

应用HRM技术对CYP2C19*2和CYP2C19*3进行双重SNP分型

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摘要 氯吡格雷是一种广泛用于预防静脉血栓形成的抗血小板药物。研究表明, 携带有CYP2C19基因功能缺失型等位基因CYP2C19*2、CYP2C19*3的病人, 其体内代谢氯吡格雷成为其活性形式的的能力降低, 导致氯吡格雷抑制血小板聚集功能减弱。文章旨在建立一种利用高分辨率熔解曲线分析(High-resolution melting curve analysis, HRM)技术在闭合单管中同时对CYP2C19*2、CYP2C19*3两个多态性位点进行简便、准确分型的方法。本实验针对两个SNP位点分别设计特异性的HRM引物, 并在两个位点引物的5'端分别加上富含AT和GC的序列, 保证两个位点的扩增产物熔解峰无重叠。利用HRM技术, 快速、灵敏地对64例随机DNA样本的CYP2C19*2、CYP2C19*3两个多态性位点进行了基因分型, 且HRM方法的分型结果与测序验证结果完全一致。因此, 利用HRM技术可以实现闭合单管中简便、准确地对CYP2C19*2、CYP2C19*3两个多态性位点同时进行基因分型。该方法有望应用于临床, 指导氯吡格雷的个体化用药。

关键词: 高分辨率熔解曲线分析 CYP2C19*2 CYP2C19*3 氯吡格雷 SNP分型

Abstract: Clopidogrel is a widely used anti-platelet agent for the prevention of arterial thrombosis. It has been suggested that clopidogrel may be less effective in inhibiting platelet aggregation among patients who are carriers of CYP2C19*2 and CYP2C19*3, two loss-of-function CYP2C19 alleles, which are associated with reduced conversion of clopidogrel to its active metabolite. The objective of this research was to develop a simple and accurate method for genotyping of CYP2C19*2 and CYP2C19*3 simultaneously in one closed-tube using high-resolution melting curve (HRM) analysis. Two amplicons bracketing CYP2C19*2 and CYP2C19*3 gene variants were designed, and AT- or GC-rich 5' tails were added to selected primers to ensure two different amplicons with non-overlapping melting curves. Sixty-four random DNA samples were all fast and sensitively genotyped by HRM analysis. This method was validated by DNA sequencing technique, and genotypes obtained using the HRM approach perfectly matched the genotypes obtained by DNA sequencing technique. Therefore, this HRM-based assay allows simple and accurate duplex genotyping of CYP2C19*2 and CYP2C19*3 simultaneously in one closed-tube. This method is expected to be applied in clinical laboratory to guide individual dosage design of clopidogrel.

Keywords: high-resolution melting curve analysis, CYP2C19*2, CYP2C19*3, clopidogrel, SNP genotyping

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