Prenatal diagnosis of cystic fibrosis: the 18-year experience of Brittany (western France)

Virginie Scotet[1,2,3]*, Ingrid Duguépéroux[1,2,3], Marie-Pierre Audrézet[1,2,3,4], Martine Blayau[5], Pierre Boisseau[6], Hubert Journel[7], Philippe Parent[8] and Claude Férec[1,2,3,4]

[1]*Inserm, U613, Brest, F-29200, France*
[2]*Univ Brest, Faculté de Médecine et des Sciences de la Santé, UMR - S613, Brest, F-29200, France*
[3]*Etablissement Français du Sang—Bretagne, Brest, F-29200, France*
[4]*C.H.U. Brest, Hop Morvan, Laboratoire de Génétique Moléculaire, Brest, F-29200, France*
[5]*C.H.R.U. Rennes, Laboratoire de Génétique Moléculaire, Rennes, F-35000, France*
[6]*C.H.R.U. Nantes, Laboratoire de Génétique Moléculaire, Nantes, F-44000, France*
[7]*C.H. Bretagne Atlantique, Unité de Génétique Médicale, Vannes, F-56000, France*
[8]*C.H.U. Brest, Hop Morvan, Service de Pédiatrie et de Génétique Médicale, Brest, F-29200, France*

Objective This study reports 18 years of experience in prenatal diagnosis (PD) of cystic fibrosis (CF) in a region where CF is frequent and the uptake of PD is common (Brittany, western France).

Method All PDs made over the period 1989–2006 in women living in Brittany were collected.

Results We recorded 268 PDs made in 1 in 4 risk couples, plus 22 PDs directly made following the sonographic finding of echogenic bowel. Most of the 268 PDs were done in couples already having CF child(ren) ($n = 195$, 72.8%). Close to one-fifth followed cascade screening ($n = 49$, 18.3%), which identified 26 new 1 in 4 risk couples among the relatives of CF patients or of carriers identified through newborn screening (NBS). The remaining PDs were mainly made in couples whose 1 in 4 risk was evidenced following the diagnosis of echogenic bowel in a previous pregnancy ($n = 22$, 8.2%). Although patients' life expectancy has considerably improved, in our population the great majority of couples chose pregnancy termination when PD indicated that the foetus had CF (95.9%).

Conclusion This study describes the distribution of PDs according to the context in which the 1 in 4 risk was discovered and highlights the real decisions of couples as regards pregnancy termination after a positive PD. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: cystic fibrosis; prenatal diagnosis; cascade screening; echogenic bowel; newborn screening

INTRODUCTION

Cystic fibrosis (CF) is one of the most common autosomal recessive disorders in Caucasian populations, with an incidence of about 1/3500 live births (Welsh *et al.*, 2001; Southern *et al.*, 2007). The severity of CF and the lack of curative treatment justified the development, in the early 1980s, of a prenatal diagnosis (PD) test, in order to avoid the birth of other affected children in families. Although the patients' life expectancy has greatly improved over recent decades, CF remains a severe and incurable disease. Therefore, the request for PD usually remains high.

Significant progress has been made in the field of PD of CF over the last 2 decades. Initially, PD consisted in assessing microvillar intestinal enzymes in the amniotic fluid cells extracted at 17 weeks of gestation (Brock, 1983; Brock, 1984; Boué *et al.*, 1986). The localisation of the gene responsible for CF in 1985 enabled indirect testing using restriction fragment length polymorphism (RFLP) markers located near this gene (Farrall *et al.*, 1986; Beaudet *et al.*, 1988; Beaudet *et al.*, 1989). Then, after the cloning of the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene in 1989, PD could be done directly by analysing its mutations (McIntosh *et al.*, 1989; Novelli *et al.*, 1990). Nowadays, PD is carried out by this technique, from chorionic villus sampling extracted at 10 weeks of gestation or from amniotic fluid cells collected at 16–17 weeks of gestation. The discovery of the *CFTR* gene with the identification of its numerous mutations has dramatically changed PD of CF. Besides the main mutation (F508del) which accounts for 66% of CF alleles, more than 1500 mutations have been reported to date in the database of the CF Consortium (Tsui, 2007).

