济宁地区发病率较低,c. 449_452del(p. T150Rfs*4)为热点突变,早期低脂肪饮食干预患儿预后较好。

3 讨论

脂肪酸氧化代谢病的发病率在不同地区和人种间差异很大,其在美国、澳大利亚、德国、意大利等国家发病率相对较高^[5-9],在亚洲国家中并不常见^[10-11]。Chace等^[12]对美国宾夕法尼亚州、北卡罗来纳州和华盛顿特区及俄亥俄州东部地区710 000名新生儿筛查结果显示,脂肪酸氧化代谢病的发病率为1/15 000;Schulze等^[13]报道德国250 000名新生儿筛查结果显示,脂肪酸氧化代谢病发病率为1/10 400;脂肪酸氧化代谢病在日本的发病率为1/30 000,在韩国的发病率为1/11 1000^[14]。本文资料显示,山东省济宁地区四种脂肪酸氧化代谢病的发病率为1/14 496,与上海市和浙江省的研究结果接近^[15-16]。其中,原发性肉碱缺乏症和短链酰基辅酶A脱氢酶缺乏症为济宁地区最常见的两种脂肪酸氧化代谢病,各占38. 10%,其他疾病所占比例较小。

本文资料中,原发性肉碱缺乏症检出率达到1/38 051。血C0水平和基因检测是筛查原发性肉碱缺乏症的主要依据^[17]。本文资料中的16例患儿均出现C0降低,其中检测到SLC22A5纯合和复合杂合突变14例,杂合突变2例,其中2例杂合突变患儿初筛时血C0降低、用药后恢复正常、停药后再次明显降低而确诊。因此,在检出一个基因位点突变时,应反复检测血C0水平,若血C0水平持续明显异常,应诊断为阳性病例,若停药后C0水平降低不明显,应检测患儿母亲血C0水平和基因突变,以排除母源性肉碱缺乏。SLC22A5基因突变类型多为错义突变,其次为无义突变及移码突变^[18]。不同种族人群的基因突变谱有一定的差

异^[19],某些种族和地区人群中存在热点突变,如高加索人常见的突变位点为c. 632A>G,意大利常见的突变位点为c. 505C>T,西非人群以c. 632A>G为主,日本以c. 1400C>G和c. 396G>A突变最常见^[20]。Lee等^[18]报道中国台湾地区以c. 760C>T(p. R254X)最常见。崔冬等^[21]报道了8例原发性肉碱缺乏症患者,6例检出突变位点c. 760C>T(p. R254X)。本文资料16例患儿检出频率最高的突变位点为c. 1400C>G(p. S467C),其次为c. 51C>G(p. F17L),与天津地区报道的结果基本一致^[22]。原发性肉碱缺乏症可在任何年龄发病,若不能及时治疗,患者有猝死的风险,早期使用肉碱替代治疗是改善患者预后的关键^[23]。本文资料中,1例患儿在新生儿期出现血糖降低,肌酸激酶增高,但家长拒绝补充左卡尼汀,随访期内出现智力和语言发育落后,该患儿存在济宁地区新发现突变位点c. 431T>C(p. L144P)与c. 695C>T(p. T232M)的复合杂合突变。c. 1400C>G(p. S467C)热点突变患儿预后较好,可能与该基因是原发性肉碱缺乏症轻微临床表型突变基因有关^[24],也与新生儿筛查后早期干预有关。

C4浓度增加是短链酰基辅酶A脱氢酶缺乏症诊断的重要标志物^[25-26]。本文资料中的16例短链酰基辅酶A脱氢酶缺乏症患者C4、C4/C2和C4/C3均异常。ACADS突变在欧洲以c. 511C>T和c. 625G>A突变最常见,美国c. 511C>T的等位基因频率为0.3%,c. 625G>A的等位基因频率为5.5%^[27-28]。常见突变是错义突变,通过改变折叠方式和细胞的寿命来影响蛋白质的生物合成^[29]。Pedersen等^[30]报道了114例短链酰基辅酶A脱氢酶缺乏症患者,c. 625G>A(p. G209S)和c. 511C(p. R147W)突变分别占所检测等位基因的67%和8%。本文资料中,16例患儿共检测到8种突变,检出频率最高的突变为c. 1031A>G(p. E344G)和c. 164C>T(p. P55L),其次为c. 682_683del(p. E228Rfs*16)和c. 989G>A(p. R330H),与山东省青岛地区报道的结果接近^[31],提示c. 1031A>G(p. E344G)突变可能是山东地区的热点突变。大多数新生儿筛查确诊的短链酰基辅酶A脱氢酶缺乏症患者无明显症状,但也有出现喂养困难及低血糖等^[32]。本文资料中的16例确诊患儿均无临床表现,体格和智力发育正常,可能是由于新生儿筛查确诊的患儿多为良

性表现的缘故,也与确诊后及时进行生活管理和喂养指导,避免了临床症状出现及应急状态下的代谢失调有关。

极长链酰基辅酶A脱氢酶缺乏症临床表现有明显的异质性,多为新生儿和婴儿早期发病,常伴有心肌受累,猝死率较高。在本文资料6例极长链酰基辅酶A脱氢酶缺乏症患者中,1例患儿早期死亡,1例患儿治疗期内急性发病。目前,ACADVL以错义突变为主要突变类型,其次为缺失突变和剪切突变^[33]。本文资料中的6例患儿有4例为c. 1349G>A(p. R450H)突变,提示该位点有较高的发生频率,为济宁地区患儿的热点突变。通过新生儿筛查确诊的患儿尽管在诊断时多无临床症状,但其发病和预后将出现较大差异,有些患儿甚至在诊断结果出来之前就已经有症状,有些患儿长期随访仍无症状^[34-35]。部分患儿会出现横纹肌溶解综合征或严重的心肌病,这些严重的表型与等位基因失活或无效有关^[36-37],因此评估患儿发病风险时,基因检测显得尤为重要。本文资料中的1例死亡患儿检出c. 488T>A(p. L163*)位点的杂合突变,该突变是本地区新发现的突变,是否存在致病性尚须进一步论证;而3例携带c. 1349G>A(p. R450H)复合杂合子的患儿虽然C14:1明显增高,使用中链脂肪酸奶粉治疗,无急性发病,发育正常,推测c. 1349G>A(p. R450H)突变可能与血C14:1生化表型存在关联,而与临床表型关联不明显。

中链酰基辅酶A脱氢酶缺乏症是澳大利亚、欧美等地区最主要的脂肪酸氧化代谢病,占50%以上,发病率为1/45 000~1/9036^[38-40]。中链酰基辅酶A脱氢酶缺乏症在我国发病率不高,本文资料显示济宁地区的发病率约为1/152 205。ACADM以错义突变为主要类型,欧美白种人最常见的突变是位于第11外显子的c. 985A>G(K329E)^[41],本文资料未检出该突变位点。该病多为良性疾病,发病多存在诱发因素,如长时间饥饿或感染性疾病等。患儿确诊后应避免饥饿,提高饮食中碳水化合物和蛋白质占比,减少脂肪摄入,以预防发病。本文资料中确诊的4例中链酰基辅酶A脱氢酶缺乏症患者随访期内体格和智力发育正常。

综上所述,济宁地区脂肪酸氧化代谢病以原发性肉碱缺乏症和短链酰基辅酶A脱氢酶缺乏症常见,极长链酰基辅酶A脱氢酶缺乏症的临床表型相对严重;通过新生儿筛查早期发现、及时干

预、规范治疗,可以在一定程度上预防发病或减轻疾病的危害。

利益冲突 所有作者均声明不存在利益冲突

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