a 21-year-old male, in an unrelated family yielded identically abnormal results in the two-dimensional electrophoresis procedure. We also noticed a marked diminution of PLS:23. Both parents (obligate carriers) in this family were studied and found to have normal serum protein 2DE patterns.


References

Harold H. Harrison
Kathy L. Miller
Dept. of Pathol., MC 1105
Univ. of Chicago Hosp. and Pritzker Sch. of Med.
3841 S. Maryland Ave.
Chicago, IL 60637

Madeleine D. Harbison
Alfred E. Slonim
Dept. of Pediatrics
525 E. 68th St.
New York, NY 10021

Pittfalls of Restriction Endonuclease Digestion for Direct Detection of Point Mutations

To the Editor:

The necessity of heeding subtle anomalies frequently encountered upon electrophoretic analyses of restriction endonuclease digestion products of genomic and polymerase chain reaction (PCR)-generated DNA templates became obvious to us recently. We wish to alert clinicians and molecular pathologists to such cues because, without such vigilance, we would have drawn an erroneous conclusion as to the cause of one patient's genetic impairment.

While routinely mapping point mutations in exon 8 of the arginine locus of a collection of hyperargininemic patients, we discovered a single base change immediately outside a restriction enzyme recognition site (TaqI), which severely inhibited cleavage of this site (1).

The consistent and reproducible results of both standard Southern mapings and endonuclease digestions of PCR-generated DNA templates of exon 8 had predicted that a point mutation, present in heterozygosity, was located directly within a unique TaqI site of exon 8 of patient 1 (Figure 1). Because of a minor aberration we noticed in the gels used to analyze the digestion products (Figure 1B), we proceeded to sequence this PCR product. We would not have done so if we had not noticed that the upper "uncut" band looked slightly less intense than the lower "cut" band of patient 1. The results of this gel suggested that this allele from patient 1 had been partially digested by TaqI. The anomaly resulted because the point mutation was actually in a base adjacent to the tetranucleotide recognition site, not directly within it. This flanking base inhibited cleavage by TaqI by ~80% on the mutated chromosome of patient 1, resulting in bands that were of unequal intensity by ethidium bromide staining.

Factors affecting the efficiency of cleavage of DNA templates by restriction endonucleases have been addressed (7-11). However, this is the first instance of inhibition of cleavage of genomic and PCR-generated DNA templates (the latter of which rules out methylation as a cause of inhibition of cleavage) and in the clinical setting of the diagnosis of a human genetic disorder.

Publications in this and other journals tout direct DNA analysis for direct detection of point mutations and polymorphisms (7-11). Generally, these methods are inexpensive, precise, simple, rapid, and reliable for diagnostic characterization of heritable diseases (7-9). Additionally, these methods yield far more information about genotype than do methods that rely on protein analyses, such as alloelectric focusing (as for apolipoprotein E polymorphism), or microvillar enzyme analysis for the prenatal diagnosis of genetic diseases such as cystic fibrosis (8, 9). The advantages of the detection of rare mutant alleles via direct DNA analysis over detection with site-specific monoclonal antibodies are well documented, particularly for the diagnosis and prognosis of alpha-1 antitrypsin deficiency (10).

Indeed, the precision of genotypic characterization allows more accurate predictions of the clinical outcome of many inherited diseases that are caused by single base changes in specific loci (12, 13). For example, in the diagnosis of cystic fibrosis, prenatal and pediatric genotype characterization has begun to predict whether patients...
creatic enzymes and special nutritional support will be required. Thus, parents may anticipate what will be required in medical intervention and lifestyle for their child (observation by authors).

However, in addition to the instance we reported (1), erroneous genotypic diagnoses were reported for two other cases involving direct DNA analysis (8). A rare variant of the apolipoprotein E gene was resolved only by digesting the PCR-amplified DNA with a second enzyme to cleave a point mutation that had been missed by the first restriction enzyme (9). Those authors would not have used a second restriction enzyme except that use of isoelectric focusing predicted this unusual variant genotype. Horn et al. (11) pointed out the limitations of using direct digestion of amplified DNA with restriction enzymes. Unless appropriate control sites of defined nucleotide sequence and of comparable template length are included, both as external digestion controls and added directly to the specimen, the investigator would not know whether the lack of digestion of the amplified DNA resulted from the absence of the polymorphic site or from a failure of the enzyme to cleave. Also, the results of an incomplete digestion of a homogenous sample can look the same as a complete digestion of a heterozygous sample. Apropos of our finding (1), the effect of a change in a flanking base would be operative in all of the latter examples.

