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LETTERS TO THE EDITOR

Whose Small Bowel Biopsy Is This? Aids from Molecular Biology: Use of Molecular Techniques for Mismatched Specimen Identification

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Accurate and correct identification is of paramount importance when processing biopsies in pathology laboratories. Although extreme care is dedicated to reducing human error in this process, such error is sometimes unavoidable and, occasionally, mislabeling occurs. This may result in harm to the patient not only due to errors in diagnosis but also to the resulting treatment, which may be inappropriate. We report here an episode of mismatched small bowel biopsies that happened in our laboratory, and the way in which it was solved through PCR technique investigation. The procedure allowed a rapid and accurate identification of the samples, avoiding any harm to the patients involved.

The first patient was a 2-year-old boy who presented with chronic diarrhea (which he had had since 6 months of age), undernourishment (11.7 kg), and abdominal swelling. There were no familial data of celiac disease. The mother noted that when she stopped feeding the child with flour-containing foods for 1 wk, improvement in the stools resulted. The child's diet had included wheat flour since the age of 5 months.

The second patient was a 5-year-old boy who presented with lower abdominal distention and weight in the 50th percentile (16,250 kg). Serology for endomisial antibodies was positive.

Both patients were submitted to peroral small bowel biopsy for villous atrophy, which at this age represents celiac disease in the vast majority of the cases. Samples were received at the pathology laboratory the same day. Mistakenly, the samples were given the same identification number. The problem arose at the time of microscopic reading, since both slides with the same number presented different microscopic features. One showed complete villous atrophy (villous/crypt ratio: < 0.5, or enteropathy grade 4), while the other had a normal villous crypt ratio (> 2.5).

METHODS

To adequately identify the two biopsies we used PCR-based DNA sequence amplification for microsatellite typing. Samples submitted for this procedure included the abovementioned biopsies and a blood sample of one of the patients. Since both biopsies were obtained from only two children, and the aim of the search was to identify each child's biopsy, we used only three microsatelites to establish the identity of the one with data from the blood sample. Selection of microsatellites was absolutely random.

Procedure

DNA extraction

The blood sample DNA extraction was performed with Chelex 100® resin, following the manufacturer's protocol. The paraffin-embedded tissue DNA extraction was performed on 10-mm-thick sections, which were sliced from each sample (equivalent to 5 to 10 mg of tissue). These were placed in a 1.5-ml microfuge tube with sterile toothpicks. The microtome and blade were carefully cleaned with xylene between each block to prevent sample-to-sample contamination and PCR carryover [1–3]. Deparaffinization of sections was performed with the procedure of Shibata et al. [4], as modified by Wright and Manos [1]. Briefly, each section was extracted twice with xylene, followed by two 100% ethanol rinses. Tis-

Table 1. Allelic variants investigated

Locus	Blood M1	Biopsy B2	Biopsy B3
D16S539	9–10	10–11	9–10
D7S820	10–13	7–10	10–13
D13S317	8–12	10–11	8–12

sues were pelleted between each extraction and dried in a 55°C heat block. Tissues were resuspended in 100–500 ml digestion buffer (100 mM Tris-HCl, ph 8; 1 mmol/liter EDTA, ph 8; 0.5% Tween 20; 0.1% Triton X-100) and proteinase K in 100 mg/ml. Samples were incubated for 3 h at 56°C. The proteinase K was heat inactivated at 95°C for 10 min and frozen at −20°C.

Microsatellite typing

Microsatellites D16S539, D7S820, and D13S317 (STR III multiplex Gene Print TM®, Promega) were amplified according to the manufacturer's protocol. The amplification products were denatured at 95°C for 5 min. Samples were run on a 4% acrylamide gel and urea for 2 h at 2000 V, stained with silver nitrate according to the DNA Silver Staining System Promega instructions, and examined under UV light for detection of the allelic variances. Lane M1 was the blood sample of one of the patients, while lanes B2 and B3 represented the small bowel biopsies of each patient.

RESULTS

The results are presented in Table 1 and Figure 1. The electrophoresis panel shows that the blood sample band (M1) was homologous to that of sample B3. Since identity was complete, there was no need to perform further studies with more microsatellites.

DISCUSSION

Molecular biology techniques enable recognition of genetic disease, chromosomal aberrations (translocations, mutations), and infectious agents, as well as human identification. The latter permits identification of specimens in cases in which the origin of the sample is in doubt. The literature contains several examples showing how the problem has been tackled. Lam et al. [5] reported on a case in which an esophageal biopsy contained a small fragment of tissue with epithelial dysplasia. Subsequent samples did not show such a finding. Because of the discrepancy between the histologic and clinical/endoscopic data, a mixture of tissue fragments from different patients was suspected. PCR microsatellite analysis of the normal and dysplastic epithelium and of subsequent biopsies showed that the tissues featuring dysplasia were not from the same patient. Giroti and

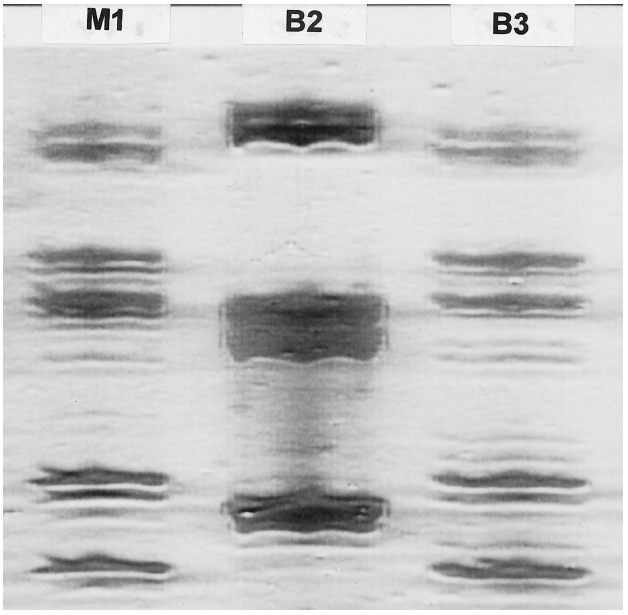

Figure 1. Electrophoresis panel showing homology between the blood sample band and that of sample B3.

