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Mutation analysis in fatal pulmonary thromboembolism—postmortem validation study and beyond

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Abstract. Sudden fatal pulmonary thromboembolism (PE) is very common in Caucasians and results in over ~120,000 deaths per year. There are both acquired and inherited risk factors for PE. The three most common mutations are Factor V Leiden G1691A, Prothrombin G20210A, and MTHFR C677T. We have developed an in-house molecular testing methodology using polymerase chain reaction (PCR) and automated DNA sequencing technologies. The method was validated on postmortem tissue samples, such as heart, spleen, and liver. Tissues were stored in *RNAlater* solution for up to 2 years. The method has also been validated on blood specimens, which were dried on staincards and stored at room temperature for up to 2 years. We obtained results for all tested specimens, including those displaying varying degrees of decomposition. The analytic sensitivity, specificity, and reproducibility show that the method is highly sensitive, and very specific for all three mutations. © 2005 Published by Elsevier B.V.

Keywords: Pulmonary thromboembolism; Genetic testing; Postmortem

1. Introduction

Sudden fatal pulmonary thromboembolism (PE) is found commonly in forensic pathology practice, usually presented as a complication of deep venous thrombosis (DVT). There are several genetic risk factors involved in the predisposition of individuals to develop DVT. The three most common mutations are Factor V Leiden G1691A, Prothrombin G20210A, and MTHFR C677T [1,2]. Molecular testing for these common

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mutations is one of the most frequently ordered molecular tests in clinical setting. In the offices of medical examiners, however, molecular testing for fatal PE is not a common practice. Among many conceivable reasons, one is the special features of the postmortem specimens. Currently there is no clinical diagnostic testing methodologies for these common mutations that are developed and validated on the postmortem specimens available to forensic pathologists. This paper describes the success of the suggested methodology for postmortem specimen.

2. Materials and methods

2.1. Test samples

1) Control DNAs with known genotypes for the tested loci. 2) Postmortem samples, including blood, heart, spleen, and liver with varying extent of decomposition. 3) Inter-lab exchange proficiency samples.

2.2. Analytic procedure

DNA was extracted using the M48 Qiagen MagAttract DNA extraction system. DNA was quantified by Quantiblot analysis (Applied Biosystems, ABI). In-house designed primers (0.4 μM) are: F5-Seq-forward (5′-GAAAATGATGCCCAGTGCTT-3′), F5-Seq-backward (5′-ttgaaggaaatgccccatta-3′), F2-Seq-forward (5′-CCGCCTGAAGAAGTGGATAC-3′), F2-Seq-backward (5′-cctgagcccagagagctg-3′), MTHFR-Seq-forward (5′-tgtcatccctattgg-cagGT-3′), MTHFR-Seq-backward (5′-TCACAAAGCGGAAGAATGTG-3′). The thermocycler conditions are: 94 °C for 5 min, 40 cycles of 94 °C 30 s, 53 °C 30 s, 72 °C 30 s, and 72 °C 7 min. PCR amplicons were detected and quantified were performed by Transgenomic WAVE system. PCR amplicons were purified by ExoSap it (USB). ABI BigDye Terminator V3.1 kit was used for direct cycle sequencing reaction. Sequencing products were detected

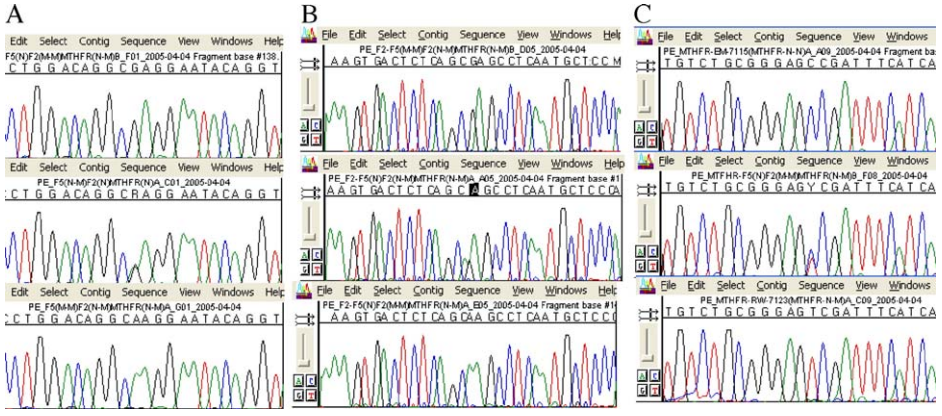

Fig. 1. Representative results. A: results for F5 1691. Top panel—wild type, middle panel—heterozygote, bottom panel—homozygote. B: results for F2 20210. Top panel—wild type, middle panel—heterozygote, bottom panel—homozygote. C: results for MTHFR 677. Top panel—wild type, middle panel—heterozygote, bottom panel—homozygote.

Table 1
Analytic characteristics

Test locus	Alleles studied	Sensitivity[a]	Specificity[b]	Reproducibility
F5	Total=30 (11 mutant alleles, 19 normal alleles)	100% 95% CI (71.5–100%)	100% (95% CI (82.3–100%)	100% (Total alleles studied=224)
F2	Total=26 (8 mutant alleles, 18 normal alleles)	100% 95% CI (63–100%)	100% 95% CI (81.5–100%)	100% (Total alleles studied=224)
MTHFR	Total=40 (17 mutant alleles, 23 normal alleles)	100% 95% CI (80.5–100%)	100% 95% CI (85.2–100%)	100% (total alleles studied=224)

[a] Analytic sensitivity is defined as the proportion of mutant alleles that are correctly typed.
[b] Analytic specificity is defined as the proportion of normal alleles that are correctly typed.

by automated genetic analyzer ABI 3100 Avant. Data were analysed by ABdI Sequencing Analysis Software 5.1 and Genecodes Sequencher software.

3. Results

Genotypes for all control samples matched the known genotypes. See representative results in Fig. 1. Genotypes for all postmortem samples were obtained. The genotypes for all sample types from the same case matched each other, which is consistent with the fact that these mutations are germ-line mutation. Genotypes for all the proficiency samples match the genotypes obtained in another clinical Genetics Diagnostic laboratory. Analytic characteristics, e.g. sensitivity, specificity, and reproducibility, have been measured (Table 1).

4. Conclusion and discussion

The molecular testing method by PCR and cycle sequencing technologies for the detection of factor V Leiden (G1691A) mutation, prothrombin (G20210A) mutation, and MTHFR (C677T) variant can be successfully applied to postmortem samples. The method is highly sensitive, specific, and can be automated. The molecular testing results are important for the diagnosis of fatal pulmonary thromboembolism due to hereditary thrombophilia mutations, as well as subsequent counselling of high-risk family members.

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