

婴儿型糖原贮积病Ⅱ型基因型表型相关性分析和出生缺陷预防

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[摘要] 目的 分析伴有肥厚型心肌病的婴儿型糖原贮积病Ⅱ型(GSDⅡ)的基因型和表型特点,以及下一胎出生缺陷的预防方法。方法 对 2016 年 12 月—2017 年 5 月西安市儿童医院心脏内科住院诊断治疗的 4 例婴儿型 GSDⅡ 病儿,进行临床和实验室检查及酸性 α-葡萄糖苷酶(GAA)活性测定,采用 Sanger 直接测序或二代测序法进行 GAA 基因突变检测,并对 2 例先证者母亲下一胎胎儿进行产前 GAA 基因突变筛查。结果 4 例病儿分别在生后 2~8 月龄出现运动发育迟缓、肌无力、心肌肥厚,3 例病儿 GAA 活性分别为 2.5、2.1、1.9 nmol/(g·min);4 例病儿基因检测发现 8 个 GAA 基因致病突变,分别为 p.R608X、p.L701P、p.R608X、p.E888X、p.W279C、p.N535Qfs*3、p.S601L、p.E888X。病儿均于确诊后 3~6 个月死亡。2 例进行了下一胎产前诊断,其中 1 例携带单个杂合突变,顺利分娩,1 例携带与先证者相同复合杂合突变,终止妊娠。结论 发病 GSDⅡ 病儿分别携带来自父母的致病突变,婴儿型 GSDⅡ 病儿症状出现早,未经酶替代治疗死亡率高。GSDⅡ 在我国尚未列入产前筛查,对于病儿母亲下一胎胎儿进行 GAA 基因检查,有助于 GSDⅡ 出生缺陷的预防。

[关键词] 糖原贮积病Ⅱ型;α-葡萄糖苷酶类;突变;基因检测;优生学

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GENOTYPE-PHENOTYPE CORRELATION IN INFANTILE GLYCOGEN STORAGE DISEASE TYPE Ⅱ AND PREVENTION OF BIRTH DEFECT WANG Tao, LEI Xi, XIAO Hongyu, ZHANG Jianfang, WANG Juanli, LIU Bailing, XU Xiaoyan, YAO Zhenyu, ZHANG Yanmin (Department of Cardiology, the Affiliated Children's Hospital of Xi'an Jiaotong University (Xi'an Children's Hospital), Xi'an, 710003, China)

[ABSTRACT] **Objective** To investigate the genotypes and phenotypes of infantile glycogen storage disease type Ⅱ (GSDⅡ) with hypertrophic cardiomyopathy and the methods for birth defect prevention in the next child. **Methods** Four children with infantile GSDⅡ who were hospitalized, diagnosed, and treated in Department of Cardiology in Xi'an Children's Hospital from December 2016 to May 2017 were enrolled. Clinical and laboratory examinations were performed and analyzed, and the activity of acidic alpha-D-glucosidase (GAA) was measured. Sanger direct sequencing or next generation sequencing was performed to identify GAA gene mutations, and prenatal GAA gene mutation screening was performed for the next fetuses of the mothers of the two probands. **Results** Four children developed motor developmental delay, myasthenia, and myocardial hypertrophy at an age of 2—8 months. The activity of GAA in three children was 2.5, 2.1, and 1.9 nmol/(g·min), respectively. Gene detection performed for four children identified 8 pathogenic mutations in the GAA gene, i.e., p.R608X, p.L701P, p.R608X, p.E888X, p.W279C, p.N535Qfs*3, p.S601L, and p.E888X. All four children died within 3—6 months after being diagnosed. Prenatal diagnosis was performed for the next child of the mothers of the two probands; one fetus carried a heterozygous mutation and was born successfully; the other carried the same compound heterozygous mutation as the proband and termination of pregnancy was performed. **Conclusion** Children with GSDⅡ carry pathogenic mutations from their parents. Symptoms of infantile GSDⅡ appear early and children who do not receive enzyme replacement therapy have a high mortality rate. Although GSDⅡ has not been included in prenatal screening in China, GAA gene test for the next fetus of the proband's mother is helpful for the prevention of GSDⅡ birth defects.

