Am J Hum Genet 36:1357–1368, 1984

DNA Analysis of First-Trimester Chorionic Villous Biopsies: Test for Maternal Contamination

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SUMMARY

We investigated the reliability of chorionic villous biopsy as a method to obtain tissues reflecting the genetic constitution of the embryo. In 12 pregnancies before elective termination, we searched for detectable maternal tissue after careful dissection of villi from small 2–5-mg specimens that yielded 7 μg of DNA per mg tissue. In Southern blotting experiments (1–2 μg DNA per lane), restriction fragment length polymorphisms (RFLPs) at an autosomal (*D14S1*) and a sex chromosomal (*DXYS1*) locus allowed recognition of maternally and embryonically derived alleles. Pure villi were obtained in six of the seven informative cases. One biopsy was not dissected satisfactorily; a mixture of embryonic and maternal DNA was found. Nonvillous tissues were mostly maternally derived in eight informative cases. Sex determination by molecular analysis (alleles at the *DXYS1* locus) agreed with the karyotypes of uncultured or cultured villi. In one continuing pregnancy, distinct RFLPs indicated maternal inheritance of the α-thalassemia 1 trait in a female embryo without detectable maternal contamination. Reliable prenatal diagnosis depends on the specimen's purity. Maternal contamination can be evaluated by DNA analyses.

INTRODUCTION

Chorionic villi obtained by transcervical biopsy in the first trimester of pregnancy have been reported to be suitable specimens for chromosomal [1–11], biochemical

Received May 11, 1984; revised June 22, 1984.

This work was supported in part by grants from the Muscular Dystrophy Association, the National Institutes of Health, and the March of Dimes Birth Defects Foundation.

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[1, 4, 5, 11, 12], and molecular [5, 13–17] diagnosis of affected offspring (for review, see [18]). The reliability of this technique is dependent on the purity of the villous tissue obtained. Because of the intimate anatomic relationship between chorionic villi and maternal decidua, both tissues are routinely sampled with this biopsy method. The maternal tissue is an unwanted contaminant and must be removed if accurate prenatal diagnosis of the embryo is to be accomplished. Attention has been drawn to possible maternal-cell contamination in cultured villi [4].

Here, we have analyzed the origin of the tissues in uncultured chorionic specimens. The presence of maternally derived tissue should be detectable by studies of genetic polymorphisms. Restriction enzyme site polymorphisms generate DNA fragments of variable lengths on Southern transfer analysis that are inherited as codominant alleles (RFLPs). We chose two independent RFLP loci because they are highly polymorphic and the alleles can be detected in small tissue samples. After careful dissection of the nonvillous tissues from the chorionic villi, we looked for the presence of remaining maternal DNA in chorionic villous samples in the informative cases where mother and embryo differed in one allele at one or both loci.

MATERIALS AND METHODS

Tissue Isolation

Chorionic villous biopsies were performed by transcervical aspiration under real-time ultrasound guidance on 12 women at 7–12 weeks of gestation immediately before elective termination [19]. One couple was referred for prenatal diagnosis of α^0-thalassemia. Both parents of different Asian ethnic backgrounds were found to be carriers of the α-thalassemia 1 trait after the birth of their first infant with hydrops fetalis. The chorionic villous biopsy was carried out at 10 weeks of gestation.

Specimens of 5–20 mg of wet wt. tissue were collected in syringes containing 5 ml of minimal essential medium (MEM). Two types of tissues were routinely found in the samples: the branching finger-like chorionic villi and smooth tissues surrounding the villi. Individual chorionic villi with several syncytial buds were selected for small size and tissue integrity, and were washed twice in MEM to remove blood and debris. Cervical mucous and decidua were carefully excised under an inverted microscope until the typical frond-like villi appeared clean of any surrounding tissue. As "nonvillous" specimens, we used the adherent tissues dissected off the chorionic villi and/or a mixture of villous and adherent tissue that could not be separately identified with certainty.

Approximately 2–5 mg of both types of tissues were used for direct DNA analysis. Remaining villous and embryonic tissues were processed for cytogenetic analysis. Whenever identified after abortion, we also collected embryonic tissue samples. To remove maternal blood, all samples were washed in TNE buffer (0.1 M sodium chloride, 0.025 M disodium EDTA, and 0.01 M of Tris-HCl adjusted to pH 8.0) and resuspended in a final volume of 0.45 ml of the same buffer.

