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Increased Frequency of the MTHFR A1298C Mutation in an Irish Population

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structural issues and can be resolved. The *p53* resequencing microarray is currently undergoing a redesign (Roche Molecular Diagnostics, personal communication). Given the cost-effectiveness of chip technology, it is reasonable to expect a next-generation Chip in which most of these limitations have been addressed. Such improvements could lead to a more useful and productive tool in cancer research and diagnostics.

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Drs. Kandel and Rohan respond:

To the Editor:

We would like to thank Drs. Allen and Chiafari for their comments. They reiterate many of the points we made in our report *(1)*. We are glad that they also had similar findings. However, they suggest that we differ in the detection of polymorphisms. In the Discussion section of our report *(1)*, we speculated that our cutoff score might be too high because we observed that a polymorphism in exon 6 had a score of 6. In their study, the GeneChip detected one of six exon 4 (codon 72) polymorphisms, whereas we were unable to detect any in 12 cases from which we obtained a PCR product. We would argue that this was not a significant difference because both sample sizes are small. In fact, it actually supports our conclusion that a combination of both microarray and sequencing is required to identify *p53* alterations, as they would have missed five polymorphisms. We also look forward to being able to use the next-generation *p53* microarray, as we stated in our report *(1)*, because this methodology definitely has a role to play in sequencing of the *p53* gene.

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Increased Frequency of the *MTHFR* A1298C Mutation in an Irish Population

To the Editor:

The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of methylene tetrahydrofolate to 5-methyltetrahydrofolate, the cosubstrate required for the remethylation of homocysteine to methionine. Mutations in the MTHFR enzyme are reported as causes of hyperhomocysteinemia *(1)*. Hyperhomocysteinemia is generally, although not universally, seen as an independent and graded risk factor for venous thrombosis and neural tube defects *(2)*. Several polymorphisms have been reported in the *MTHFR* gene, but two particular mutations generate the most interest, the recently described A1298C *(3)* and the most-characterized C677T *(4)*. The A1298C polymorphism in the *MTHFR* gene encodes for a glutamate to alanine substitution and leads to a decrease in enzyme activity. Combined heterozygosity for the C677T/A1298C polymorphisms in some studies *(5)* is associated with higher homocysteine concentrations and decreased plasma folate.

Amplification Refractory Mutation System (ARMS) PCR determination of the *MTHFR* C677T mutation has been described by Hessner et al. *(6)*. To determine the frequency of the A1298C mutation in the Irish population, we developed a reliable and rapid ARMS PCR method. We compared the results with those obtained with the standard method for detection, PCR followed by restriction fragment length polymorphism (RFLP) analysis *(3)*.

Our cohort consisted of 120 blood donors, none of whom had experienced any past or current thrombotic events or had a family history of thrombosis. Informed consent was obtained from all study participants. Total genomic DNA was isolated from blood leukocytes, and *MTHFR* A1298C was analyzed by PCR-RFLP *(3)*.

ARMS PCR was also used to determine the frequency of this mutation. A typical ARMS PCR set-up for the wild-type reaction consisted of 200 ng of genomic DNA, 2.5 mM $MgCl_2$, 0.4 mM each deoxynucleotide triphosphate (Invitrogen, BioSciences), 2.5 μL of 10× buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl; Invitrogen], 1.5 U of Platinum Taq polymerase (Invitrogen), and 50 mL/L dimethyl sulfoxide (Sigma-Aldrich). ARMS PCR primers used in the wild-type reaction were as follows: A1298C forward consensus primer (5′-CCTTTGGGGAGCTGAAGGACTACTAC-3′); A1298C wild-type reverse primer (5′-CAAAGACTTCAAAGACAGTC-3′); cystic fibrosis 22 (CF22) forward primer (5′-AAACGCTGAGCCTCACAAGA-3′), and CF22 reverse primer (5′-TGTCACCATGAAGCAGGCAT-3′; Sigma-Aldrich). The mutant reaction