[KEY WORDS] Glycogen storage disease type Ⅱ; alpha-Glucosidases; Mutation; Genetic testing; Eugenics

糖原贮积病Ⅱ型(GSDⅡ)又称庞贝病,发病率约为 1/40 000^[1],是一种罕见的常染色体隐性遗传病。其发病机制为位于 17q25.3 染色体上编码酸性 α-葡萄糖苷酶(GAA)的基因发生突变,使溶酶体内 GAA 缺乏,致糖原不能转化为葡萄糖,从而在骨骼肌、心肌和平滑肌等组织细胞内大量沉积^[2]。本研究对 2016 年 12 月—2017 年 5 月于西安市儿童医院心内科确诊的 4 例伴有肥厚型心肌病的 GSDⅡ 病儿的临床特点、GAA 活性以及 GAA 基因进行分析,总结临床表现、酶学特点和基因变异的相互关系,并对下一胎进行遗传咨询和产前诊断。现将结果报告如下。

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1 对象与方法

1.1 研究对象

2016 年 12 月—2017 年 5 月西安市儿童医院心内科确诊的 GSD II 住院患儿 4 例,男 3 例,女 1 例,发病年龄为 2~8 月龄,确诊年龄为 2~9 月。4 例患儿均因呼吸道感染就诊,有明显的运动发育迟缓或伴语言发育落后,主要为四肢无力、肌张力低下,累及四肢、躯干肌,面肌未受累。4 例呼吸肌无力,均有心肌受累;舌体肥大 2 例;4 例伴有肝大。4 例肌酸激酶(CK)、谷丙转氨酶(ALT)、谷草转氨酶(AST)均升高,达正常值 3~10 倍。4 例进行了心电图检查,提示 PR 间期 80~90 ms,双心室肥厚。1 例肌电图检查示肌源性损害。4 例患儿心脏 B 超均示左心室增大,左心室壁弥散性增厚,左心室后壁内膜增厚,提示肥厚型心肌病。患儿分别来自 4 个不同家庭,父母均非近亲婚配,无遗传病家族史,因呼吸道感染、心脏超声发现心肌肥厚就诊。临床资料包括病史、心电图、超声心动图、心脏核磁共振、心肌酶、肝肾功及 GAA 酶活性检查和基因检测。本研究经西安市儿童医院伦理委员会批准同意,患儿家长均签署了知情同意书。

1.2 研究方法

1.2.1 GAA 酶活性测定 外周血白细胞用生理盐水重悬,取 20 μ L 白细胞悬液(约 2 μ g 蛋白)加入 80 μ L 反应体系(1 mmol/L 4-甲基伞形酮- α -D-葡萄糖苷, pH 4.3 的 50 mmol/L 醋酸钠缓冲液, 1 g/L 的 Triton X-100, 10 μ mol/L 阿卡波糖)中, 37 $^{\circ}$ C 水浴反应 2 h。加入 pH 10.7 的 0.1 mol/L 甘氨酸-氢氧化钠缓冲液 150 μ L 终止反应。检测反应体系荧光(BioTek SynergyH4 多功能酶标仪)激发波长 360 nm,发射波长 450 nm。空白对照为相同条件,反应体系中不加蛋白。配置不同浓度的 4-甲基伞形酮,绘制标准曲线,按照标准曲线计算出产物浓度。蛋白浓度用 Bradford 法测定。

1.2.2 基因组 DNA 的提取和 GAA 基因突变分析 基因组 DNA 的提取:采集患儿及其父母外周血各 2 mL,用血液基因组 DNA 提取试剂盒(天根生化科技(北京)有限公司)提取基因组 DNA,放至 -80 $^{\circ}$ C 低温冰箱保存备用。高通量测序(患儿 2):先将证者的基因组 DNA 送至北京信诺百世医学检验所进行心血管系统疾病相关基因 panel 的靶向捕获二代测序,对二代测序所分析出的候选基因突变位点在家系内进行 Sanger 测序验证。Sanger 测序(患儿

1、3、4):依据 NCBI GAA 基因(NM_000152.3)序列,采用 Primer 5.0 软件设计引物,扩增 GAA 基因的 20 个外显子序列。引物由上海生工生物工程有 限公司合成。PCR 反应条件为:95 $^{\circ}$ C 5 min, 95 $^{\circ}$ C 30 s, 55 $^{\circ}$ C 30 s, 72 $^{\circ}$ C 30 s,共进行 30 个循环,最后 72 $^{\circ}$ C 延伸 7 min。PCR 扩增产物用 20 g/L 琼脂糖凝胶电泳检测,之后采用 ABI 3500dx 仪器进行双向测序。产前基因筛查:针对家庭内有明确基因诊断的患儿母亲在孕 7~12 周采集绒毛膜,或者于孕 16 周后行羊膜穿刺,抽取羊水,提取基因组 DNA。按照上述 Sanger 测序的方法,对相应的突变位点所在的外显子进行 PCR 扩增及 Sanger 测序,并对测序结果进行分析。