Five-ml samples of maternal blood were collected in EDTA anticoagulant tubes; mononuclear cells were separated from whole blood on Ficoll gradients (Lymphocyte Separation Medium, Bionetics, Kensington, Md.) and resuspended in 1 ml of TNE buffer.

DNA Isolation

Total cellular DNA was isolated as described [20], with the following modifications. Tissue disruption and lysis of the nuclear membranes were achieved by incubating the

samples for 6–8 hrs at 45°C in the presence of 1% sodium dodecyl sulfate and Proteinase K (Boehringer-Mannheim, Indianapolis, Ind., final concentration of 1 mg/ml in samples containing intact tissues and 0.1 mg/ml in leukocyte samples). The crude nucleic acid solutions were extracted once with an equal volume of pH 8.0 Tris-HCl buffered, TNE-equilibrated redistilled phenol (Bethesda Research, Gaithersburg, Md.), once with a 1:1 vol of buffered-equilibrated phenol and chloroform (1:24 isoamylalcohol), and once with chloroform alone. After ethanol precipitation, the samples were treated with 0.2–0.4 mg/ml of RNase A (Sigma, St. Louis, Mo.), followed by a second Proteinase K incubation (0.1 mg/ml for 1 hr at 45°C). Phenol-chloroform extractions and ethanol precipitations were repeated. The DNA samples were then resuspended in 0.1–0.5 ml of TE buffer (0.01 M Tris-HCl, pH 7.4, 0.1 mM EDTA). This procedure generates high molecular weight DNA that can be digested to completion without further purification. From 2 to 5 mg tissue samples we achieved an average yield of 7 μg (2–10 μg) DNA per approximate mg of wet wt. tissue.

Endonuclease Cleavage, Southern Transfer, and Hybridization

DNA concentrations of the samples were determined using the DNA-binding fluorescent dye 4′-6-diamidino-2-phenyl-indole dihydrochloride hydrate (DAPI) [21]. Aliquots of 2–10 μg of DNA were digested with 2–4 U/μg DNA with restriction endonucleases *Eco*RI and *Taq*I (New England Biolabs, Beverly, Mass.) according to the manufacturer's recommendations. Cleavage to completion was monitored in parallel reactions using the bacteriophage λ or a pBR plasmid as DNA markers [22]. When necessary, incubations were continued with additional enzyme until a uniform spread of fragments by molecular weight was observed in test gels.

