USEFULNESS OF LINKED DNA PROBES FOR PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS : REPORT OF A CASE IN A 1 : 4 RISK PREGNANCY

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FONTAINE F., VASSEUR F., SAVARY J.B., MENAIS M., ROUSSEL M., DEMINATTI M.M. — Usefulness of linked DNA probes for prenatal diagnosis of cystic fibrosis : report of a case in a 1 : 4 risk pregnancy. *(In English).*
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SUMMARY : Prenatal diagnosis of cystic fibrosis was performed with linked DNA probes in a couple with a 1 : 4 risk. The limits and the future of molecular prenatal diagnosis are discussed.

KEY-WORDS : Cystic fibrosis. — Prenatal diagnosis. — DNA markers.

FONTAINE F., VASSEUR F., SAVARY J.B., MENAIS M., ROUSSEL M., DEMINATTI M.M. — Utilité de sondes d'ADN liées pour le diagnostic prénatal de la fibrose kystique : observation d'une grossesse avec un risque : 4 *(En Anglais).*
Ann Génét, 1988, **31,** n° 2, 102-104.

RÉSUMÉ : Un diagnostic anténatal de mucoviscidose a été effectué dans une famille à risque, par les méthodes de la biologie moléculaire. Les limites et les perspectives de la méthode sont discutées.

MOTS-CLÉS : Mucoviscidose. — Diagnostic anténatal. — Marqueurs d'ADN.

INTRODUCTION

Cystic fibrosis (CF) is a frequent autosomal recessive genetic disease in Caucasian populations (frequency 1/2000 newborns). The basic biochemical defect is still unknown but genetic markers (restriction fragment length polymorphisms, R.F.L.P.'s) very closely linked to the CF locus were recently described : fragments of the *met* protooncogene (White et al. 1985 ; White et al., 1986 ; Spence et al., 1986), anonymous DNA fragments pJ3.11 (Wainwright et al., 1985) and 7C22 (Scambler et al., 1986). All are localized on the chromosome 7, pJ3.11 and *met* flanking the CF locus (Beaudet et al., 1986).

Familial studies with these markers result in typing each parental chromosome 7 when the phase can be determined (Vasseur et al., 1988). In fully informative families, typing of the fetus allows prenatal diagnosis of CF.

PATIENTS AND METHODS

Mrs L. presenting an affected child with CF was seen for a prenatal diagnosis of the disease. Term of pregnancy was too late for a chorion villus sampling (CVS). Seven R.F.L.P.'s were studied in each member of the family in order to determine whether the family was fully or partly informative.

DNA was extracted from leucocytes and cultivated amnion cells according to standard methods, and hydrolyzed with appropriates restriction enzymes (*Msp*I, *Taq*I, *Eco*RI, BanI, *Pst*I). Restriction fragments were separated by agarose gel electrophoresis, alkali blotted on nylon membranes and hybridized with ^{32}P oligolabelled probes (Feinberg and Vogelstein, 1983 ; Feinberg and Vogelstein, 1984).

RESULTS

Seven markers (R.F.L.P.'s) were investigated in the family (table I). Two of them : *met*D/*Taq*I and pJ3.11/*Taq*I were not informatives, both parents being homozygote 1-1 (fig. 1). Alleles detected by five other R.F.L.P.'s allowed phase determination and typing of parental chromosomes 7 (Vasseur et al., 1988).

Among informative R.F.L.P.'s at the *met* locus, only *met*H/*Msp*I, was analyzed on fetal DNA, because *met*H/*Taq*I, *met*D/*Ban*I gave the same allelic

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TABLE I. — **R.F.L.P.'s tightly linked to the CF locus.**

R.F.L.P.	Size of alleles (kilobases) allele 1	allele 2	Reference
*met*H/*Msp*I	2.3	1.8	White et al., 1985
*met*H/*Taq*I	7.5	4	
*met*D/*Taq*I	6	4.3	White et al., 1986
*met*D/*Ban*I	7.6	6.8	Spence et al., 1986
pJ3.11/*Msp*I	4.2	1.8	Wainwright et al., 1985
pJ3.11/*Taq*I	6.3	3.1	
7C22/*Eco*RI	7.2	5.1	Scambler et al., 1986

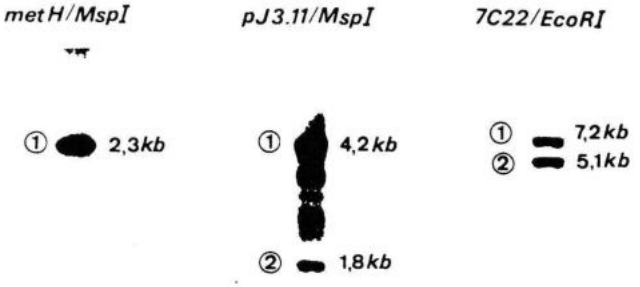


Fig. 2. — Southern blot analysis of fetal DNA from cultivated amnion cells.

