Comparative-high resolution melting: a novel method of simultaneous screening for small mutations and copy number variations.

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Efficient and cost-effective screening for DNA sequence changes, both small mutations and copy number variations (CNVs), is a crucial aspect for routine genetic diagnostics as well as for basic research. In this study we present a development and evaluation of comparative-high resolution melting (C-HRM), a new approach for the simultaneous screening of small DNA changes and gene CNVs. In contrast to other methods, relative quantification in C-HRM is based on the results obtained during the melting process and calculations of the melting peak height ratio in the multiplex reaction. Validation of the method was conducted on DNA samples from 50 individuals from Duchenne muscular dystrophy (DMD) families, 50 probands diagnosed with familial adenomatous polyposis and a control group of 36 women and 36 men. The results of analyses conducted on fragments of the *DMD* and *APC* genes correspond completely (100 %) with the results of previous studies. C-HRM sensitivity in CNV detection was assessed through the analysis of mixed DNA samples with different proportions of a deletion carrier and wild type control. The results are presented as a linear regression with R2 of 0.9974 and imply the capability of the method to detect mosaics. C-HRM is an attractive and powerful alternative to other methods of point mutations and CNV detection with 100 % accuracy in our studied group.