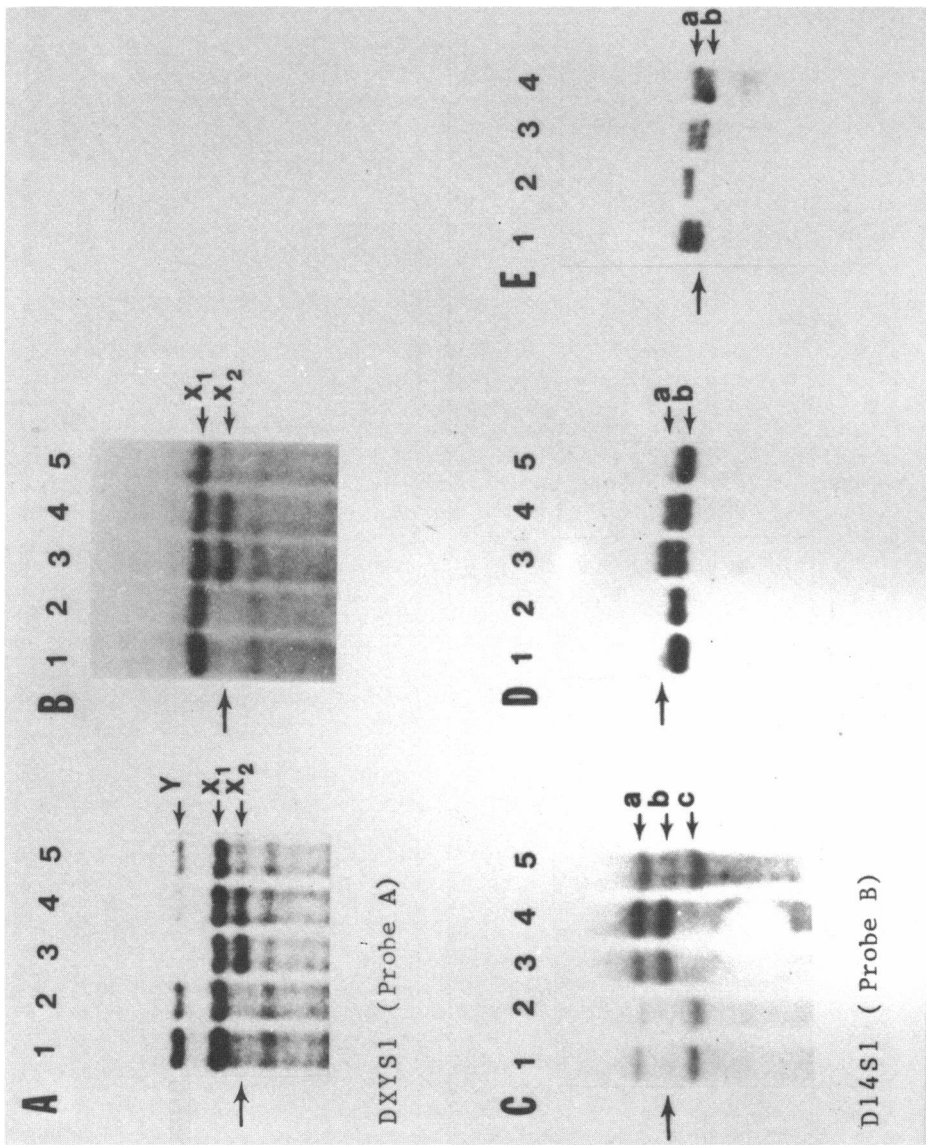
After electrophoresis in horizontal 0.5%–0.6% agarose gel in TAE buffer (0.04 M Tris-acetate, pH 7.8, 0.01 M sodium acetate, and 1 mM EDTA), uv-nicked, single-stranded DNA fragments were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, N.H., BA 85) by passive diffusion in 3 M sodium chloride, 0.3 M trisodium citrate.

Plasmid p*DP31* [23], probe A in this study, plasmid p*AW101* [22], probe B, and plasmids p*SV0α1* [24] and *4p7-7* [25] were ^{32}P-labeled by nick-translation [26] with the following modifications: 4 U of *E. coli* polymerase I (Kornberg polymerase grade I, Boehringer-Mannheim) per 200 ng of probe with 30–40 μCi of α[^{32}P]dCTP and α[^{32}P]dATP (3,000 Ci/mmol, Amersham, Arlington Heights, Ill.) in a 2-hr reaction at room temperature without addition of DNase.

Filters were preincubated for 6–20 hrs at 66°C–68°C in 0.75 M sodium chloride, 0.075 M trisodium citrate, 5 × Denhardt's solution, 0.05 M sodium phosphate buffer, pH 6.5, 0.2 mg/ml yeast RNA, 0.1 mg/ml sheared and denatured salmon sperm DNA, 1% glycine, and 1 mM EDTA. The hybridization buffer was supplemented with 7% dextran sulfate (Pharmacia, Piscataway, N.J.) and 2–5 ng of radiolabeled probe per ml of solution, and hybridization was allowed to proceed for 14–24 hrs at 66°C–68°C. Filters were subsequently washed at increasing stringencies with a final wash in 0.015 M sodium chloride, 0.0015 M trisodium citrate at 68°C, and exposed at −70°C on XAR-5 films with intensifying screens. With this procedure, single-copy sequences can be detected after autoradiography for 18–30 hrs using less than 0.5 μg of genomic DNA per sample.

Chromosome Preparation

Uncultured villi from the original biopsy samples were directly karyotyped by a method to be reported separately. An average of 15 metaphase cells were analyzed in each case. In addition, 2–10 metaphase cells from cultures of embryonic tissues, whenever available, and/or cultures of chorionic villous mesenchymal cores were analyzed to confirm the karyotypes [27].


