

应用高通量测序技术对昆明地区人群珠蛋白生成障碍性贫血基因的筛查研究

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摘要: 目的 探讨昆明地区人群珠蛋白生成障碍性贫血 (thalassemia) 基因变异情况, 为昆明地区珠蛋白生成障碍性贫血防控工作提供理论依据。方法 对2018年昆明市政府“十大惠民项目”中25家医疗机构收集的5787例婚前、孕前优生及健康体检样本采用高通量测序技术检测, 进行珠蛋白生成障碍性贫血基因 ($\alpha + \beta$) 301型筛查应用情况分析。结果 ①通过高通量测序检测发现, 在5787例样本中, 共检出465例阳性, 阳性率为8.04%。其中 α -检出285例, β -检出131例, 复合型检出10例, 异常血红蛋白变异类型检出17例, 其他变异型检出22例。② α -珠蛋白生成障碍性贫血携带者检出285例, 以 $-\alpha^{3.7}/\alpha\alpha$ 基因型最为常见, 为152例 (53.33%)。③ β -珠蛋白生成障碍性贫血携带者检出131例, 以Hb E杂合子、Codon 17(A > T) 基因型最为常见, 各占26.72%; ④ $\alpha\beta$ 复合型珠蛋白生成障碍性贫血变异类型检出10例, 占2.15%; 异常血红蛋白变异类型检出17例, 占3.66%; 检出其他变异型22例, 占4.73%。结论 昆明地区人群珠蛋白生成障碍性贫血变异基因类型较为复杂, 高通量测序技术可更全面检测珠蛋白生成障碍性贫血变异基因类型。

关键词: 珠蛋白生成障碍性贫血; 高通量测序技术

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Study on Screening of Thalassemia Genes by Next-generation Sequencing in Kunming Area

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Abstract: Objective To explore the variations of thalassemia genes in Kunming and provide theoretical basis for prevention and control. **Methods** 5787 samples of premarriage, prepregnancy healthy birth testing and healthy physical examination population, which from 25 medical institutions in Kunming with thalassemia gene ($\alpha + \beta$) 301 types were screened by Next-generation sequencing technology in the “Ten Projects Benefiting the People” of Kunming government in 2018, and the clinical data were analyzed by bioinformatics and statistical analysis. **Results** ① Through high-throughput Sequencing, 465 out of 5787 samples were positive, with a positive rate of 8.04%, and among them, 285 cases of α -thalassemia, 131 cases of β thalassemia, 10 cases of compound type, 17 cases of abnormal hemoglobin variation and 22 cases of other variations were detected. ② There were 285 cases of α -thalassemia and 152 cases (53.33%) were most common with $-\alpha^{3.7}/\alpha\alpha$ genotype. A total of 131 cases of β -thalassemia were detected, and Hb E heterozygote and Codon 17(A > T) genotype were the most common, accounting for 26.72% respectively. ③ There were 10 cases (2.15%) of the variation types of α/β -complex thalassemia. 17 cases (3.66%) abnormal hemoglobin variation were detected, and 22 cases (4.73%) of other variants were detected. **Conclusion** The carriers rate of thalassemia in Kunming was 8.04%. Due to the variation gene types of thalassemia were more complex, Next-generation sequencing can detect rare thalassemia variation gene types more comprehensively.

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Keywords: thalassemia; nextgeneration sequencing

珠蛋白生成障碍性贫血(thalassemia)是基因缺陷导致身体无法合成足量的血红蛋白而引起贫血的一种疾病,临床上主要分为轻型、中间型和重型贫血^[1-3],其中重型贫血除骨髓移植外尚无有效的治疗措施,需终身护理与治疗,给患者家庭及社会带来沉重负担,而预防重型贫血患儿出生是最为有效的措施^[4-5]。目前,高通量测序技术(next-generation sequencing, NGS)因自动化程度高、通量高、成本低及具备检测新发变异及复杂变异等优势被广泛应用于基因检测^[7]。本研究基于高通量测序技术首次对昆明地区人群进行珠蛋白生成障碍性贫血基因($\alpha + \beta$)301型筛查,对昆明地区人群携带率情况进行分析,为昆明地区珠蛋白生成障碍性贫血防控工作提供理论依据,现报告如下。

1 材料与方法

1.1 研究对象 对2018年昆明市政府“十大惠民项目”中来自25家医疗机构婚前、孕前优生及健康体检的5787例样本,进行高通量测序检测。所有检测者均充分知情,本研究通过我院伦理委员会审批。

