

ORIGINAL ARTICLE

Cell Line Controls for the Genotyping of a Spectrum of Human Single Nucleotide Polymorphisms in the Clinical Laboratory

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SUMMARY

Background: Genotyping for clinically important single nucleotide polymorphisms (SNPs) is performed by many clinical routine laboratories. To support testing, quality controls and reference materials are needed. Those may be derived from residual patient samples, left over samples of external quality assurance schemes, plasmid DNA or DNA from cell lines. DNAs from cell lines are commutable and available in large amounts.

Methods: DNA from 38 cell lines were examined for suitability as controls in 11 SNP assays that are frequently used in a clinical routine laboratory: *FV* (1691G>A), *FII* (20210G>A), *PAI-1* 4G/5G polymorphism, *MTHFR* (677C>T, 1298A>C), *HFE* (H63D, S65C, C282Y), *APOE* (E2, E3, E4), *LPH* (-13910C>T), *UGT1A1* (*28, *36, *37), *TPMT* (*2, *3A, *3B, *3C), *VKORC1* (-1639G>A, 1173C>T), *CYP2C9* (*2, *3, *5). Genotyping was performed by real-time PCR with melting curve analysis and confirmed by bi-directional sequencing.

Results: We find an almost complete spectrum of genotypic constellations within these 38 cell lines. About 12 cell lines appear sufficient as genotypic controls for the 11 SNP assays by covering almost all of the genotypes. However, hetero- and homozygous genotypes for *FII* and the alleles *TPMT**2, *UGT1A1**37 and *CYP2C9**5 were not detected in any of the cell lines.

Conclusions: DNA from most of the examined cell lines appear suitable as quality controls for these SNP assays in the laboratory routine, as to the implementation of those assays or to prepare samples for quality assurance schemes. Our study may serve as a pilot to further characterize these cell lines to arrive at the status of reference materials.

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Supplementary Table.

Table 1. Primers and Probes.