We recommend as a means of resolving questionable endonuclease digestion results demonstrated on analytical gels the routine inclusion of commercial controls (e.g., Custom Cuts®; BioVentures Inc., Murfreesboro, TN) or homemade templates at least 200 bp (base pairs) long that contain the appropriate enzyme sites. We do not recommend the inclusion of biotinylated nucleotides (e.g., ~20 bp) because the efficiency of cleavage differs for short and longer templates (1). The routine inclusion of such control templates in analyses of frequently occurring mutations (e.g., cystic fibrosis) of amplified products offers significant advantages over direct sequencing of PCR-generated templates in cost efficiency, simplicity, and time. If the precise location of an anomalous base relative to the restriction site is required, direct sequencing of the template must be done. For less frequent issues (e.g., hyperargininemia), direct sequencing of ambiguous digestion results of PCR products may be the more efficient route.

In conclusion, in most instances, direct DNA analysis provides clinicians with advantageous methods for the precise, complete, and rapid genotypic characterization of point mutations that cause inherited diseases. Caveat emporer: these analyses should be performed while remaining alert to anomalies in analytical gels, which can signal incorrect conclusions.

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References
Increases in CA-125 Concentrations in Children

To the Editor:

I have read the letter by Schöna et al. (1) about the increase of serum concentrations of CA-125 in children with different diseases. The authors were surprised by the finding of increased concentrations in 23 of the 250 children and speculated about several mechanisms that could be involved in such an increase, specifically, hormonal, iatrogenic, and liver dysfunction mechanisms. However, they failed to mention the mechanism that could explain most, if not all, of the abnormal values: the serous effusions.

Schöna et al. (1) observed the greatest percentage of increased CA-125 concentrations in the group of children with heart diseases: 16 of the 23 patients with above-normal CA-125 had severe heart failure. These patients probably had associated pleural, peritoneal, and (or) pericardial effusions, which are known to be associated with increased concentrations of CA-125 (2–5). We have found abnormal concentrations of CA-125 in 98.4% of the patients with benign liver disease who have ascites; the CA-125 values were very high in many cases, and the volume of the ascitic fluid was significantly correlated with the CA-125 concentrations in serum (6). CA-125 has been found immunohistochemically on mesothelial cells in the fetal and adult pleura, the pericardium, and the peritoneum (7), which accounts for the increased values seen in patients with diseases affecting these tissues.

Also, some of the children studied by Schöna et al. had undergone heart surgery. Peritoneal trauma after abdominal surgery has been found to be responsible for increases in CA-125 in serum, with maximum values occurring about 2–4 h after the operation and above-normal concentrations persisting for as long as three months (8). Similarly, surgical trauma to pericardium and mediastinal structures, and the accompanying effusion, could be responsible for the increase of CA-125 in the children who underwent heart surgery.

The remaining seven patients reported by Schöna et al. to have increased concentrations of CA-125 include patients with liver cirrhosis (probably three cases), portal vein thrombosis with esophageal bleeding (portal hypertension), and a ventriculo-peritoneal shunt. All of these diseases have in common the existence of peritoneal fluid and thus could explain the abnormal concentrations of CA-125.

Serous effusions are a very frequent cause of increased concentrations of CA-125 in serum and probably account for the abnormal values observed by Schöna et al. (1) in children.

References

Julio Collazos
Section of Intern. Med. Hospital de Galdacano 49860 Vizcaya, Spain

An author of the letter cited responds:

Like Collazos, we also thought that the increased concentrations of CA-125 in children might be explained by serous effusions. However, we were not able to give definitive data because our Letter was reporting a retrospective analysis and sometimes roentgenograms were missing. What we nevertheless know definitively, from the ultrasonic examinations of the patients’ hearts, was that none of the children had pericardial effusions. Furthermore, in 10 of the 16 children with heart diseases, the chest roentgenogram excluded pleural effusions.

The CA-125 concentrations in those children with severe heart failure who underwent operations were always measured in samples obtained before surgery. In the remaining children with various diseases, the possibility (serous effusion) could not be ruled out.

In summary, we think that the increase of CA-125 in children, especially in those with severe heart failure, was probably not caused by serous effusions and should therefore be further investigated.

W. Jäger
Dept. of Obstet. and Gynecol. Univ. of Erlangen Universitätstr. 21 8520 Erlangen, FRG