2 结 果

2.1 GAA 酶活性

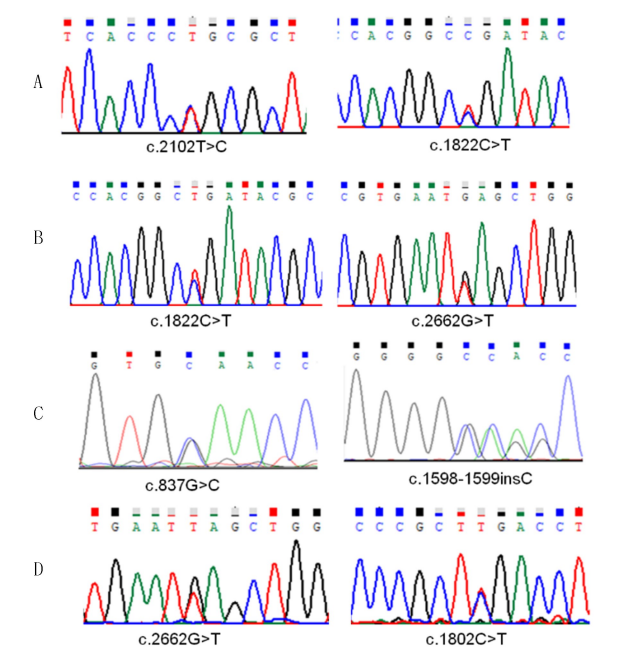
患儿 1、2 和 3 GAA 的活性分别为 2.5、2.1 和 1.9 nmol/(g \cdot min),患儿 4 未行 GAA 活性测定。见表 1。

2.2 GAA 基因突变分析

4 例患儿均为复合杂合突变,分别来自于患儿父亲和母亲(见表 1)。在检测到的 8 个突变中有 5 个错义突变,3 个插入突变,根据生物信息分析软件 SIFT、PolyPhen2 及 Mutation_Taster 致病性分析以及美国 ACMG 指南^[3]预测,其中 7 个突变为致病性突变,患儿 1 携带的 p.L701P 突变预测为可疑致病,结合患儿 GAA 活性的检测结果,确定为致病性的突变。而在已经检测出的突变位点中,p.L701P、p.W279C、p.N535Qfs * 3 突变位点在 HGMD 数据库及相关文献中均未见报道。4 例患儿均携带复合杂合突变,符合常染色体隐性遗传,突变测序峰图见图 1。

2.3 治疗、随访以及产前 GAA 基因突变检测

1 例患儿接受 Myozyme GAA 酶替代治疗 1 个月,其余 3 例患儿均未接受酶替代治疗。4 例患儿确诊后 2~6 个月内均死亡,死亡原因为肺部感染、肺不张合,导致呼吸循环衰竭。2 例患儿母亲孕下一胎,于妊娠期行羊水穿刺胎儿 GAA 基因检测,患儿 3 母亲孕 13 周绒毛膜致病基因检测确诊胎儿携带 GAA c.837G>C 和 c.1598-1599insC 复合杂合突变,终止妊娠。患儿 1 母亲孕 18 周羊水穿刺 GAA 检测确定胎儿携带一个杂合突变,遗传咨询结果考虑胎儿不会发病,现已经足月娩出,出生体质量约 3.4 kg,健康。



A、B、C、D 分别为病儿 1、2、3、4。
图 1 4 例病儿 GAA 突变位点测序图

3 讨 论

GSDⅡ分为早发型（婴儿型）及晚发型（青年型及成人型）。晚发型 GSDⅡ多青少年起病，表现为肌力减退和呼吸肌受累等症状，罕见心肌受累^[4]。早发型婴儿期起病，症状严重，心肌受累的超声心动图表现为以左心室心肌肥厚为主的双心室心肌向心性肥厚，多在 1 岁以内死于心力衰竭。由于婴儿型早期临床表现非特异、进展迅速、诊断治疗时间窗短^[5]，早期快速做出正确诊断对病儿的治疗和预后有重大意义。本组报道的 4 例病儿发病年龄均在 1 岁前，伴有肥厚型心肌病和骨骼肌受累，故为早发型 GSDⅡ。

GAA 基因突变影响 GAA 的合成、磷酸化修饰、转运以及分泌，突变性质决定残余 GAA 活性，GAA 活性越低，发病年龄越早，临床表现越严重^[6]。婴儿型病儿皮肤成纤维细胞的 GAA 活性常低于正

常对照的 1%，而晚发型残余酶的活性可达 1%～40%^[7]。国内有报道幼年起病的晚发型 GSDⅡ型病儿，但无心肌肥厚表现^[8]。而本组 4 例病儿均有明显的心肌肥厚，心功能有不同程度的降低，血肌酸激酶升高较显著，提示该 4 例病儿属于典型的婴儿型 GSDⅡ。