A 1 2 3 4 5
Y
X1
X2
DXYS1 (Probe A)
B 1 2 3 4 5
X1
X2
C 1 2 3 4 5
a
b
c
D14S1 (Probe B)
D 1 2 3 4 5
a
b
E 1 2 3 4
a
b

RESULTS

Identification of Informative Cases

In order to identify mother-embryo pairs that differed in restriction fragment length alleles, DNA was extracted from embryonic tissues obtained after abortion and from maternal blood. Embryonic tissues could be identified only in seven cases because of the early stage of some of the pregnancies. Maternal blood was available in all but one case. For this initial screening, we prepared nitrocellulose filters with 1–2 μg of *Eco*RI- and *Taq*I-cleaved DNA samples using the Southern blot methodology adapted to small samples described above.

Two cloned unique human DNA sequences of unknown function that detect restriction fragment length polymorphisms (RFLPs) served as molecular probes. Probe A recognizes homologous sequences on the human X and Y chromosomes at the *DXYS1* locus [23]. After cleavage with *Taq*I, male DNA (for example, the male embryo shown in fig. 1*A*, lane 2) will always produce a 14.6-kilobase pair (Kbp) Y-specific fragment (*Y*) in addition to one of the two possible X-specific allelic fragments: 11.8 Kbp (X_1) or 10.6 Kbp (X_2). The presence of maternal tissues in villous specimens can be detected only if the mother is heterozygous for the X-specific alleles X_1 and X_2 (as in fig. 1*A* and *B*, lanes 3). The informative maternal allele, that is, the fragment that has not been transmitted to the embryo (fragments X_2 in fig. 1*A* and *B*), can be detected if the embryo is 46,XY male (as shown in fig. 1*A*, lane 2) or homozygous female (as shown in fig. 1*B*, lane 2). In four of the 12 cases, the alleles of the *DXYS1* locus were informative.

Alleles at the highly polymorphic autosomal locus *D14S1* on chromosome 14 [20] were detected in *Eco*RI-cleaved DNA samples with probe B [22]. Because the exact fragment sizes are not relevant here, we arbitrarily named the alleles *a*, *b*, and *c* in decreasing order of size. The letters designate the relative fragment sizes within each family set and do not denote any specific fragment length alleles. One of the alleles in the embryo is inherited from the mother (fragments *a*, *b*, and *a*, respectively, in fig. 1*C–E*, lanes 2). Provided that the mother is heterozygous and her second allele is distinct from the paternally derived allele of the embryo, admixture of maternal tissue can be recognized. Informative maternally derived fragments are *b*, *a*, and *b*, marked by arrows in figure 1*C–E*. Whereas six of the 12 cases were informative at this locus, in other cases, the sets of alleles were apparently identical or could not be resolved.

FIG. 1.—Autoradiograms (filters exposed for 16–24 hrs) of matched DNA samples (1–2 μg per lane) from case 1 (*B* and *D*), case 4 (*A*), case 5 (*C*), and case 7 (*E*). In each case, the origins of the DNA samples are the following: *lanes 1* = villous tissues, *lanes 2* = embryonic tissues, *lanes 3* = maternal leukocytes, *lanes 4* = nonvillous tissues (see MATERIALS AND METHODS), *lanes 5* = admixture of approximately 80% embryonic DNA and 20% maternal DNA. *Taq*I-cleaved DNA samples in *A* and *B* were analyzed with probe A, p*DP31* [23], which detects a 14.6-Kbp Y-specific fragment (*Y*) and 11.8 Kbp (X_1) and 10.6 Kbp (X_2) X-specific fragments in addition to lower-size fragments of weaker intensity caused by a repetitive sequence within the probe. In *Eco*RI-cleaved DNA samples (*C–E*) studied with probe B, p*AW101* [22], the various fragments were arbitrarily named. In each independent set of matched DNA samples, *a*, *b*, and *c* (if necessary) represent the larger to the smaller fragments, as described in the text. *Arrows on the left side point to* the informative maternal alleles.

Purity of Chorionic Villous Samples

For each informative case, filters were prepared with DNAs from chorionic villi (lanes 1 in fig. 1), embryonic tissue (lanes 2), maternal leukocytes (lanes 3), nonvillous tissue (lanes 4), and a mixture of 80% embryonic and 20% maternal DNA samples (lanes 5). Figure 1*A–D* illustrates the patterns observed in three different informative cases. Case 1 was informative with both probes (fig. 1*B* and *D*). The same set of alleles was present in embryonic (lanes 2) and villous tissues (lanes 1). Purity of the chorionic villous samples is suggested by the absence of the maternally derived fragments X_2 (fig. 1*A* and *B*), *b* (fig. 1*C*), and *a* (fig. 1*D*), while in the simultaneously analyzed mixed samples (lanes 5), all three alleles can be detected. Whenever embryonic tissue or maternal blood was not available, a limited study was carried out.