In addition to the parents of previously affected children, PD is offered to 1 in 4 risk couples who can be identified in several ways: through cascade screening performed in families (Super *et al.*, 1994), through prenatal screening designed to detect the 1 in 4 risk couples in a given population (Mennie *et al.*, 1992; Brambati *et al.*, 1993) or following the detection of echogenic bowel upon routine ultrasound examinations during pregnancy (Dicke and Crane, 1992; Scotet *et al.*, 2002a).

*Correspondence to: Virginie Scotet, Inserm U613, Génétique Moléculaire et Génétique Épidémiologique, 46 Rue Félix Le Dantec, BP 62025, 29220 Brest Cedex 2, France.
E-mail: virginie.scotet@univ-brest.fr

Copyright © 2008 John Wiley & Sons, Ltd.

The aim of this study was to report the 18-year experience in PD of CF in Brittany, a region of western France where CF is frequent and where the uptake of PD is common (period 1989–2006).

POPULATION AND METHOD

Study population

We retrospectively identified the PDs of CF made over the period 1989–2006 in couples living in Brittany (western France). Located at the western end of Europe, Brittany is a region of 3 million inhabitants where the incidence of CF is among the highest in the world (1/2800) (Scotet *et al.*, 2002b). This area was also one of the first in Europe to implement a newborn screening (NBS) programme for CF (pilot programme set up in 1989) (Scotet *et al.*, 2000). The PDs were mainly collected by the two genetic laboratories which perform PD of CF in Brittany (in Brest and Rennes), but a third laboratory of a neighbouring area (in Nantes) was also requested in order to be sure to cover the south-east part of the region.

Prenatal diagnosis of CF

In our region, we have been able to perform first-trimester PD by DNA analysis since 1987, initially using RFLPs and since late 1989 by direct CFTR probing. The test is performed by looking for the *CFTR* mutations identified in both parents in the chorionic villus sample extracted at 10 weeks of gestation or the amniotic fluid cells collected at 16–17 weeks of gestation (Férec *et al.*, 1993). In the case of a positive PD, the couple may choose to terminate the pregnancy.

PD of CF is offered to 1 in 4 risk couples during a genetic counselling consultation. The message given to those couples by the genetic counsellor is the following: "Today CF is still a lethal genetic disorder and we have no specific treatment to cure those children. However, in 2007, the life expectancy at birth of the CF children is close to 40 years, and is somewhat higher for those carrying at least one mild mutation (i.e. about 15% of the CF population). The decision to terminate the pregnancy or not belongs to the parents".

Initially, PD was only offered to couples who already had at least one CF child. Thereafter, PD could also be offered to the new 1 in 4 risk couples identified through cascade screening in families. Cascade screening consists in analysing the *CFTR* gene in the relatives and in their partners. If the couples prove to have a 1 in 4 risk of a CF foetus, genetic counselling is offered to them and the possibility of PD is then made available. In Brittany, genetic counselling and cascade screening are widely offered to families related to a CF patient or to families in whom the NBS programme has identified a carrier child. PD can also be offered to couples with no history of CF, but in whom the 1 in 4 risk is highlighted following the sonographic finding of foetal echogenic bowel during pregnancy. In France, a systematic medical follow-up of pregnancies is performed and includes three obligatory sonographic examinations (one per trimester). It is generally during the second one (done at 22 weeks of gestation) that echogenic bowel may be detected. This may be an early sign of meconium ileus and therefore justifies analysis of the *CFTR* gene. In Brittany, the first analyses for this indication were done in 1991 (Scotet *et al.*, 2002a). Another way to identify 1 in 4 risk couples is prenatal screening, but such a strategy has never been implemented in Brittany.

