imprecision, although it is impossible to exclude a slight downward assay drift of 0.1% over the 56-week study period.

In conclusion, whole blood stored at −70 °C for 1 year is stable for later measurement of HbA1c by HPLC in individuals without diabetes. This eliminates the need to analyze fresh blood in epidemiology and potentially could permit HbA1c to be measured in existing cohorts with stored whole blood.

References


Additional Data for Oligonucleotide Arrays of the p53 Gene in DNA from Formalin-Fixed, Paraffin-Embedded Tissue

To the Editor:

The report published by Cooper et al. (1) motivated us to report our experience with the Affymetrix® GeneChip™ (Chip). In its current state, we agree that the Chip cannot be used as a stand-alone test for mutational analysis of the p53 gene. However, it is still a sensitive tool for reducing the need for full-length sequencing. Validation studies in our laboratory have demonstrated that the Chip can detect most mutations in as few as 5% of a cell population; other claims run to as low as 2%.

The limitations of the Chip include a failure to detect insertions, large deletions, and >6 bp of intronic sequence in any direction. Some limitations are attributable to sequence tiling (2). Insertions and large deletions were not tiled at all. As for the intronic sequences, only the splicing junctions were considered to be critical, thus limiting the utility of the Chip.

Failure to detect some missense mutations appears to be attributable to the quality of the DNA and the number of times a sequence has been tiled on the Chip. As noted by the authors (1), DNA recovery from paraffin-embedded tissue (PET) is dependent on many variables, including the age of the block, fixation, and isolation conditions. The quantity of tumor tissue present in the section can impact the ability to detect a mutation, particularly if the tissue is predominantly healthy, nondiseased stroma. Staining of tissue samples before isolation introduces inhibitors of the amplification process. To optimize detection, we suggest manual microdissection using a stained slide as a guide for dissecting the unstained slides. This process improves the ratio of tumor tissue in the isolation; a minimum of 5–10 μm of unstained sections should provide sufficient DNA for analysis.

The focus of the report, however, was to describe the performance of the Chip on PET samples. Our experience has been that poor performance for exon 4 and the lack of polymorphism detection can be explained predominantly by issues concerning DNA recovery from PET. In many cases, the Chip could not interrogate exon 4 because of amplification failure (~32%). This amplification product is the largest (366 bp) in the multiplex, and given the compromised template, poor results are not unexpected. Alternative amplification conditions and/or exon-specific amplification can be used to overcome sample quality issues.

The investigators (1) also noted that the Chip lacks the ability to detect polymorphisms in PET. From a sampling of data collected over the past 2 years in our laboratory, 62 cancer samples were identified as originating from PET. Of the 62 samples, 34% (21 cases) were identified with a polymorphism in exon 4, 6, 7, or 8. The scoring assigned to these polymorphisms was consistently a “5” or a “6” and was confirmed by sequencing in most instances. The Chip identified one of six R72P polymorphisms in PET.

Lastly, we would like to address the application of a general algorithm for scoring cutoffs. Our primary cutoffs are set at 15 for the determination of mutation status; these are based on validation studies against sequencing, the “gold standard”. Of the 62 samples that we analyzed using both Chip and sequencing, 37 mutations (missense, nonsense, or splice site) were detected by Chip, and 23 of these were detected by both methods. Six samples were determined to be wild type by both methods. Additionally, indications of other mutations were present in 16 samples. Of these, 44% (7) contained signals that were above the local background but were below the general cutoff scores of both methods. Our group and others have produced data that support the use of specific interpretive scoring of Chip positions to maximize interpretive accuracy.

In conclusion, we believe that the limitations noted by Cooper et al. (1) are related to interpretive as well as
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. Such improvements could lead to a more useful and productive tool in cancer research and diagnostics.

References


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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 in sequencing of the p53 gene.

Reference


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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 MgCl2, 0.4 mM each deoxynucleotide triphosphate (Invitrogen, Bio-Sciences), 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′-CCCTTTGGGAGCTGAAGGACTACTAC-3′); A1298C wild-type reverse primer (5′-CAAGAC-TCTAAAGACAGTC-3′); cystic fibrosis 22 (CF22) forward primer (5′-AAAAGCTGACACCCAAGA-3′), and CF22 reverse primer (5′-TGTACCAGTGAACAGGACAT-3′; Sigma-Aldrich). The mutant reaction