TECHNICAL NOTE

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Fixed Human Tissues: A Resource for the Identification of Individuals

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ABSTRACT: Polymorphic genetic loci of the deoxyribonucleic acid (DNA) present in formalin-fixed, paraffin-embedded tissues were successfully analyzed by utilizing the polymerase chain reaction. Using this analysis, with three different polymorphic loci [human leucocyte antigen (HLA) DQ alpha, low-density lipoprotein receptor, and parathyroid hormone], fixed tissues representing 14 different individuals were genotyped and could be distinguished from each other. The techniques were further applied to the fixed autopsy tissues of a man in which a question of paternity arose postmortem. Since many individuals have surgical procedures or autopsy, these readily available fixed tissues represent an additional resource for the identification of individuals.

KEYWORDS: pathology and biology, tissues (biology), human identification, genetic typing, polymerase chain reaction, forensic identification, paraffin-embedded tissue, human leucocyte antigen, paternity testing, deoxyribonucleic acid (DNA) typing

Individuals differ in their genetic compositions. Recent advances in technology have allowed the identification of individuals based on these differences between their deoxyribonucleic acid (DNA) sequences. These methods detect polymorphisms between restriction fragment lengths (RFLP) or the numbers of tandem repeats (VNTR) which are variable between individuals [*1–3*]. The autoradiographic patterns produced after such analysis has been commonly called "DNA fingerprints." Applications of these powerful techniques have been accepted as evidence in many legal settings [*4–6*].

Unfortunately, these fingerprint techniques require large amounts of relatively intact DNA for analysis. An alternative technique which can analyze smaller amounts of de-

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graded DNA is the polymerase chain reaction (PCR) [7,8]. The PCR can amplify and detect polymorphisms from small specimens such as single hairs [9] or sperm [10]. In addition, DNA from very old specimens, such as mummies, has been analyzed [11,12].

The DNA present in formalin-fixed, paraffin-embedded tissues can similarly be analyzed by PCR techniques [13–15]. This DNA is usually degraded in size and therefore unsuitable for conventional DNA fingerprints. Although fresh specimens taken directly from an individual are probably optimal for genotypic analysis, in isolated cases this may not be possible as the individual may be missing or dead. Some scenarios with this problem are unidentified bodies or body fragments, and paternity cases involving deceased parents.

In these instances, genotyping might still be possible if the individual received biopsy, surgery, or autopsy. The fixed tissues from such procedures are commonly stored in hospitals for many years, and great effort is made to ensure that such tissues are matched to the correct patient. In this study we demonstrate that individuals can be genotyped after amplification of their fixed tissues by the PCR. In addition, we illustrate the application of this technique with a paternity case involving a deceased father.

Materials and Methods

Tissue Samples

The fixed tissues from 14 unrelated individuals from the Los Angeles County and University of Southern California Medical Center from 1988 to 1989 were selected for analysis. All selected individuals had biopsy or surgical specimens and autopsy tissues available.

Polymerase Chain Reaction

DNA was extracted [14] from single 10-μm slices from each of the 10% formalin-fixed, paraffin-embedded specimens. This extracted DNA was amplified by the PCR [7] with a thermal stable DNA polymerase (AmpliTaq, Perkin Elmer Cetus, Norwalk, Connecticut) with primers specific for the human leucocyte antigen (HLA) DQ alpha region [16–18] for 34 cycles. The materials and methods used were essentially identical to a now commercially available kit (AmpliType, Cetus Corp., Emeryville, California). The amplification mixture contained biotinylated primers, such that the 242 base pair HLA product was biotin labeled [17]. The amplification products were hybridized and washed against allelic specific HLA oligomer probes which were immobilized on a nylon filter. The hybridized HLA products were visualized using a streptavidin-horseradish peroxidase conjugate and 3,3′,5,5′-tetramethylbenzidine (TMB) chromagen. The colored reaction products were photographed using a Polaroid camera.