1.2 仪器与试剂 高通量测序技术:基因扩增仪、MGISEQ-2000高通量测序仪。试剂:DNA提取试剂和扩增试剂由广州美基生物和深圳华大临床检验中心提供。操作严格按仪器设备标准操作程序以及试剂盒说明书进行。

1.3 方法 采集受检者外周血2~3ml, EDTA-K₂抗凝,采用磁珠法提取外周血样本中基因组DNA,进行目的DNA基因扩增,高通量测序后,采用生物信息分析软件Gene detection software for Thalassemia V1.0对测序数据进行珠蛋白生成障碍性贫血基因($\alpha + \beta$)301型生信分析。

1.4 统计学分析 计数资料以例数或百分率表示。

2 结果

2.1 高通量测序检测结果 在5787例样本中共检出465例携带者,阳性率为8.04%。其中 α -检出285例,构成比61.29%; β -检出131例,构成比28.17%;复合型检出10例,构成比2.15%;异常血红蛋白变异类型检出17例,构成比3.66%;其他变异型检出22例,构成比4.73%。

2.2 α -珠蛋白生成障碍性贫血基因检测结果 285例 α -变异中,共检出13种基因变异类型,包括6种缺失型和7种非缺失型,其中以 $-\alpha^{3.7}/\alpha\alpha$ 基因型最为常见,为152例(53.33%), $-\alpha^{SEA}/\alpha\alpha$ 检出87例(30.53%), $-\alpha^{4.2}/\alpha\alpha$ 检出23例(8.07%),Hb Westmead杂合子检出12例(4.21%),Hb Constant Spring杂合子检出3例(1.05%); $-\alpha^{3.7}/$

$\alpha^{3.7}$, $--^{THAI}/\alpha\alpha$, $-\alpha^{3.7}/--^{SEA}$, Hb Phnom Penh杂合子、Hb Quang Sze杂合子、Initiation codon(-T)杂合子、Initiation codon(T>C)杂合子和IVS I-1 AGGT>AGAT donor杂合子各检出1例,各占0.35%。

2.3 β -珠蛋白生成障碍性贫血基因检测结果 131例 β -变异中,共检出13种基因变异类型。Hb E杂合子、Codon 17(A>T)杂合子各检出35例,各占26.72%;Codon 41/42(-TTCT)杂合子检出22例(16.79%);IVS-II-654(C>T)杂合子检出20例(15.27%);-50(G>A)杂合子检出6例(4.58%);-28(A>G)杂合子检出4例(3.05%);Codon 41(-C)杂合子、Codon 71/72(+A)杂合子各检出2例,各占1.53%;-72(T>A)杂合子、-88(C>T)杂合子、Codon 5(-CT)CCT(Pro)>C杂合子、Initiation codon ATG>ACG杂合子各检出1例,各占0.76%;Chinese Gamma(A gamma delta beta)缺失杂合子检出1例(0.76%)。以Hb E杂合子和Codon 17(A>T)杂合子基因型最为常见。

2.4 复合型珠蛋白生成障碍性贫血基因检测结果 10例复合型变异基因型中,检出7种基因变异类型。其中 $-\alpha^{4.2}/\alpha\alpha$ 并发Hb Q-Thailand杂合子检出3例(30%); $--^{SEA}/\alpha\alpha$ 并发Hb Hekinan II杂合子检出2例(20%); $-\alpha^{3.7}/\alpha\alpha$ 并发Init CD ATG>TG杂合子、 $--^{SEA}/\alpha\alpha$ 并发Codon 41/42(-TTCT)杂合子、 $--^{SEA}/\alpha\alpha$ 并发Hb E杂合子、 $-\alpha^{3.7}/\alpha\alpha$ 并发Hb E杂合子、 $-\alpha^{4.2}/\alpha\alpha$ 并发Codon 41/42(-TTCT)杂合子各检出1例,各占10%。

2.5 异常血红蛋白变异基因检测结果 17例异常血红蛋白携带者中,检出14种基因变异类型。Hb G-Taipei杂合子、Hb Hamilton杂合子、Hb J-Bangkok杂合子各检出2例,各占11.76%;Hb Broomhill杂合子、Hb Fuchu-I杂合子、Hb G-Honolulu杂合子、Hb Groene Hart杂合子、Hb J-Lome杂合子、Hb Melusine杂合子、Hb Olivet杂合子、Hb San Bruno杂合子、Hb Shizuoka杂合子、Hb Singapore杂合子、Hb Zurich-Albisrieden杂合子各检出1例,各占5.88%。