As summarized in table 1, distinct informative alleles were identified in seven cases, either at both loci and with concordant results (cases 1–3) or at one of the two loci (cases 4–7). In the first six cases, villous tissues appeared pure after microscopic dissection, and we did not detect any maternal DNA. In case 7, dissection was recognized to be unsatisfactory and molecular analysis revealed the presence of both embryonic and maternal DNA in the "villous" tissue (presence of fragments *a* and *b* in fig. 1*E*, lane 1).

Origin of the Nonvillous Tissues

The presence of the second informative maternal allele in nonvillous tissues proves a maternal contribution. Embryonic tissue is detected by the presence of the paternally derived informative allele; for example, the Y-specific fragment in case 4 (fig. 1*A*, lane 4). The absence of fragment *c* in case 5 (fig. 1*C*, lane 4) suggests that the nonvillous tissue sample was purely maternal in origin. If both types of tissues were present, three alleles should be detected (fig. 1*A*). In cases 1 and 7 (fig. 1*B*, *D*, and *E*), the nonvillous tissues appeared mostly maternal in origin, but the presence of a small amount of embryonic tissues cannot be ruled out.

As summarized in table 1, maternal tissues were detected in eight nonvillous samples (cases 1–8), in four of them with both probes. In case 8, the Y-specific fragment was clearly visible in the male villous sample and in the mixing experiment, but was absent in the nonvillous sample. While seven cases were informative for detection of an embryonic allele, its presence was recognized in only two of them (cases 4 and 9). Based on the relative intensities of the bands in the mixed samples, we estimated the proportion of embryonic tissues as less than 50%. In five informative cases, the nonvillous tissues appeared exclusively maternal in origin.

Molecular Sex as Determined with Probe A

Karyotypes were established on direct preparations and on cultured embryonic tissues or cultured villous mesenchyme. The detection of X- and Y-specific alleles agreed with the chromosome analysis in all cases (table 1).