The low-density lipoprotein receptor (LDLr) and the parathyroid hormone (PTH) were also amplified and analyzed as previously described (except 36 PCR cycles were used) [10,19]. Autoradiography of the dot blot after hybridization to the ^{32}P-labeled allelic specific oligomer probes was used to detect the alleles present.

Case Report

A 46-year-old man was killed in an accident; an autopsy was performed. Afterwards, in the estate settlement, his girlfriend claimed that the man was the father of her seven-year-old daughter. Since no other tissues were available except for the fixed tissues routinely obtained at autopsy, PCR analysis was performed for the analysis of HLA DQ alpha, LDLr, and PTH polymorphisms. A similar analysis was performed on buccal cells [20] obtained from the girlfriend and her daughter.

TABLE 1—*Autopsy and biopsy findings of 14 individuals.*

Individual	Age, Years	Sex	Race[a]	A or B[b]	Interval,[c] days	Major Diagnosis	Tissues Analyzed for DNA typing	Postmortem, h
1	61	M	H	A	10	lung, carcinoma	esophagus, prostate	30
				B		carcinoma	lung	
2	48	F	C	A	10	septic shock	pituitary, lymph nodes	66
				B		vasculitis	skin	
3	37	F	H	A	135	cerebrovascular accident	heart, thyroid, lymph nodes	46
				B		keratosis	bowel	
4	59	M	C	A	16	septic shock	colon, aorta	146
				B		necrosis	bowel	
5	5 months	F	B	A	10	AIDS	stomach, esophagus	17
				B		pneumocystis carinii pneumonia	lung	
6	46	F	H	A	140	ovary, carcinoma	pituitary, breast	85
				B		no significant lesion	appendix	
7	45	F	B	A	14	adult respiratory distress syndrome	thyroid	64
				B		ulcer	stomach	

8	75	F	C	A B	12	probable pneumonia trichilemmal cyst	parathyroid, pituitary scalp	87
9	23	F	H	A B	35	pneumonia intraepidermal pustule	larynx, colon skin	28
10	52	F	O	A B	240	hemorrhagic shock adenocarcinoma	intestine rectum	36
11	47	M	C	A B	60	bronchopneumonia no significant lesion	pancreas, pituitary colon	27
12	63	M	C	A B	23	coronary artery thrombosis no significant lesion	intestine, breast parathyroid prostate	96
13	58	M	H	A B	47	pancreas, carcinoma adenocarcinoma	pancreas pancreas	24
14	39	F	C	A B	20	lung, carcinoma adenocarcinoma	omentum, pancreas, breast pericardium	7

[a]B = black, C = caucasian, H = hispanic, and O = oriental.
[b]A is autopsy; B is biopsy.
[c]Interval between biopsy and autopsy.

Results

Paired-fixed premortem and postmortem specimens from 14 unrelated individuals were examined to validate the procedures. The DNA present in fixed biopsy and autopsy tissues from a variety of organs and postmortem intervals up to 146 h from the 14 individuals were analyzed successfully (Table 1). The HLA DQ alpha, PTH, and LDLr loci were amplified by the PCR, and the alleles were identified after hybridization with allelic specific oligomers (Table 2). In all 14 cases, the genotypes from the autopsy and biopsy tissues from each individual were concordant. The 14 individuals could not be completely distinguished from each other by the analysis of one single polymorphic locus. Only 4 individuals could be uniquely identified by their HLA DQ alpha locus, and none of the 14 individuals could be uniquely identified by their PTH or LDLr loci. However, the combined HLA, PTH, and LDLr genotypes were completely different among the 14 individuals.

The DNA extracted from buccal swabs from the mother and her daughter, and the fixed autopsy tissues of the suspected father were subjected to PCR analysis (Figs. 1 and 2, Table 3). The mother was HLA 1.1,1.1; PTH Aa; and LDLr 1,1. Her daughter was HLA 1.1,1.3; PTH AA; and LDLr 1,1. The suspected father was HLA 1.3,4; PTH Aa; and LDLr 1,1. This analysis did not exclude the suspected father, and based on the frequencies of these alleles in the general population, the probability of this occurring by chance was 11% (random man not excluded).