Mutation analysis

Identification of mutations in parents of a CF child is a pre-requisite to offer those families PD for subsequent pregnancies. We have proposed the following molecular strategy: (1) a screening with a kit looking for the 30 most common *CFTR* mutations in our population leading to a mutation detection rate of 85% (various kits are marketed), (2) then if only one or no mutation is identified, a screening of the *CFTR* gene by denaturing high-performance liquid chromatography (DHPLC)/sequencing (Le Maréchal *et al.*, 2001) and, if necessary, a search for large rearrangements in the gene (Audrézet *et al.*, 2004). Such a strategy allows identifying 98.5% of the *CFTR* mutations in our population (Scotet *et al.*, 2002b).

Data collection

For each PD, the following data were recorded: the date of the examination, the place of residence of the mother/couple at the time of diagnosis, the result of the PD, the genotype of the foetus, the pregnancy outcome and the reason for PD (i.e. how the 1 in 4 risk was discovered).

Statistical analysis

Statistical analysis was conduced using EPI-INFO software (version 6.04, CDC, Atlanta, GA, USA). The significance level was set at $p \leq 0.05$.

First, we described the distribution of PDs according to the context in which the 1 in 4 risk was discovered. Then, we reported the proportion of foetuses CF-affected, heterozygous or without *CFTR* mutations (this, globally and for each indication), and compared it to the theoretical distribution (25, 50 and 25%) using the Chi-square test. We also described the outcome of the pregnancies when CF affected, and calculated the proportion of couples who opted for a pregnancy termination after a positive PD.

For all analyses, we distinguished the PDs made in the 1 in 4 risk couples from those made directly following a finding of echogenic bowel. The couples in whom echogenic bowel is diagnosed during pregnancy and who prove to be 1 in 4 risk couples have a risk of CF of up to 100% for the pregnancy concerned. In this case, PD generally enables to confirm the diagnosis of CF in the

foetus. In such pregnancies, the risk of CF is therefore higher than the theoretical 25%; this is why these PDs were presented separately from the others.

<div align="center">RESULTS</div>

Over the 18-year period (1989–2006), we recorded 268 PDs of CF made in 1 in 4 risk couples living in Brittany, plus 22 PDs made directly following the sonographic finding of echogenic bowel during pregnancy in couples living in that region.

Prenatal diagnosis of CF in 1 in 4 risk couples

A total of 268 PDs were made in 165 1 in 4 risk couples living in Brittany, 2 of whom had a twin pregnancy. The number of PDs made per woman ranged from one to five over the study period. As illustrated in Table 1 and in Figure 1, nearly three quarters of these PDs were done in couples who already had one or more CF children ($n = 195$, 72.8%). Close to 20% were consecutive to cascade screening in families ($n = 49$, 18.3%), whereas 8.2% were done in couples whose 1 in 4 risk was identified following the detection of echogenic bowel in a previous pregnancy ($n = 22$). Finally, two PDs were made in one couple without previous history of CF but who lived in a town of Brittany where the incidence of CF is known to be particularly high. This couple was particularly aware of the severity of the disease and requested PD in order to reduce its anxiety.

Over the 18-year period, PD led to identify 74 CF-affected foetuses (27.7%) and 193 healthy ones [including 137 heterozygous (51.3%) and 56 without *CFTR* mutations (21.0%)], which is consistent with the theoretical distribution (Chi-square = 1.4, $p = 0.499$). Moreover, there was one false-negative result in our area in 1989, at the time of indirect analysis using RFLP markers. In this case, PD indicated that the foetus was heterozygous whereas it was CF-affected (genotype: F508del/F508del). This led to the birth of another affected child to a couple who already had one CF child aged 5 years at that time.