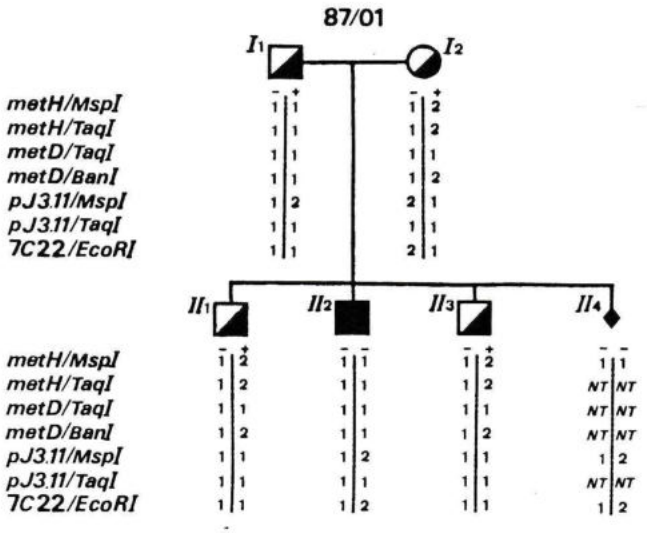
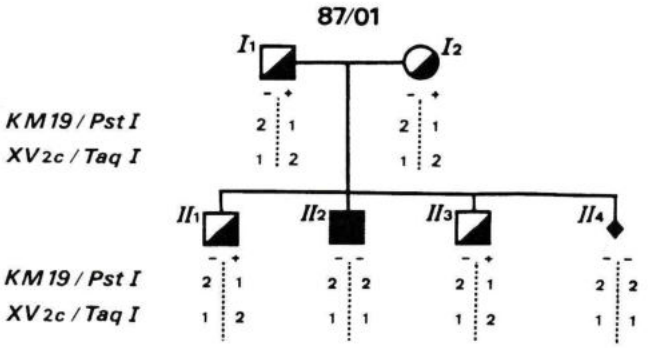


Fig. 1. — DNA genotyping for family 87/01. Heterozygote : ◪ ◔. Affected : ■ ◆. CF chromosome : −. Normal chromosome : +. NT : R.F.L.P. not tested.



R.F.L.P.	Size of alleles (kilobases)		Reference
KM19/*Pst*I	7.8	6.6	B. Williamson, personnal communication
XV2c/*Taq*I	2.1	1.4	Estivill et al., 1987

Fig. 3. — New RFLP's and genotyping of 87/01 family with KM19 and XV2c. DNA II-4 was extracted from skin biopsy of the fetus after termination of pregnancy. Legend is as figure 1.

distribution. Two more informative R.F.L.P.'s at other loci than the *met* protooncogene, were also analyzed : pJ3.11/*Msp*I and 7C22/*Eco*RI.

Fetal DNA (fig. 2) showed an 1-1 allelic configuration with *met*H/*Msp*I : the fetus had inherited the maternal CF gene. This result was supported by the fetal 7C22/*Eco*RI allelic configuration 1-2. With pJ3.11/*Msp*I, the fetal configuration was 1-2 ; the fetus had inherited allele 1 from his father, together with the CF gene.

Haplotypes of the fetus showed he had inherited both parental mutated genes.

Pregnancy was terminated and anatomopathology of the fetus showed a meconial ileus which is present in most CF fetuses (Müller et al., 1985). later, when probes XV2c (Estivill et al., 1987) and KM19 (Williamson, personnal communication) flanking the CF locus at 20 kb, became available, family 87/01 was screenes again. The results (fig. 3) were in total agreement with the genotyping of the family and the prenatal diagnosis performed a few months ago.

DISCUSSION

Prenatal diagnosis was achieved within on week after securing fetal DNA, and was done on cultivated amnion cells. Delay of results can be considerably shortened if DNA is prepared directly from amnion cells without culture, or from C.V.S.

One of the limits of this analysis is the opportunity of typing parental chromosomes. This requires the analysis of an affected child in the family (Fontaine et al., 1986), although Law et al. (1987) reported two cases of prenatal diagnosis in families with no more alive affected child, but these were very particular cases.

When DNA analysis shows a fully informative family (both CF parental chromosomes can be identified) prenatal diagnosis can be achieved. In partly informative families (only one CF parental chromosome can be identified), detection of the CF chromosome in the fetus leads to an ambiguous result, the fetus being either affected or heterozygote. In

non informative families, no parental chromosome can be identified : prenatal diagnosis is impossible with these methods.

According to Spence et al. (1987) 74 % of families with a 1 : 4 risk are fully informative for both parents, 1 % are not informative, the remaining being partly informatives. These results implie screening as many R.F.L.P.'s as possible in order to type parental chromosomes.

Another limit of the method is the occurrence of a crossing-over between the markers and the CF locus. Crossing-over may interfere in phase determination leading to false parental chromosome typing. This risk of error is lower in families where sibs (CF or not) are screened, giving additional information on phase determination, than in families presenting only two parents and an affected child. Crossing over may also have occured in meiosis leading to the conception of the fetus. If the recombination fraction is 0.01 for CF to *met*, and for CF to pJ3.11, in our data, the odds in favour of an affected fetus (II_4) were 33:1.

Accuracy of prenatal diagnosis will be improved with markers for which the recombination fraction for CF is lower : XV2c, CS7 (Estivill et al., 1987), KM19 (Williamson, personnal communication).

When the phase can be determined with markers flanking the CF locus at 20 kb (i.e. KM19 and XV2c), if recombination is a function of genetic distance, even in the most disadvantagous configuration (the family bearing only two parents and an affected child, and each marker being partly informative), odds in favour of an affected fetus would be 300 : 1.

Moreover, the new markers XV2c, KM19 and CS7 show important linkage desiquilibrium with CF. Estivill et al. (1987) have shown that 94 % of CF chromosomes bear the haplotype XV2c - allele 1/CS7 - allele 2, and for CS7 alone 70 out of 71 chromosomes tested bear CS7 - allele 2. According to Williamson (personnal communication) 90 % of CF chromosomes would be associated with the haplotype KM19 - allele 2/XV2c - allele 1. One can assume that prenatal diagnosis and carrier testing will soon be based rather on the presence or absence of an haplotype showing an important linkage desiquilibrium with CF than on the study of familial segregation of DNA markers.

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