Discussion

In this study, we demonstrated that individuals can be genotyped from the DNA present in their formalin-fixed, paraffin-embedded tissues. The genetic composition of an individual is extremely stable, and as expected, tissues taken before death and at autopsy from an individual maintained concordant genotypes. Although somatic mutations can occur in neoplastic tissues, these changes tend to cluster in oncogenes or tumor suppressor genes, and no discrepancies were observed with five tumor tissues in this study. Nevertheless, it may be wise to avoid neoplastic tissues to prevent this remote but theoretical possibility.

TABLE 2—*HLA DQ alpha, low-density lipoprotein receptor, and parathyroid hormone typings of formalin-fixed, paraffin-embedded tissues.*

Individual	HLA-DQ alpha	PTH[a]	LDLr[b]
1	3,4	Aa	1,1
2	3,4	aa	1,2
3	1.1,4	AA	1,2
4	4,4	AA	2,2
5	1.2,2	aa	1,1
6	3,4	AA	1,2
7	1.2,4	AA	1,1
8	1.1,1.2	Aa	2,2
9	3,4	Aa	1,2
10	1.1,1.2	Aa	1,2
11	3,4	AA	1,1
12	4,4	Aa	1,2
13	4,4	Aa	2,2
14	1.3,4	Aa	1,2

[a]PTH = parathyroid hormone.
[b]LDLr = low-density lipoprotein receptor.

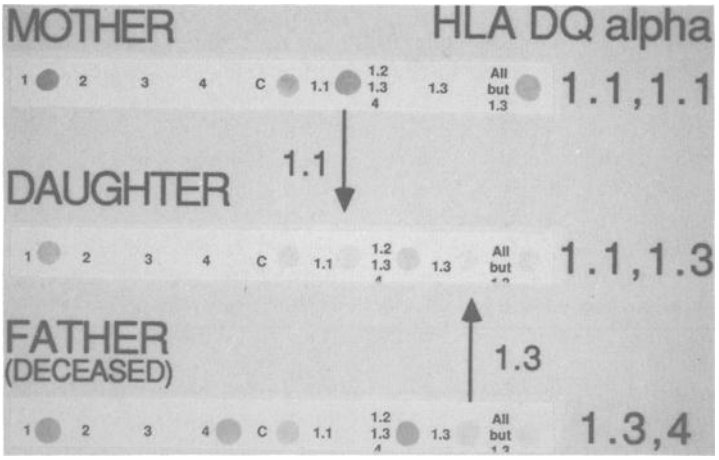

FIG. 1—*The HLA DQ alpha PCR analysis is shown. The dots represent colored dye produced after hybridization with the biotinylated PCR products. Based on the pattern of dots, the mother was 1.1,1.1, the daughter was 1.1,1.3, and the deceased putative father was 1.3,4. Paternity is not excluded as the daughter's 1.1 allele must have come from the mother, and the 1.3 allele may have come from the putative father. The color had faded from the daughter's 1.3 dot at the time of photography.*

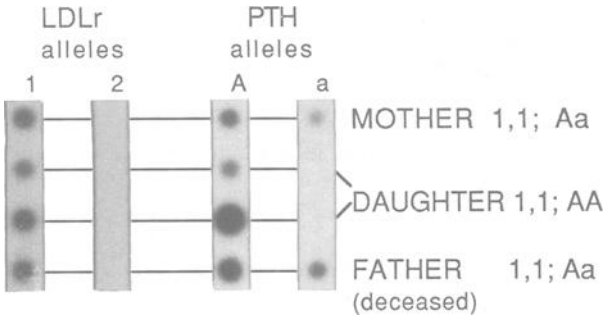

FIG. 2—*The low-density lipoprotein receptor (LDLr) and parathyroid hormone PCR results are shown. The dots represent the alleles after autoradiography. Based on the pattern of dots, the mother was 1,1;Aa, the daughter was 1,1;AA, and the deceased putative father was 1,1;Aa. Paternity is not excluded as the daughter's LDLr-1 and PTH-A alleles may have come from the putative father.*