GAA 基因的突变型与临床表型相关，在该组病儿 1 中发现的 GAA 基因 c.1822C>T, p.R608X 杂合突变为 HGMD 报道的已知致病性突变^[9-10]；而 c.2102T>C, p.L701P 杂合突变既往未见报道，生物信息分析软件 SIFT、PolyPhen2 及 Mutation_Taster 均预测该突变可能有害，病儿 1 临床发病，证实了 p.L701P 为致病突变。病儿 2 携带的 GAA 基因 c.1822C>T, p.R608X 杂合变异，为 HGMD 报道的与 GSDⅡ相关的已知变异，人群样本数据筛查在个例病人中检出上述变异^[11-14]，该变异为无义变异，造成终止密码提前出现，提示该变异可能对蛋白多肽链的正常合成有重要影响，该变异在外显子组整合数据库（ExAC）中的频率为 0.00002479，定义为致病性变异。KOBAYASHI 等^[15]通过将重组 GAA（rGAA）对病人进行体内置换治疗，发现可以减轻早期病人的症状。病儿 2 携带的另外一个 GAA 基因 c.2662G>T, p.E888X 杂合变异为 HGMD 报道的与 GSD 相关的已知变异^[15-18]。p.E888X 在 Ex-AC 中的频率为 0.00002，为致病性变异。本组病儿中有 2 例携带 p.E888X，进一步证实该突变为热点突变。病儿 3 GAA 外显子 4 和 11 分别检测到一个杂合突变位点 c.837G>C, p.Trp279Cys 和 c.1598-1599insC, p.N535Qfs * 3，这两个位点在 HGMD 数据库及相关文献中均未见报道。c.837G>C 突变导致 GAA 蛋白的第 279 位氨基酸由色氨酸（Trp）变为半胱氨酸（Cys），通过 <http://genetics.bwh.harvard.edu/pph2/> 预测其为可能致病。c.1598-1599-insC 为移码突变，导致 GAA 蛋白翻译到第 537 位氨基酸时终止，形成一个不成熟的蛋白截短体，影响

表 1 病儿 GAA 基因突变结果

病儿	核苷酸改变	氨基酸改变	突变位置	杂合/纯合	突变类型	变异来源	致病性分析
病儿 1	c.1822C>T	p.R608X	E12	杂合	错义突变	母亲	致病性变异
	c.2102G>C	p.L701P	E14	杂合	插入突变	父亲	疑似致病性变异
病儿 2	c.2662G>T	p.E888X	E18	杂合	错义突变	母亲	致病性变异
	c.1822C>T	p.R608X	E12	杂合	插入突变	父亲	致病性变异
病儿 3	c.837G>C	p.W279C	E4	杂合	错义突变	母亲	致病性突变
	c.1598-1599insC	p.N535Qfs * 3	E11	杂合	插入突变	父亲	致病性突变
病儿 4	c.1802C>T	p.S601L	E12	杂合	错义突变	母亲	致病性突变
	c.2662G>T	p.E888X	E18	杂合	插入突变	父亲	致病性突变

蛋白正常功能的发挥,通过 <http://www.mutation-taster.org/> 预测该位点为极有可能致病。家系验证结果显示,受检者的父亲和母亲分别携带 c.1598-1599insC 和 c.837G>C,符合遗传共分离规律。患儿 4 样本分析显示 GAA 基因有 2 个杂合突变,即 c.1802C>T, p.S601L 杂合突变以及 c.2662G>T, p.E888X 杂合突变。这两个位点均为 HGMD 报道与 GSD II 相关的疑似致病性突变^[19]。

GSD II 为常染色体隐性遗传,患儿分别携带来自父母的致病突变方可发病,绒毛、羊水和胎儿血均可提取胎儿 DNA 用于产前遗传病诊断。妊娠 7~12 周可以选择绒毛膜,缩短诊断周期。妊娠 14~22 周可以选择羊水穿刺,羊水取材安全性高、结果准确。如果妊娠超过 22 周,可以用胎儿血培养。胎儿血培养成功率高,染色体核型形态好、分裂相多^[20]。本组患儿中 2 例母亲孕下一胎,1 例妊娠 13 周取得绒毛膜进行 GAA 测序后发现胎儿携带 2 个致病位点,终止妊娠。1 例在妊娠 18 周行羊水穿刺,GAA 基因检测确定胎儿只携带一个致病基因,继续妊娠,顺利分娩正常新生儿。

综上,婴儿发病 GSD II 患儿分别携带来自父母的致病突变,患儿症状出现早,未经酶替代治疗死亡率高。GSD II 在我国尚未列入产前筛查,对于患儿母亲孕下一胎胎儿进行 GAA 基因检查,有助于 GSD II 出生缺陷的预防。

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