406a High Throughput Genotyping of Cyp2c9 Snps Using Biotinylated Ddntps and Maldi-Tof MS

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We report here high throughput SNP genotyping of the CYP2C9 gene using SPC-SBE (Kim et al., Nucleic Acids Res., 2002), a MALDI-TOF mass spectrometry-based genotyping method. SPC-SBE involves multiplex single base extension (SBE) using biotinylated dideoxynucleotides, and subsequent separation of DNA extension fragments using solid phase capture (SPC). Previously, simultaneous screening of the p53 gene for 30 mutation sites by the SPC-SBE approach has been shown (Kim et al., Nature Reviews Genetics, 2003), however, analysis of SNPs at a similar multiplexing level has not been reported.

CYP2C9 is an important isoenzyme of the cytochrome P450 family that is involved in the metabolism of about 10 % of therapeutically important drugs. 2C9 is responsible for metabolism of drugs such as S-warfarin, tolbutamide and many NSAIDs (non-steroidal anti-inflammatory drugs). 2C9 is highly polymorphic and around 250 SNPs have been reported for in the dbSNP database. These polymorphisms may result in large interindividual differences in drug metabolism mediated by the enzyme. To explore the high multiplexing capability of SPC-SBE, we chose 40 SNPs of 2C9 in the vicinity of exons 2, 6 and 7 including non-synonymous SNPs in codons 144 (C/T; Arg/Cys), 359 (A/C; Ile/Leu) and 360 (C/G, Asp/Glu).

A SBE primer was designed for each of the SNPs with sufficient mass difference between primers to allow complete resolution of all 4 possible extension products. Human genomic DNA was amplified by multiplex PCR to produce amplicons of the SNP containing regions and then simultaneous genotyping was done for all SNPs. SBE reaction was carried out by mixing SBE primers with PCR products and biotinylated ddNTPs to generate 3'-biotinylated extension products. These products were captured using streptavidin coated magnetic beads and the unused primers washed away. The extension products were subsequently released and analysed by MALDI TOF MS yielding a spectrum with extension product peaks only.

Location of each SNP is identified by the mass of corresponding extension product and its genotype by difference in mass between primer and extension product. SPC-SBE allows separation of extension products from unused primers by using biotinylated terminators, which increases the number of SNPs that can be genotyped in one assay and facilitates the sample purification procedure of MALDI-TOF MS. Further, the approach provides improved accuracy in heterozygote detection since the smallest mass difference between any two extension products of a primer is 16 Da (between biotin-ddATP and biotin-ddGTP) as compared to 9 Da using regular dideoxynucleotides (between ddTTP and ddATP). The results presented here demonstrate that SPC-SBE allows high throughput separation of DNA extension fragments and is a robust method for the SNP genotyping.