Globally, PD enabled the birth of 191 healthy children in 1 in 4 risk couples (one intra-uterine death and one elective abortion occurred in the group of 193 healthy foetuses). The 74 pregnancies for which the diagnosis proved to be positive were followed by 70 terminations,
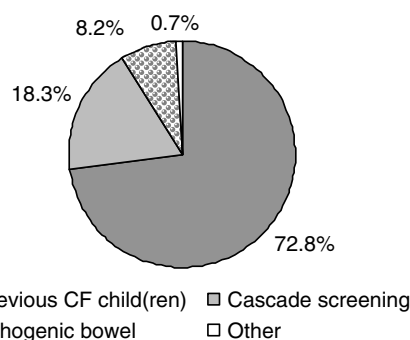

Figure 1—Reasons for prenatal diagnoses made over the period 1989–2006 in 1 in 4 risk couples living in Brittany

one miscarriage and three births of CF children. These births occurred in three couples who deliberately chose to continue the pregnancy (for one of them it was for religious convictions). Each of these couples already had an affected child with a classic form of CF who were, respectively, aged 6, 12 and 13 years at the time of the positive PD [genotypes: F508del/N1303K ($n = 2$) and F508del/F508del].

The proportion of couples who opted for a pregnancy termination after a positive PD was therefore 95.9% in our population (70/73 by excluding the miscarriage). For the large majority of these couples ($n = 68$, 97.1%), the foetus carried a genotype associated with a classic form of CF, whereas in two couples the genotype was associated with a mild form (F508del/R347L and F508del/R553G).

Prenatal diagnosis in the parents of one or more CF children

As mentioned above, parents of CF children used PD 195 times over the study period, which represented 72.8% of all the PDs made during that period in 1 in 4 risk couples living in Brittany. These PDs were done in 124 couples and led to the identification of 55 CF foetuses (28.4%), 96 carrier foetuses (49.5%) and 43 foetuses without *CFTR* mutation (22.2%) (data excluding the diagnostic error described above). The two twin pregnancies of the series occurred in two couples who already had one CF child. The PD identified a CF foetus and a carrier one in the first couple, and two carrier foetuses in the second one. The outcome of the 55 pregnancies with a CF-affected foetus was 51 terminations, one intra-uterine death and three births (described above).

Table 1—Distribution of prenatal diagnoses made over the period 1989-2006 in 1 in 4 risk couples living in Brittany

Reason for PD	PDs		Couples		No. of PDs per couple	Positive PDs		Terminations	
	No.	Freq. (%)	No.	Freq. (%)		No.	Freq. (%)	No.	Freq. (%)
Previous CF child(ren)	195	72.8	124	75.1	1.6	55	28.2	51	92.7
Cascade screening	49	18.3	26	15.8	1.9	13	26.5	13	100.0
Echogenic bowel	22	8.2	14	8.5	1.6	6	27.3	6	100.0
Other	2	0.7	1	0.6	2.0	0	0.0	0	—
Total	268	100.0	165	100.0	1.6	74		70	

More than 60% of those PDs were made in couples whose first CF child was identified through the NBS programme ($n = 121$, 62.1%). In those couples, the mean interval between the identification of the screened CF child and the uptake of PD was 3.0 years (range: 0.7 to 7.3).

Prenatal diagnosis in couples whose 1 in 4 risk was identified through cascade screening

Among the 49 PDs made in couples whose 1 in 4 risk was identified through cascade screening, 87.8% were done in couples related to a CF patient ($n = 43$), whereas 12.2% were done in couples related to a carrier child identified through NBS ($n = 6$).

Extended testing in the families of CF patients led to the identification of 21 new 1 in 4 risk couples who used PD over the study period. The relationship with the proband is presented in Figure 2. Those couples opted for PD 43 times over the study period. A total of 12 CF foetuses were identified (27.9%) and pregnancy was terminated in all cases. Over the same period, the birth of a CF child occurred in one couple in whom the mother was the aunt of a CF child. Cascade screening was performed in this family, but the *CFTR* mutation present in her husband ($3272-26A > G$) did not belong to the panel of mutations searched for at that time.

