Figure 1 Agarose gel (2.5%) electrophoresis of PCR products generated from DNA isolated from paraffin wax embedded liver (lanes 1–4) and brain (lanes 5–8) tissue. Lane M, Φ x174 DNA markers; lanes 1 and 5, blank controls (no DNA added); lanes 2–4, 237 bp AAT gene fragment amplified from liver tissue; lanes 6–8, 227 bp APOE gene fragment amplified from brain tissue.

Using the method describe herein, it is possible to obtain amplification products reproducibly from DNA isolated from paraffin wax embedded liver and brain tissue. PCR products up to around 240 bp in size can be generated consistently, which in the majority of cases permits further genetic analysis to be performed. We used the 227 bp APOE PCR prod-

uct that was amplified from five paraffin wax embedded brain tissue samples for genotyping⁶ and the results obtained matched those determined previously using DNA prepared from frozen (-20°C) brain tissue, that had been in storage following necropsy, in all five cases (data not shown). As this method is so simple and rapid, it should now be possible to analyse large numbers of preserved samples at the molecular level.

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Postmortem diagnosis of Factor V Leiden from paraffin wax embedded tissue

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Abstract

Activated protein C resistance resulting from Factor V Leiden is an important inherited thrombophilia disorder which is found in 3.5% of people in the UK. The genetic defect can be detected using the PCR and the diagnosis can be made post-mortem from paraffin wax embedded tissue. The presence of Factor V Leiden should be sought in all cases of unexplained sudden death resulting from venous thromboembolism.

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Keywords: Factor V Leiden, thromboembolism, thrombophilia disorder.

Activated protein C resistance has been recognised recently as an important risk factor predisposing to venous thromboembolism.¹ The disorder results from a specific missense mutation (G1691A) in exon 10 of the coagula-

tion factor V gene, which is located in the sequence encoding the activated protein C cleavage site.² Recent studies have shown that the mutant factor Va (Factor V Leiden) is 10 times less susceptible to deactivation by this natural anticoagulant.³ Factor V Leiden can be detected by means of the PCR as the nucleotide base change eliminates a cleavage site for the restriction enzyme, MnlI.² Recently, we have demonstrated the importance of diagnosing this disorder retrospectively from postmortem paraffin wax embedded tissue.

Case report

A 24 year old man sustained a soft tissue laceration to the right leg following a road traffic accident. The wound was debrided and sutured and the patient was prescribed diclofenac sodium (Voltarol) as analgesia. One week later, he presented with lower abdominal pain and appendicitis was diagnosed provision-

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Figure 1 Detection of Factor V Leiden by PCR: amplified 147 bp fragment digested with *Mnl*I for a normal subject (N), patient P who is heterozygous for the mutation and a homozygous control (C). M = molecular size markers (*pBR322* digested with *Hae*III).

ally. Laparotomy was performed and revealed a short section of thickened distal ileum. This was resected without complication, but two days after the procedure the patient developed dyspnoea and subsequently collapsed and suffered a cardiac arrest. Postmortem examination revealed massive pulmonary embolus. Histology of the distal ileum showed typical Diaphragm disease as described in association with use of non-steroidal anti-inflammatory drugs.⁴

POLYMERASE CHAIN REACTION

To detect Factor V Leiden, several 15 µm sections of paraffin wax embedded myocardial tissue were placed in a 0.5 ml Eppendorf tube and 100 µl sterile water was added together with 100 µl proteinase K buffer solution (25 µl 5% Tween, 250 µl 20 mg/ml proteinase K, 100 µl 1 M Tris HCl (pH 8.3), and sterile water to 500 µl). The solution was covered with 100 µl mineral oil and incubated at 55°C for 42 hours before boiling for 10 minutes at 100°C and microcentrifugation. Ten microlitres of the supernatant was used for each PCR. The oligoprimers and PCR conditions described by Beauchamp *et al*⁵ were used to detect the mutation.

An aliquot of the product was incubated with two units of the restriction enzyme *Mnl*I for three hours at 37°C prior to electrophoresis on an 8% polyacrylamide gel and staining with ethidium bromide. Two restriction sites for this enzyme are present in normal subjects, giving fragments of 85, 37 and 25 base pairs (bp). The Leiden mutation eliminates one of these sites, however, and bands of 122 and 25 bp are obtained. Patient P was confirmed to be heterozygous for this defect (fig 1).

Discussion

Heterozygous Factor V Leiden is found in 3.5% of the population of the UK.⁵ It can be identified in about 20% of subjects with de novo venous thrombosis and accounts for about 40% of familial thrombophilia.⁶ The genetic defect is an important cofactor for thromboembolism associated with use of oral contraceptives⁷ and pregnancy.⁸ Homozygous subjects and those patients who co-inherit a second thrombophilia disorder are probably at significantly higher risk.^{9 10}

Postmortem detection of Factor V Leiden from fresh or paraffin wax embedded tissue should be considered in all cases of unexplained sudden death where venous thromboembolism is suspected. Any tissue containing nucleated cells can be used as a source of DNA—for example, liver or lung tissue obtained by a “tru-cut” biopsy. Retrospective diagnosis enables family studies and genetic counselling to be performed. The technique may also allow important studies to be undertaken on archival material—for example, to determine the significance of Factor V Leiden as a risk factor for maternal death resulting from thromboembolism in pregnancy.

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