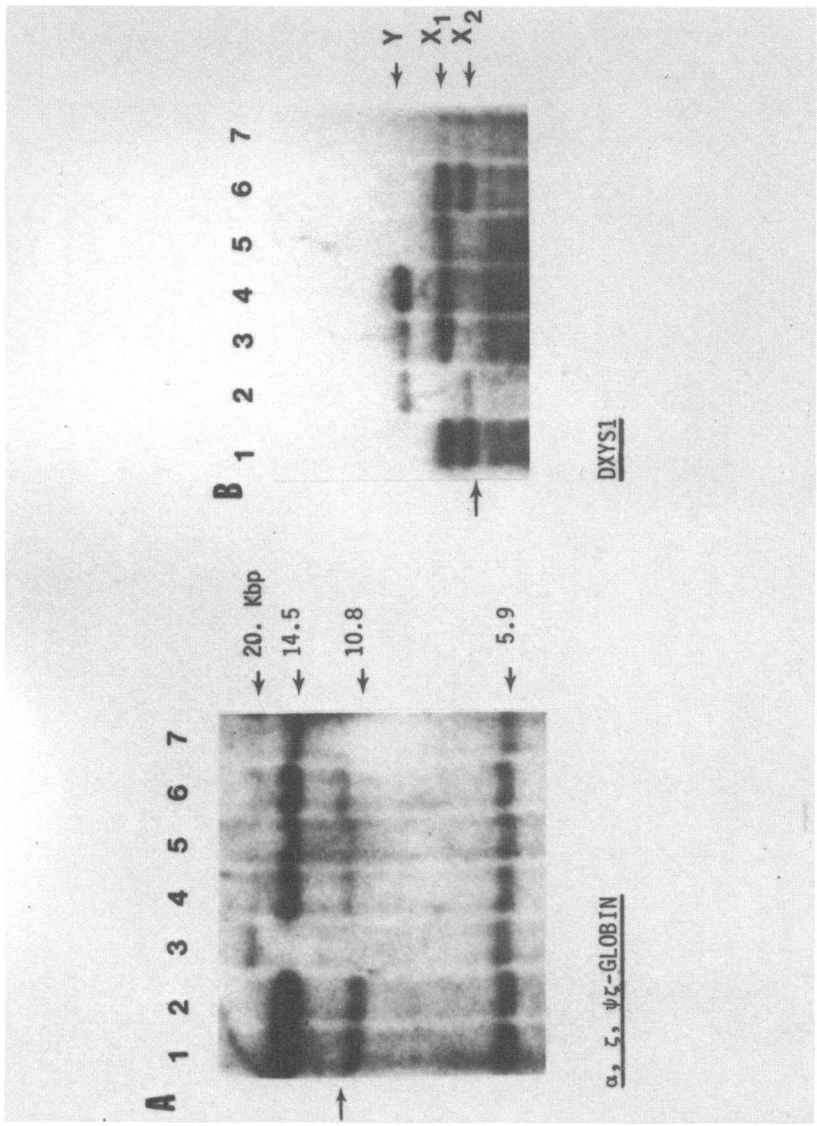
TABLE 1

ORIGIN OF THE TISSUES IN UNCULTURED CHORIONIC VILLOUS SPECIMENS DETERMINED BY DNA POLYMORPHISM ANALYSIS AND CYTOGENETIC STUDIES

CASE NO.	FETAL KARYOTYPE		MOLECULAR SEX	*DXYS1* (PROBE SEX							*D14S1* (PROBE B)						
				RESULTS				INTERPRETATION			RESULTS				INTERPRETATION		
	Direct from villi	Cultured villi or embryos		Villi	Embryo	Mother	Nonvilli	Evidence of maternal DNA in villi	Origin of nonvilli		Villi	Embryo	Mother	Nonvilli	Evidence of maternal DNA in villi	Origin of nonvilli	
									Mat.	Embr.						Mat.	Embr.
1	46,XX	46,XX	F	X_1	X_1	X_1, X_2	X_1, X_2	−	+	ni	*b*	*b*	*a, b*	*a, b*	−	+	ni
2	46,XX	46,XX	F	X_1	na	X_1, X_2	X_1, X_2	−	+	ni	*a, b*	na	*a, c*	*a, c*	−	+	−
3	46,XY	46,XY	M	*Y*, X_2	*Y*, X_2	X_1, X_2	na	−	na	na	*a, c*	*a, c*	*b, c*	*b, c*	−	+	−
4	46,XY	46,XY	M	*Y*, X_1	*Y*, X_1	X_1, X_2	(*Y*), X_1, X_2	−	+	+	ns	*a, b*	*a, b*	ns	ni	ni	ni
5	na	46,XY	M	*Y*, X_1	*Y*, X_1	X_1	X_1	ni	+	−	*a, c*	*a, c*	*a, b*	*a, b*	−	+	−
6	46,XY	46,XY	M	*Y*, X_2	*Y*, X_2	X_2	X_2	ni	+	−	*a, c*	*a*, (*b*), *c**	*b, c*	*b, c*	−	+	−
7	na	46,XX	F	X_2	X_2	X_2	X_2	ni	ni	ni	*a, b*	*b*	*a, b*	*a, b*	+	+	ni
8	46,XY	46,XY	M	*Y*, X_2	na	X_2	X_2	ni	+	−	ns	na	*a*	na	ni	na	na
9	46,XY	46,XY	M	*Y*, X_1	na	na	(*Y*), X_1	ni	ni	+	*a, b*	na	na	*a, b*	ni	ni	ni
10	46,XX	46,XX	F	X_2	X_2	X_2	X_2	ni	ni	ni	ns	*a*	*a*	ns	ni	ni	ni
11	69,XXX	na	F	X_1, X_2, X_2	na	X_1, X_2	ns	ni	ni	ni	*a, b*	na	*a, b*	*a, b*	ni	ni	ni
12	na	46,XX	F	X_2	na	X_2	X_2	ni	ni	ni	ns	na	*a*	na	ni	na	na

NOTE: *Y*, X_1, X_2, *a*, *b*, and *c* are the various fragments detected by probes A and B and described in the figure legends and in the text. M = male; F = female; ni = not informative; na = not available (insufficient tissue or DNA); ( ) = weak allele; ns = not studied (would have been uninformative); Mat. = maternal; Embr. = embryonic.

* Embryonic tissue was umbilical cord and presumably carried some placental tissues with it.


