RFLPs TIGHTLY LINKED WITH CYSTIC FIBROSIS : VALUE OF PROBES AT THE D7S23 LOCUS IN PRENATAL DIAGNOSIS

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VASSEUR F., FLACTIF M., SAVARY J.B., TURPIN Dominique, DEMINATTI M.M. – RFLPs tightly linked with cystic fibrosis : value of probes at the D7S23 locus in prenatal diagnosis.

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SUMMARY : In a 1:4 risk family, the usefulness of probes at the D7S23 locus for prenatal diagnosis of cystic fibrosis is discussed by comparison with probes at the MET, D7S8, and D7S18 loci that did not allow accuracy in this family.

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

VASSEUR F., FLACTIF M., SAVARY J.B., TURPIN Dominique, DEMINATTI M.M. – Polymorphismes de restriction liés à la fibrose kystique : valeur des sondes au locus D7S23 pour le diagnostic prénatal. *(En Anglais).*

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RÉSUMÉ : Chez un couple à risque, l'utilisation des sondes du locus D7S23 dans le diagnostic prénatal de mucoviscidose est discutée par rapport aux sondes situées aux loci MET, D7S8 et D7S18 dont la fiabilité était insuffisante dans cette famille.

MOTS-CLÉS : Mucoviscidose. – Diagnostic prénatal. – Marqueurs d'ADN.

Genetic markers (restriction fragments length polymorphisms, RFLP's) tightly linked with cystic fibrosis (CF) have been described in 1985, simultaneously by several groups : DOCRI 917 (Tsui et al., 1985), the met oncogene (White et al., 1985), anonymous DNA fragment pJ3.11 (Wainwright et al., 1985), respectively at 15, 1 and 1 centimorgan (cM) away from the CF locus. Later, Scambler et al. (1986) described an anonymous DNA fragment 7C22 at 5cM from the CF locus. All these markers have been used for prenatal diagnosis of CF (Farrall et al., 1986a) and carrier detection (Farrall et al., 1986b), with an accuracy depending upon their recombination fraction versus CF. New markers at the D7S23 locus defined by probes XV2c and KM19, physically very close to the CF locus, were later described (Estivill et al., 1987). These probes show a strong linkage disequilibrium with the CF mutation and are of great interest for prenatal diagnosis and carrier detection of CF.

MATERIAL AND METHODS

DNA was extracted from leukocytes or cultivated amnion cells according to standard protocols previously described (Fontaine et al., 1988), digested with appropriate restriction endonucleases according to the manufacturer's protocol, separated by agarose gel electrophoresis, alkali blotted on nylon membranes and hybridized with ^{32}P oligolabelled probes (Feinberg and Vogelstein, 1983, 1984).

RESULTS

Haplotypes of nine families presenting an affected child with CF are given in table I. From our experience three periods may be distinguished : some families in 1986 and the first six months of 1987 were investigated with probes at the MET (met H, met D), D7S8 (pJ3.11), and D7S18 (7C22) loci. Later, when probes at the D7S23 locus, XV2c and KM19, were available, some families were screened again. At present time, families are analyzed with the most tightly linked probes XV2c, KM19 and pJ311 that map to the other side of CF, the most likely order being D7S18-MET-D7S23-CF-D7S8 (Estivill et al., 1987).

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TABLE I. – **Haplotypes of 9 families presenting an affected child with cystic fibrosis. pJ3.11 : pJ3.11/MspI, KM19 : KM19/PstI, XV2c : XV2c/TaqI, met H : met H/MspI or TaqI, met D : met D/TaqI or met D/BanI, 7C22 : 7C22/EcoRI. (a) alleles in brackets could not be attributed because of a crossing over in the family. NI : not informatif - NT : not tested.**