| Gene | Sequence name | Sequence | Final conc. ^d | Ref. |
|---------------------------|---|---|--------------------------|-----------------------------|
| <i>APOE</i> | ApoE CA1 | TTG AAG GCC TAC AAA TCG GAA CTG | 0.5 µmol/L | [22] |
| | ApoE CA 2 | CCG GCT GCC CAT CTC CTC CAT CCG | 0.5 µmol/L | [22] |
| | Apo E4-112A ^a | CTG CAG GCG GCG CAG GCC CGG CTG GGC GC | 0.2 µmol/L | [22] |
| | Apo E4-112M ^b | ACA TGG AGG ACG TGC GCG G | 0.2 µmol/L | [22] |
| | Apo E2-158A ^a | GCT GCG TAA GCG GCT CCT CCG CGA TGC CG | 0.2 µmol/L | [22] |
| | Apo E2-158M ^c | GAC CTG CAG AAG CGC CTG GC | 0.2 µmol/L | [22] |
| <i>CYP2C9</i> | Cyp 2C9 e3F1 | AGG ATG GAA AAC AGA GAC TTA C | 0.1 µmol/L | [23] |
| | Cyp 2C9 e3R1 | GGT CAG TGA TAT GGA GTA GGG | 0.3 µmol/L | [23] |
| | Cyp 2C9 e7F1 | ACC GGA GCA CCT GCA TAC AA | 0.1 µmol/L | [23] |
| | Cyp 2C9 e7R1 | CTA TGA ATT TGG GGT CTT CG | 0.3 µmol/L | [23] |
| | C9 sensor*2 ^a | GAG GAC CGT GTT CAA GAG GAA G | 0.2 µmol/L | [23] |
| | C9 anchor*2 ^b | GGA ATT TTG GGA TGG GGA AGA GGA GCA T | 0.2 µmol/L | [23] |
| | C9 sensor*3*5 ^a | GTC CAG AGA TAC CTT GAC CTT CT | 0.2 µmol/L | [23] |
| | C9 anchor*3*5 ^c | CCA CCA GAC TAC CAC ATG CAG TGA CCT GT | 0.2 µmol/L | [23] |
| <i>FII</i> | F2-for | CCG CTG GTA TCA AAT GGG | 0.5 µmol/L | [24] |
| | F2-rev | CCA GTA GTA TTA CTG GCT CTT CCT G | 0.5 µmol/L | [24] |
| | F2-wt ^a | CTC AGC GAG CCT CAA TG | 0.1 µmol/L | [24] |
| | F2-anchor-L ^c | TCC CAG TGC TAT TCA TGG GCA GCT C | 0.3 µmol/L | [24] |
| <i>FV</i> | M-5 for | TGC CCA GTG CTT AAC AAG ACC A | 0.1 µmol/L | [24] |
| | M-3 rev | CTT GAA GGA AAT GCC CCA TTA | 0.5 µmol/L | [24] |
| | F5-mut ^a | GGC GAG GAA TAC AGG TAT | 0.2 µmol/L | [24] |
| | F5-anch ^c | TGT CCT TGA AGT AAC CTT TCA GAA ATT CTG | 0.4 µmol/L | [24] |
| <i>HFE</i> | H63D -for | ACA TGG TTA AGG CCT GTT GC | 0.5 µmol/L | NG 008720.2 ^e |
| | H63D -rev | CAG CTG TTT CCT TCA AGA TGC | 0.2 µmol/L | [25] |
| | H63D Sensor ^a | CGG CGA CTC TCA TCA TCA TAG AAC ACG AAC A | 0.1 µmol/L | [26] |
| | H63D Anchor ^b | CTG GTC ATC CAC GTA GCC CAA AGC TTC AA | 0.3 µmol/L | [26] |
| | C282Y-for | TGG CAA GGG TAA ACA GAT CC | 0.5 µmol/L | [25] |
| | C282Y-rev | CTC AGG CAC TCC TCT CAA CC | 0.5 µmol/L | [25] |
| | C282Y Mu t ^a | AGA TAT ACG TAC CAG GTG GAG | 0.2 µmol/L | [25] |
| C282Y Anchor ^b | CCC AGG CCT GGA TCA GCC CCT CAT TGT GAT CTG GG | 0.5 µmol/L | [25] | |
| <i>LPH</i> | Lac I PH for | TGA GTG TAG TTG TTA GAC GG | 0.5 µmol/L | [15] |
| | Lac I PH rev | CAA CCT AAG GAG GAG AGT | 0.5 µmol/L | [15] |
| | Lac I PH FL ^a | GCC TCT GCG CTG GCA ATA CAG ATA | 0.4 µmol/L | [15] |
| | Lac I PH LC ^b | ATA ATG TAG CCC CTG GCC T | 0.4 µmol/L | [15] |
| <i>MTHFR</i> | MTHFR 677 for | CGA AGC AGG GAG CTT TGA GG | 0.1 µmol/L | [27] |
| | MTHFR 677 rev | AGG ACG GTG CGG TGA GAG TG | 0.3 µmol/L | [27] |
| | MTHFR 677 FL ^a | CGG GAG CCG ATT TCA TCA | 0.1 µmol/L | [27] |
| | MTHFR 677 LC640 ^c | CGC AGC TTT TCT TTG AGG CTG ACA | 0.3 µmol/L | [27] |
| | MTHFR 1298 for | GGG AGC TGA AGG ACT ACT AC | 0.2 µmol/L | [11] |
| | MTHFR 1298 rev | TGT GAC CAT TCC GGT TTG | 0.6 µmol/L | [11] |
| | MTHFR 1298 FLmut ^a | GTG AAG CAA GTG TCT TTG AA | 0.2 µmol/L | [11] |

Table 1. Primers and Probes (continued).