TABLE 3—*Paternity case.*

Individual	LDLr[a]	PTH[b]	HLA DQ alpha
Mother	1,1	Aa	1.1,1.1
Daughter	1,1	AA	1.1,1.3
Deceased putative father	1,1	Aa	1.3,4

[a]LDLr = low-density lipoprotein receptor; two alleles, 1 or 2.
[b]PTH = parathyroid hormone; two alleles, A or a.

A greater source of discrepant findings may be the mislabeling of the fixed tissues. However, great care is routinely taken in the accurate labeling, recording, and storage of human specimens. The formalin-fixed, paraffin-embedded tissue blocks present in hospitals therefore represent a well-documented resource of human tissues. Since many individuals receive surgery, biopsy, or autopsy, these stored tissues may provide a source of genetic material for forensic analysis when an individual is not otherwise available. Common surgical procedures include tonsillectomy, appendectomy, cholecystectomy, hysterectomy, and endocervical biopsy.

The analysis of a highly polymorphic genetic locus is most useful for the purpose of identification. The HLA DQ alpha locus consists of 21 different genotypes which can be identified with the system of primers and probes utilized in this study. The primers replicate a 242 base pair HLA DQ alpha region, and differences between the alleles, consisting of differences in one or more base pairs, can be distinguished after sequence specific hybridization. The pattern of dots generated by the test allows classification of the individual to 1 of the 21 different HLA DQ alpha genotypes.

The ability of a test to distinguish between individuals depends on the frequency of the marker in the population. Although the HLA DQ alpha locus is highly polymorphic, its analysis alone, as in this study, may be insufficient to distinguish between large numbers of individuals. Fortunately, other polymorphic loci exist and can be analyzed by the same general method. In this study, in order to completely distinguish the 14 different individuals, two other polymorphic loci were studied. One locus, the low-density lipoprotein receptor (LDLr), has two alleles designated LDLr-1 and LDLr-2. The frequency of each allele is approximately 50% [*21*]. Using this analysis, individuals were classified as homozygous LDLr-1 or LDLr-2, or heterozygous. Similarly, a second locus, the parathyroid hormone (PTH), has two alleles designated PTH-A and PTH-a with respective haplotype frequencies of approximately 75 and 25%.[4] The 14 individuals in this study could be completely distinguished by genotyping at these three different unlinked polymorphic loci.

Similarly, other polymorphic loci could be analyzed by the PCR to obtain greater specificity. There are several limitations, however, of the use of fixed tissues for genetic analysis. Not all fixed tissues are suitable for amplification by the PCR, and a minority of fixed tissues fail to produce detectable PCR products [*22*]. The exact etiology for this failure is unknown, but it may involve differences in fixation. Fixatives other than 10% buffered formalin and fixation for longer than three days impairs amplification (Ref *23* and personal observation). In addition, longer sequences (greater than 600 base pairs) generally cannot be amplified [*15*].

To illustrate an application of these techniques, a paternity case involving a deceased putative father was examined. The question of paternity arose only after the suspected father was buried, and only the fixed tissues from his autopsy were available for analysis. The genotype of the individual, when compared with those of the mother and child, was consistent with paternity. Based on the frequencies of the alleles in the general population, the probability of a random man not excluded was 11%.

In summary, we have demonstrated that fixed tissues can be genotyped by the PCR. Numerous forensic applications of these techniques on fixed tissues can be envisioned since in many legal settings the individual in question may be missing or dead. Paraffin-embedded specimens represent a large resource of stored and cataloged human tissues. These widely available tissues, which in essence fix an individual in time and place, can extend the utility of genetic forensic analysis.

[4]Li, Honghua, personal communication.

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