Family	Probe	Parents (−/+)	Parents (−/+)	Affected Children	Affected Foetuses	Unaffected children			
8601	pJ3.11	1 2	2 2	1 2					
	met H	1 2	1 2	1 1					
	met D	NI		NI					
	7C22	2 2	2 1	2 2					
8602	pJ3.11	1 1	1 2	1 1		1 2			
	met H	1 1	2 1	1 2		1 1			
	met D	1 2	1 1	1 1		2 1			
	7C22	NI		NI		NI			
8701	pJ3.11	1 2	2 1	1 2	1 2	1 1	1 1		
	KM19	2 1	2 1	2 2	2 2	2 1	2 1		
	XV2c	1 2	1 2	1 1	1 1	1 2	1 2		
	met H	1 1	1 2	1 1	1 1	1 2	1 2		
	met D	1 1	1 2	1 1	NT	1 2	1 2		
	7C22	1 1	2 1	1 2	1 2	1 1	1 1		
8702	pJ3.11	1 2	2 2	2 1		2 2			
	KM19	2 1	2 2	2 2		1 2			
	XV2c	1 1	1 2	1 1		1 2			
	met H	2 1	1 1	1 2		1 1			
	met D	2 1	2 1	2 2		1 1			
	7C22	NI		NI		NI			
8703	pJ3.11	NI		NI	NI				
	KM19	2 2	2 1	2 2	2 2				
	XV2c	1 2	1 1	1 1	1 1				
	met H	2 2	2 1	2 2	2 2				
	met D	2 2	2 1	2 2	NT				
	7C22	2 1	1 1	1 2	NT				
8705	pJ3.11	1 2	1 2	1 1		1 2			
(a)	KM19	2 2	2 1	2 2		2 1			
	XV2c	1 1	1 2	1 1		1 2			
	met H	(2 1)	(2 1)	2 2		(2 2)			
	met D	(1 1)	(1 2)	1 1		(1 1)			
	7C22	(1 2)	(1 2)	(1 2)		(1 2)			
8706	pJ3.11	NI		NI		NI	NI		
	KM19	2 1	2 2	2 2		2 2	2 1		
	XV2c	2 1	1 2	1 2		2 2	1 1		
8801	pJ3.11	2 2	2 1	2 2					
	KM19	2 2	2 1	2 2					
	XV2c	2 2	1 1	1 2					
8802	pJ3.11	1 2	1 2	1 1		1 2	1 2	1 2	1 2
	KM19	2 1	1 1	1 2		1 1	2 1	1 1	1 1
	XV2c	1 2	2 2	2 1		2 2	1 2	2 2	2 2

All families, but one (8801), appeared to be fully informative either with the use of one marker or with the combination of two partly informative markers, as discussed elsewhere (Vasseur et al., 1988). Among the families tested with probes at the MET, D7S8 and D7S18 loci, one (8703) raised a problem of accuracy. Patient I2 of family 8703 was already pregnant as she was seen for a prenatal diagnosis of CF. The pregnancy was too advanced for chorion villi sampling (CVS), and foetal DNA was extracted from cultivated amnion cells. As shown in figure 1 seven RFLP's defined by the available probes, were used. Phase could be determined with two partly informative markers : 7C22/EcoRI and *met* H/MspI or TaqI or *met*D/BanI. Despite its 5 % recombination fraction versus CF, 7C22/EcoRI was the only marker able to type the paternal mutated chromosome (associated with allele 2). In order to improve accuracy in prenatal diagnosis, recently described probes at the D7S23 locus (Estivill et al., 1987) were requested. Family 8703 was typed again as shown in figure 2 and phase could be determined. Foetal DNA was analyzed with these probes and showed (fig. 2) an affected foetus. The parents did not wish termination of pregnancy : the child is now born and his CF status has been confirmed.