Kashyap [6] confirmed the biopsy origin of a patient with breast carcinoma diagnosis who claimed the possibility of an error in identification of the specimen by the laboratory. In this case, they compared material of the histologic slide with that of the paraffin bloc and a blood sample of the patient. Through PCR for HLA-DQA1, they showed that the specimen was indeed from the patient. Shibata et al. [7] were confronted with the problem of having the same identification number for a lymph node containing metastatic breast carcinoma and a muscle biopsy. At the same time, a lymph node of the mastectomy specimen was missing. PCR amplification of genes for HLA DQ and low-density lipoprotein receptor allowed adequate identification, indicating that the lymph node belonged to the patient with the mastectomy.

Worsham et al. [8] solved a problem of mixed tissues from the specimens of two patients. One was from a man with a gastric biopsy showing an inflammatory process plus a small fragment of a poorly differentiated adenocarcinoma. Endoscopy findings were negative for the presence of a malignant lesion. In the same group of biopsies there was a sample of stomach from a woman whose endoscopic results suggested a malignant ulcer, which showed a poorly differentiated adenocarcinoma. Through FISH for chromosomes X and Y, the authors demonstrated that there had been a contamination of the specimen from the patient without cancer. The same authors solved a similar problem using PCR for microsatellites in prostate biopsies of two patients.

The conflict created with our two cases was solved through the use of microsatellite analysis. Microsatellites are short bases of DNA sequences (usually dinucleotides) repeated a variable number of times and ar-

ranged in fixed positions within each chromosome; they follow a mendelian inheritance. Since microsatellites are polymorphic, they are very useful as markers because the number of repeated sequences is highly variable among nonrelated individuals [9].

In summary, PCR for microsatellites accurately defined the small bowel biopsies we were dealing with. This resulted in accurate diagnosis and treatment: the patient with total (grade 4 enteropathy) villous atrophy was put on a gluten-free diet while the other was not.

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REFERENCES

1. Wright DK, Manos MM. Samples preparation from paraffin-embedded tissues. In: Innis MA, Gelfand DH, Snisky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. San Diego, CA: Academic Press, 1990;153–158.
2. Kwok S. Procedures to minimize PCR-product carryover. In: Innis MA, Gelfand DH, Snisky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. San Diego, CA: Academic Press, 1990;142–145.
3. Kwok S, Higuchi R. Avoiding false positives with PCR. Nature 1989;339:237–238.
4. Shibata DK, Arnheim N, Martin WJ. Detection of human papilloma virus in paraffin-embedded tissue using the polymerase chain reaction. J Exp Med 1988;167:225–230.
5. Lam KH, Mooi WJ, Sleddens HFBM, Dinjens WNM. On tiny tissue samples, common sense, and molecular pathology. Hum Pathol 2000;31:1006.
6. Giroti R, Kashyap VK. Detection of the source of mislabeled biopsy tissue paraffin block and histopathological section on glass slide. Diagn Mol Pathol 1999;7:331–334.
7. Shibata D, Namiki T, Higuchi R. Identification of a mislabeled fixed specimen by DNA analysis. Am J Surg Pathol 1990;14:1076–1078.
8. Worsham MJ, Wolman SR, Zarbo RJ. Molecular approaches to identification of tissue contamination in surgical pathology sections. J Mol Diagn 2001;3:11–15.
9. Solari AJ. Ligamiento. Genes ligados al sexo y fenómenos de compensación de dosis. Genética Humana. Fundamentos y Aplicaciones en Medicina. Buenos Aires: Editorial Médica Panamericana, 1999;149–173.

Idiopathic Arterial Calcification Presenting with Cardiac Failure and Sudden Death in an 11-Year-Old Girl

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An 11-year-old girl presented with breathlessness, pleural effusion, and ascites. Direct questioning revealed that she had started to become unwell about 2 years previously with repeated syncopal episodes and gradually decreasing exercise tolerance. There was nothing else of note in her medical history and there was no family history of cardiac disease. On admission, she was in clinical cardiac failure and an echocardiogram demonstrated a dilated left ventricle with poor function but no structural abnormalities. Cardiac catheterization indicated raised left ventricular end diastolic pressure with low transpulmonary gradient. With a working diagnosis of dilated cardiomyopathy, she was managed with dobutamine infusion and placed on the waiting list for cardiac transplantation; however, 2 weeks later she collapsed with a cardiac arrest. Resuscitation was carried out and cardiac output restored, but she remained hypotensive and required intensive dobutamine support for several days until she died following a further cardiac arrest.

A limited postmortem examination (heart only at parental request) was carried out. The heart weighed 249 g (>97.5th centile for age and sex, 193 g), but was otherwise externally unremarkable. The pericardium, pulmonary, and systemic venous drainage was normal. The left ventricle was dilated (6 cm maximum diameter) and the free left ventricular wall showed extensive areas of acute hemorrhagic infarction and further pale areas consistent with fibrosis. The valvular apparatus was macroscopically normal. The coronary arteries showed a normal anatomical pattern but were firm to palpation. On sectioning, the major branches of the coronary arteries were patent with no significant macroscopically apparent luminal narrowing but were difficult to cut, consistent with calcification. Microscopic examination confirmed focal acute infarction of the left ventricular