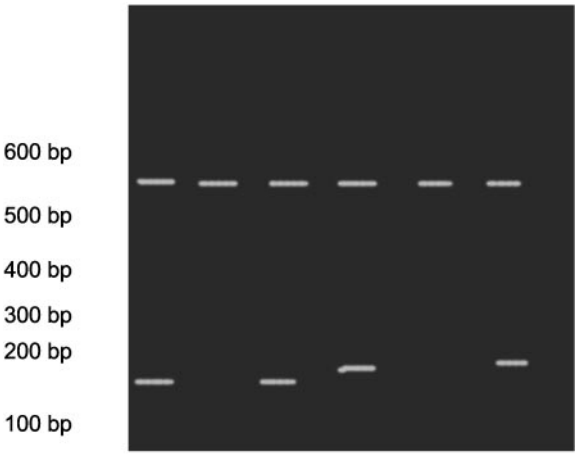

Fig. 1. ARMS PCR for *MTHFR* A1298C.

Reactions are shown in pairs. Products of the wild-type reaction are analyzed in the first lane of each pair, products of mutant reaction are analyzed in the second lane of each pair. Each pair contains the CF22 internal control, which generates a 578-bp product. *Lane 0*, 100-bp DNA ladder (Invitrogen, Bio-Sciences); *lanes 1* and *2*, presence of the 120-bp product in *lane 1* indicates wild type for the mutation; *lanes 3* and *4*, presence of 120-bp product in *lane 3* and 127-bp product in *lane 4* indicate heterozygous carrier of the mutation; *lanes 5* and *6*, presence of the 127-bp product in lane 6 indicates a homozygous carrier of the mutation.

had the same components as above, with the replacement of A1298C wild-type reverse primer with A1298C mutant reverse primer, which had the following sequence: 5′-GGTAAAGAACAAAGACTTCA-AAGACACTGTG-3′ (Sigma-Aldrich).

ARMS PCR conditions were 95 °C for 5 min; 40 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s; and 72 °C for 5 min. The wild-type reaction produces a 120-bp product, whereas the mutant reaction produces a 127-bp fragment (Fig. 1). The CF22 primers were used as internal control primers and produce a 578-bp fragment in each reaction. Statistical analysis was performed using the *Z*-test for two independent proportions. Statistical significance was set at $Z > 1.96$.

Of the 120 healthy Irish participants, 56 were heterozygous carriers, giving a genotype frequency of 46.7%, whereas 11 (14.2%) were homozygous for A1298C. Of the *MTHFR* C677T/A1298C genotype combinations, 28 participants (23.3%) were double heterozygotes. The prevalence of 1298CC homozygotes in this Irish study is significantly higher than that reported for most European populations, including a UK study ($Z = 1.97$) *(7)*, an Italian cohort ($Z = 2.51$) *(8)*, and an American study ($Z = 1.99$) *(9)*. Only two studies from Northern Scotland *(10, 11)* have reported a higher prevalence.

The results of the ARMS PCR were in complete concordance with the results obtained by standard PCR-RFLP. The ability of the ARMS PCR to distinguish between genotypes for the A1298C mutation is much higher than that of the standard method.

We thank Dr. A Davern, Dublin Blood Transfusion Centre, for the blood samples. We would like to also thank Drs. Valeria Capra (Laboratorio del Servizio di Neurochirugia, Instituto Scientifico G. Gaslin, Genova, Italy) and Nurit Rosenberg (Department of Hematology, Institute of Thrombosis and Hemostasis, The Chaim Sheba Medical Center, Tel-Hashomer, Israel) for the A1298C positive controls.

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Interference in Measurement of Potassium Caused by Bacterial Contamination of an Analyzer

To the Editor:

We encountered a problem with potassium measurement after periods of standby on a Beckman Synchron LX20 Pro. Initial potassium measurements were rejected because of excessive reference drift, and potassium results for quality-control (QC) samples were as much as 2.5 mmol/L above the previous mean. Replicate analyses of control material immediately after standby revealed a decreasing trend in potassium concentration, whereas simultaneously