A
1 2 3 4 5 6 7
20. Kbp
14.5
10.8
5.9
α, ζ, ψζ-GLOBIN
B
1 2 3 4 5 6 7
Y
X1
X2
DXYS1

Prenatal Diagnosis

A chorionic villous biopsy was performed at 10 weeks of gestation for prenatal diagnosis of α^0-thalassemia. The findings are shown in figure 2*A*. *Bam*HI-cleaved DNA samples were simultaneously analyzed with α- and ζ-globin probes [24, 25]. Both parents (lanes 4 and 6) were carriers of the α-thalassemia 1 trait with a deletion of both α-globin genes on one chromosome. The 20-Kbp fragment carrying one ψζ-globin gene is derived from the deleted chromosome, and the 14.5-Kbp fragment, from the normal chromosome (a 1:2 ratio of intensity of this band compared to normal controls in lanes 1 and 2); in the homozygous state (hydrops fetalis in lane 3), all four α-globin genes are deleted and the 20-Kbp fragment corresponds to the two remaining ψζ-globin genes [28]. In addition, both parents carried different RFLPs in the intergenic ζ-globin region [29]: their *Bam*HI ψζ-specific fragments are different and larger than the common 10.8-Kbp fragment found in the general population (controls in lanes 1 and 2). The pattern of alleles seen in the villous DNA sample (lane 5) was identical to that of the father; thus, the embryo inherited a normal chromosome carrying two α-globin genes from the father and the α-thalassemia 1 trait from the mother. The nonvillous tissue DNA sample exhibited the maternal pattern of alleles (lane 7). In this lane, the informative > 10.8 Kbp fragment that was clearly visible on the original autoradiogram was partially wiped out by a transfer defect. The two ζ-globin genes (fragments of 5.9 Kbp) are present in all individuals and were used as internal controls.

The sex was determined to be female by analysis with probe A (fig. 2*B*, lane 5) and agreed with the 46,XX results of direct chromosome analysis. There was no evidence of maternal contamination of the villous DNA, as indicated by absence of the maternally derived X_2 fragment.

DISCUSSION

We have carried out a molecular analysis of 12 chorionic villous samples obtained by first-trimester aspiration biopsy before elective termination. The analysis of DNA extracted directly from uncultured tissues accurately reflects their genetic constitutions. Our results indicate that villous tissue is embryonic in origin if carefully identified and immediately dissected clean under the microscope. The six informative cases with satisfactory dissection were shown to be free of detectable maternal contamination. In one case, tissues were disintegrating before dissection. The biopsy specimen had been maintained in phosphate-buffered saline, and we found later that collection of samples in a pH-equilibrated tissue-

FIG. 2.—Autoradiograms (filters exposed for 20 hrs) for prenatal diagnosis of α-thalassemia 1 trait. *Lanes 1 and 2* = control DNA samples from a mother and her son with normal α-globin gene clusters; *lanes 3* = homozygous α^0-thalassemia control; *lanes 4* = father; *lanes 5* = villous DNA; *lanes 6* = mother; *lanes 7* = nonvillous biopsy tissue DNA sample. In *A*, *Bam*HI-cleaved DNA samples (~4 μg per lane) were analyzed with probes p*SV0α1* [24] and *4p7-7* [25], which detect the α-, ζ-, and ψζ-specific fragments (14.5, 5.9, and ~10.8 Kbp, respectively); the 20-Kbp fragment is associated with the α-thalassemia 1 trait [28]. In *B*, *Taq*I-cleaved DNA samples (~2 μg per lane) were analyzed with probe A as described in figure 1. *Arrows on the left side point to* the informative maternal alleles absent in the villous DNA samples.

culture transport medium better preserves tissue integrity. The dissection might not be difficult if the specimen contains multiple fragments of intact and clean villi but becomes tedious and requires experience and care when the tissue sample is very small, hemorrhagic, or is tightly intermixed with decidual tissues.

As nonvillous tissues, we chose tissues adherent to the villi, or a mixture of both that could not be dissected clean. The presence of maternal tissue was proven in eight informative cases, and in five cases, it was exclusively maternal in origin. Proper identification and dissection of chorionic villi is an essential step to avoid contamination of villous specimens by maternal tissues.