Cascade screening is also offered to families in whom a carrier is identified through the NBS programme. Five new 1 in 4 risk couples were detected in this way in Brittany (four parents of a carrier child and one uncle). They opted for PD six times over the study period. One CF foetus was identified and the pregnancy was terminated.

Globally, cascade screening enabled detection of 26 new 1 in 4 risk couples who used PD over the 18-year period. The PDs made in these couples led to the birth of 36 healthy children (27 carrier foetuses and nine without *CFTR* mutation) and to the detection of 13 CF foetuses (all these pregnancies were terminated).
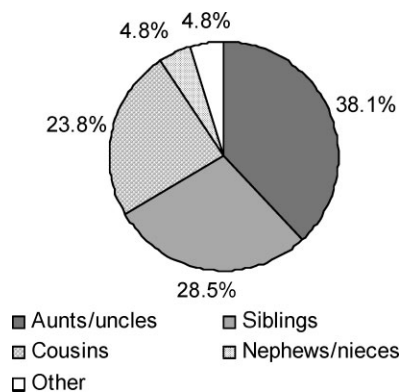

Figure 2—Cascade screening in families of CF patients: relationship with the proband of the new 1 in 4 risk couples identified over the study period

Prenatal diagnosis in couples whose 1 in 4 risk was identified following the sonographic finding of echogenic bowel in a previous pregnancy

The discovery that a couple has a 1 in 4 risk of CF may follow diagnosis of foetal echogenic bowel during pregnancy. Such couples can request PD for subsequent pregnancies. In our region, a total of 14 couples whose 1 in 4 risk was discovered in this way used PD 22 times for subsequent pregnancies over the study period. These PDs represented 8.2% of the PDs made over the period 1989–2006 in couples living in Brittany. Among the 22 foetuses, six had CF (27.3%) and all these pregnancies were terminated.

Prenatal diagnosis of CF directly following the sonographic finding of echogenic bowel

In addition to these 268 PDs, 22 others were done directly following the detection of echogenic bowel upon ultrasound examination during pregnancy. All 22 foetuses had CF. Eighteen of the 22 couples chose pregnancy termination (81.8%), whereas four decided to continue the pregnancy leading to the birth of four CF children. Over the same time, another birth of a CF child occurred in one couple in whom echogenic bowel was diagnosed but in whom the second *CFTR* mutation was not identified at the time of examination (Q220X). This mutation did not belong to the panel of mutations systematically searched for at that time.

DISCUSSION

The present study reports the long experience in the field of PD of CF of a region where this disease is frequent and where the uptake of PD is particularly common. Over the 18-year period, a total of 268 PDs were made in 1 in 4 risk couples living in Brittany. This enabled the birth of 191 healthy children in high-risk couples who would perhaps not have considered having other children without this opportunity. To our knowledge, few teams have reported their experience in this field (Baranov *et al.*, 1992; Jedlicka-Köhler *et al.*, 1994; Casals *et al.*, 1996).

This study was based on the collection over a long period of all the PDs of CF made in couples living in one region. One cannot exclude that some diagnoses have been missed. Nevertheless, the particular situation of Brittany means that collection of PDs here is probably exhaustive. Located at the western end of Europe, Brittany is a large peninsula which has, for a long time, been considered as a geographically and culturally isolated region. Its geographical situation means that the data are well centralised by the two genetic laboratories of the region. Nevertheless, we validated the information by contacting a third laboratory and by cross-checking with other data sources (medical genetics units of the region).