2.6 其他变异类型基因检测结果 22例其他变异基因型中,检出14种基因变异类型。HBA2:c.46G>A(Gly>Ser)杂合子检出5例(22.73%);HBB:c.*+129T>A杂合子检出4例(18.18%);HBB:c.-107A>C杂合子检出2例(9.09%);HBA1:c.*+41delC杂合子、HBA1:c.16G>A(Ala>Thr)杂合子、HBA1:c.203C>T(Thr>Ile)杂合子、HBA1:c.300G>C(Lys>Asn)杂合子、HBB:c.-132G>A杂合子、HBB:c.*+132C>T杂合子、HBB:c.-146G>T杂合子、

HBB:c.-180G>C 杂合子、HBB:c.-71G>T 杂合子、HBB:c.-39T>G 杂合子各检出 1 例 (4.55%) ; HBA2:c.95+5_HBA2:c.95+28delGGCTCCCTCCCCTGCTC CGACCCG 纯合子检出 1 例 (4.55%)。根据人类血红蛋白变异数据库 (HbVar) 和人类基因突变数据库 (HGMD) 查询可知这 14 种基因变异类型均无明显致病性。

3 讨论

珠蛋白生成障碍性贫血主要分布于地中海沿岸、东南亚、热带及亚热带地区,在我国主要集中于南方省份,其中云南省为高发地区之一^[7-9]。在珠蛋白生成障碍性贫血防控方面,云南省前期主要采用聚合酶链式反应 (polymerase chain reaction, PCR)、反向斑点杂交法 (reverse dot blot, RDB) 等传统技术^[10]对昆明地区妊娠期妇女、新生儿进行检测。随着医学技术飞速发展,高通量测序技术 (NGS) 已成为用于珠蛋白生成障碍性贫血全面防控的新检测手段^[4]。本研究采用 NGS 对昆明地区人群进行珠蛋白生成障碍性贫血基因 ($\alpha + \beta$) 301 型进行检测,检出阳性率为 8.04%,高于前期报道的妊娠期妇女检出率 5.92%^[11]和新生儿珠蛋白生成障碍性贫血检出率 7.75%^[12],推测与前期使用检测方法的局限性有关。

本研究 α -珠蛋白生成障碍性贫血基因变异类型以 $-\alpha^{3.7}/\alpha\alpha$ 基因型最为常见, β -珠蛋白生成障碍性贫血基因变异类型以 Hb E 杂合子、CD17(A>G) 杂合子基因型占比最高,同昆明地区妊娠妇女、新生儿检出的 α -及 β -基因变异类型报道的基因变异类型排序一致^[11-12],表明昆明地区人群 α -基因类型最常见的为 $-\alpha^{3.7}/\alpha\alpha$,该基因型的检出对防控工作起着积极作用; β -基因变异类型频次最高的 Hb E 杂合子与 CD17(A>G) 杂合子,数据显示昆明地区与我国南方其他地区 β -基因型突变常见类型有所不同^[2,13]。通过 NGS 检测后发现昆明地区人群中异常血红蛋白变异类型、复合型珠蛋白生成障碍性贫血变异类型以及其他变异类型均有检出,提示昆明地区珠蛋白生成障碍性贫血基因变异类型较为复杂,可能与昆明地区地理、气候、少数民族等影响因素的共同作用有关^[14]。

本研究首次采用高通量测序技术检测后,珠蛋白生成障碍性贫血的阳性率明显高于前期报道的阳性率,提示昆明地区存在稀有、罕见变异珠蛋白生成障碍性贫血基因,因此,在昆明地区珠蛋白生成障碍性贫血防控工作方面,除了以常见珠蛋白生成障碍性贫血防控为主,同时也要兼顾稀有、罕见变异珠蛋白生成障碍性贫血,以免造成漏诊。昆明地区珠蛋白生成障碍性贫血基因变异类型较为复杂,

NGS 可以更全面检测到罕见、稀有的珠蛋白生成障碍性贫血基因变异类型,采用 NGS 技术作为珠蛋白生成障碍性贫血高风险地区筛查手段,对丰富珠蛋白生成障碍性贫血基因谱具有重要的应用价值。

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