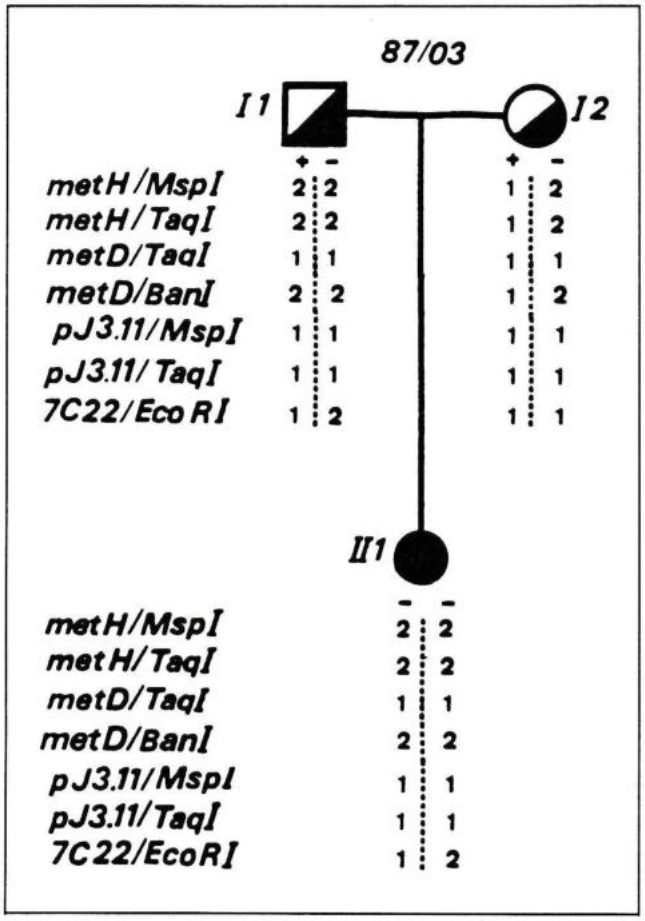

Fig. 1. — Haplotypes of family 8703 with probes at the MET (met H, met D), D7S8 (pJ3.11) and D7S18 (7C22) loci.

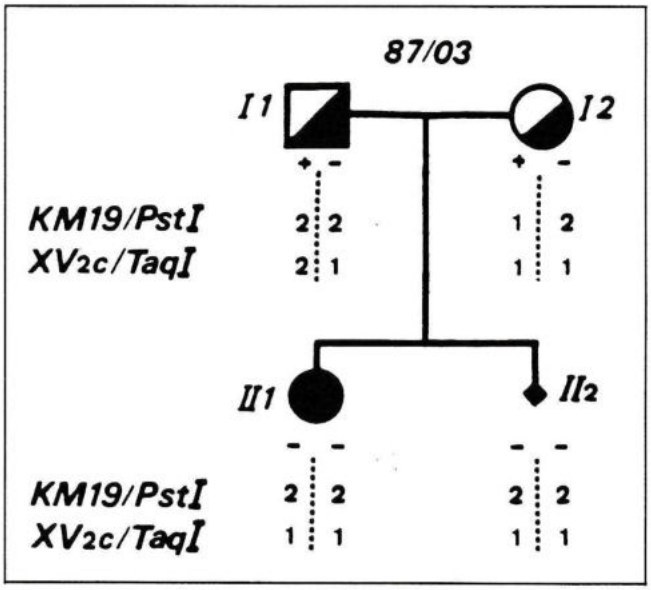

Fig. 2. — Haplotypes of family 8703 with probes at the D7S23 locus (XV2c and KM19), and of the foetus II_2 showing his CF status.

DISCUSSION

Unlike family 8701 previously described (Fontaine et al., 1988), prenatal diagnosis of CF in family 8703 is an example of a disadvantageous situation, i.e. a familial configuration in which no unaffected sib could be typed in order to detect a recombination event. The use of two partly informative markers (MET, D7S18), and a relative high recombination fraction (5 %) for one of them, hampered both family typing and prenatal diagnosis accuracy.

Assuming the recombination value for MET and D7S18 as θ for maximal lod score, respectively 0,01 (White et al., 1986) and 0,05 (Scambler et al., 1986), accuracy would have been 0,884 (odds 7,6 : 1) in case of an affected foetus. If the recombination value is taken at the lower limit of the confidence interval accuracy falls to 0,549. Recent figures given by Lathrop et al., (1988), i.e., maximal lod score with $\theta = 0{,}023$ for MET and 0,052 for D7S18, do not significantly modify our value (0,857).

Reliability of prenatal diagnosis was greatly improved with the use of KM19 and XV2c in family 8703. Assuming a recombination fraction of 0,0001 for KM19 to CF (R. Williamson, personnal communication) and XV2c being about 40 kb away from KM19, and supposing that recombination is a linear function of genetic distance, a 100-fold improvement in accuracy was reached with probes of the D7S23 locus, with a likelihood of 0,9988 (odds 832 :1).

Linkage disequilibrium between the CF mutation and markers at the D7S23 locus (Estivill et al., 1987, 1988) is of great interest in prenatal diagnosis and carrier detection of CF because most families become informative.

In our data, haplotype KM19 allele 2/XV2c allele 1 was found associated with 11 out of 14 CF chromosomes and only with one normal chromosome. A11, except one family, appeared to be fully informative. With probes at the MET D7S8 and D7S18, 67 % of families are fully informatives (Farrall et al., 1986a, Novelli et al., 1987). Additional typing with probes at the D7S23 locus renders more than 90 % of families fully informative (Estivill et al., 1988).

Linkage disequilibrium may also be used to estimate the risk of an individual to be carrier or not, according to the presence or absence of the « risk » haplotype as discussed by Estivill et al., (1988). This estimation may be of importance for the decision of prenatal diagnosis by use of microvillar enzymes testing (Brock, 1983, 1985) in couples with a prior risk less than 1:4.

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