| Gene | Sequence name | Sequence | Final conc. ^d | Ref. |
|---------------|--|--|--------------------------|-----------------------------|
| <i>MTHFR</i> | MTHFR 1298 LC705 ^b | TCT TTG TTC TTT ACC TCT CGG GAG AAC C | 0.6 µmol/L | [11] |
| <i>PAI-1</i> | PAI-1 for | AGC CAG ACA AGG TTG TTG ACA C | 0.4 µmol/L | [28] |
| | PAI-1 rev | CAG AGG ACT CTT GGT CTT TCC C | 0.2 µmol/L | [28] |
| | PAI-1 sens 5G ^a | TGA CTC CCC CAC GTG TCC | 0.2 µmol/L | [28] |
| | PAI-1 anchor ^c | ACT CTC TCT GTG CCC CTG AGG ACT CT | 0.2 µmol/L | [28] |
| <i>TPMT*2</i> | TPMT *2 for | CAT GTT CTT TGA AAC CCT ATG AA | 0.2 µmol/L | [29] |
| | TPMT *2 rev | AGT ACA GAG AGG CTT TGA CCT C | 0.2 µmol/L | [29] |
| | TPMT *2 anchor ^a | CCA AGT TCA CTG ATT TCC ACA CCA ACT ACA C | 0.2 µmol/L | [30] |
| | TPMT *2 sensor ^c | GTG TCC CCG GTC TGC AAA C | 0.2 µmol/L | [30] |
| <i>*3B</i> | TPMT *3B for | ACG CAG ACG TGA GAT CCT AA | 0.05 µmol/L | [30] |
| | TPMT *3B rev | GTA TAG TAT ACT AAA AAA TTA AGA CAG CTA AAC | 0.2 µmol/L | [30] |
| | TPMT *3B sensor ^a | ATG ATT TGG GAT AGA GGA GCA TTA G | 0.2 µmol/L | NG 0121137 ^e |
| | TPMT *3B anchor ^c | GCC ATC AAT CCA GGT GAT CGC AAA TG | 0.2 µmol/L | NG 0121137 ^e |
| <i>*3C</i> | TPMT *3C for | AAT CCC TGA TGT CAT TCT TCA TAG T | 0.2 µmol/L | [29] |
| | TPMT *3C rev | CTT TCA CAT CAT AAT CTC CTC TCC | 0.2 µmol/L | [29] |
| | TPMT *3C sensor ^a | CTT TTC TGT AAG TAG ACA TAA C | 0.2 µmol/L | [29] |
| | TPMT *3C anchor ^b | TCA AAA AGA CAG TCA ATT CCC CA | 0.2 µmol/L | [29] |
| <i>UGT1A1</i> | UGT1A1 for | GTC ACG TGA CAC AGT CAA AC | 0.1 µmol/L | [32] |
| | UGT1A1 rev | CAG CAT GGG ACA CCA CTG | 0.4 µmol/L | [32] |
| | UGT1A1 (TA) ₇ sens-TT ^a | TTG CCA TAT ATA TAT ATA TAT AAG | 0.1 µmol/L | [32] |
| | UGT1A1 anchor ^b | AGG GCG AAC CTC TGG CAG GA | 0.3 µmol/L | [32] |
| <i>VKORC1</i> | C1173T VKORC1 for-2 | AGA GAC TTA CTT AAG GTC TAA GAT GAA | 0.4 µmol/L | [31] |
| | C1173T VKORC1 rev | GCC CGA GAA AGG TGA TTT CC | 0.4 µmol/L | [31] |
| | C1173T VKORC1 sens-wt ^a | AGA TCA TCG ACC CTT GGA CTA GG | 0.2 µmol/L | [31] |
| | C1173T VKORC1 anch ^c | GGG AGG TCG GGG AAC AGA GGA T | 0.2 µmol/L | [31] |
| | G-1639A VKORC1 for | CAG CAT CTG GAG AGG GAG G | 0.4 µmol/L | NG 011564.1 ^e |
| | G-1639A VKORC1 rev | GCC AGG CTT GTC TTA AAC TCC | 0.4 µmol/L | NG 011564.1 ^e |
| | G-1639A VKORC1 sens ^a | AAA CAA CCA TTG GCC GGG TG | 0.2 µmol/L | NG011564.1 ^e |
| | G-1639A VKORC1 anch ^b | GTG GCT CAC GCC TAT AAT CCT AGC ATT | 0.2 µmol/L | NG011564.1 ^e |

^a 3' labeled with Fluorescein.^b 5' labeled with LC-Red 705, 3' phosphorylated.^c 5' labeled with LC-Red 640, 3' phosphorylated.^d final concentrations used in real time PCR.^e GenBank accession number.