If the nature of the biopsy specimen is uncertain, genetic markers can be used to assess the embryonic origin of the tissues. We used molecular probes to analyze two independent RFLP loci; seven of the 12 cases studied had informative alleles at one or both loci. Although no detailed gene frequency data are yet available for the two loci studied, the highly polymorphic *D14S1* locus was more useful in our analyses (six of the 11 cases studied had different alleles at this locus). By contrast, alleles at the *DXYS1* locus will be informative only in approximately 37% of the cases, if one assumes equal frequencies of the two X-specific alleles. Four of the 11 cases we studied had informative alleles. Despite being less informative in the recognition of maternal contamination, Y-chromosome-specific probes can have an important role in prenatal diagnosis of an X-linked disorder. DNA samples could be analyzed simultaneously for the presence of a Y-specific fragment to diagnose the sex and for the specific marker allele associated with the disease gene. As illustrated in this report, Y-specific fragments can be sought using probe p*DP31* and a *Taq*I digest or other Y-specific probes as they become available [15]. Whether sex determination by DNA analysis should be carried out in conjunction with direct chromosome analysis of villous metaphases depends on the quality and the amount of tissues available and will be further clarified by current investigations.

We have adapted the Southern-blotting methodology to small biopsy specimens. The amount of tissue required for a molecular diagnosis is minimal, only 2–5 mg. Thus, DNA analysis can be done along with enzymatic or cytogenetic procedures requiring 10–20 mg of tissues. The yield of high molecular weight DNA suitable for digestion is sufficient (average 7 μg/approximate mg of wet tissues) to carry out several assays on the same biopsy sample. We used only 1–2 μg of restriction enzyme-cleaved DNA per sample to detect single-copy sequences. Using small genomic DNA samples defined the limit in the efficiency of the detection system itself. In our mixing experiments, we were able to detect approximately 20% or more of maternal DNA using 1–2 μg of digested genomic DNA. Whether or not a maternal allele was detectable in an approximately 20% maternal/80% embryonic DNA mixture depended on the quality of the blot and on the alleles involved; for example, the maternal X_2 band is more conspicuous in lane 5 of figure 1*A* than in lane 5 of figure 1*B*, and the maternal allele (*b*) is more obvious in figure 1*C*, lane 5, than the allele (*a*) is in figure 1*D*, lane 5. Because of this variability, we would not feel confident to consistently detect maternal contamination at a level below 20% in 1–2 μg DNA samples. Although the presence of less than 20% of maternal DNA, if unrecognized, might not interfere with the search for a specific allele associated with an inherited disease,

it might render quantitative biochemical assay results uninterpretable for accurate prenatal diagnosis.

Several reports [1, 4, 5, 11–18] have indicated that molecular and enzymatic diagnoses can be performed on chorionic villous samples. We carried out a prenatal diagnosis in a continuing pregnancy at risk for α^0-thalassemia and detected two distinct informative polymorphisms. We found no evidence of maternal contamination of the villous sample and showed that the female embryo inherited the α-thalassemia 1 trait from her mother.

The reliability of the results depends heavily on the purity of the villous tissues. We have shown that only a few milligrams of villi are required for an accurate diagnosis and more than one DNA probe can readily be used. Restriction fragment length polymorphisms will often distinguish maternal and embryonic DNA so that, when one is concerned about the purity of the sample, an unacceptable degree of maternal contamination can be recognized or excluded. Most often, as our data demonstrate, careful dissection of aspirated villi can provide fetal tissue free of interfering amounts of maternal tissue.

ACKNOWLEDGMENTS

We are indebted to Drs. John Hobbins and William Lieber and to the obstetrics staff and faculty of the perinatal unit and Sterling Clinic of Yale-New Haven Hospital for their help in performing the chorionic villous biopsies; to Drs. W. Roy Breg and Michael Watson and to Joan Samuelson and Deborah Boles for cytogenetic analyses; to Dr. Pauline Brenholz for referring the couple at risk for α^0-thalassemia; to Dr. Haig Kazazian, Jr., and Corinne Boehm who diagnosed the carrier status of this couple and confirmed our findings on the prenatal diagnosis; to Dr. Bernard Forget for invaluable advice, for probes *4p7-7* and p*SV0α1*, and for the control DNA of an infant with hydrops fetalis; to Dr. Arlene Wyman for probe p*AW101* and Dr. David Page for probe p*DP31;* and to Stephenie Blakemore for expert technical assistance.

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