Despite the progress accomplished over recent decades in the field of CF and the consequent increase in patients'

life expectancy, most at-risk couples request PD in our region. In 2004, De Braekeleer *et al*. assessed the reproductive behaviour of 124 couples with a CF child treated in a CF centre in Brittany. They showed that the large majority of those couples were in favour of PD (95%), and that 76% would terminate the pregnancy if PD indicated that their foetus has CF (De Braekeleer *et al*., 2004). Similarly, Sawyer *et al*. analysed the reproductive attitudes of a cohort of parents with a CF child diagnosed through NBS in Australia (Sawyer *et al*., 2006). Globally, 82% of those couples declared that they would use PD in a subsequent pregnancy, and 56% that they would opt for pregnancy termination if their foetus has CF. The situation is different in the US where parents of CF children choose PD only for 20–25% of subsequent pregnancies (Mischler *et al*., 1998).

The present study confirms that, despite the improvement in patients' life expectancy, the great majority of 1 in 4 risk couples (95.9%) opt for pregnancy termination in the case of a positive PD in our region (with a rate even higher than that reported by De Braekeleer *et al*.). This observed percentage is based on an experience over 18 years, whereas the previous studies have usually transcribed what would be the behaviour of couples in such situations (projections of behaviour). Therefore, this study reflects the real decisions of couples as regards pregnancy termination after a positive PD. The high percentage of terminations observed in our population should be re-examined in the coming years, as decisions of families will probably be influenced by the fact that CF is no more exclusively a paediatric disease and that patients' life expectancy is increasing. With this improvement in life expectancy, ethical problems will arise in the field of PD of CF. Indeed, we will be confronted with more delicate decisions, notably regarding the legitimacy of PD in couples in whom one of the members carries a mild or atypical mutation.

PD of CF has dramatically changed since its development. Initially, it was only proposed to the couples with one or more CF children. Thereafter, cascade screening in families and ultrasound examination of pregnancies has also enabled detection of new 1 in 4 risk couples susceptible to request PD. In our region, while the majority of the PDs were done in couples who already had CF children (72.9%), close to one-fifth followed cascade screening which is widely offered to families. Cascade screening has thus enabled detection of 26 new 1 in 4 risk couples who used PD over the study period. This led to the birth of 36 healthy children to those couples who systematically chose to terminate the pregnancies in which PD identified a CF foetus.

PD in 1 in 2 risk couples is also becoming a reality. Indeed, as more and more CF patients reach adulthood nowadays, the proportion of patients who plan pregnancy is increasing. We are therefore confronted to a growing number of CF patients who request an analysis of the *CFTR* gene in their partner (about 12 cases per year in our experience). This will soon lead us make PD in 1 in 2 risk couples.

Among all the PDs collected over the study period, the test misled only once (error rate: 0.4%). This false-negative result occurred at the beginning of 1989, when PD was made indirectly using RFLP markers. The reliability of PD was significantly improved with the direct analysis of *CFTR* mutations from the end of 1989 and we have observed no false-negative result in our region since application of this strategy.

In order to be able to offer reliable PD, a family segregation analysis must be performed to validate the paternity and to check the transmission of the paternal and maternal mutations. This aims to discount rare cases of *de novo* mutation (i.e. new mutation not inherited from either parent) or of uniparental disomy (i.e. inheritance of both chromosomes from only one parent). *De novo* mutations are rare but not entirely exceptional because we recorded two cases (estimated frequency: 1/1000). The first one was found in a CF boy diagnosed through NBS in Brittany. This child received the F508del allele from his mother, whereas the paternal mutation remained unidentified for a few years. The existence of a *de novo* mutation was evidenced when the *CFTR* gene was analysed in the boy's young sister (she carried the same paternal chromosome but was not CF-affected). The second one was identified in a CF patient carrying the $3272-26A > G/296+1G > T$ genotype. The $296+1G > T$ mutation was not found in his father, whereas the paternity was validated. The phenomenon of uniparental disomy, which is also rare, was found once in our experience, in a girl carrying the F508del/F508del genotype. The F508del mutation was not found in her mother (Le Caignec *et al*., 2007). These two phenomena can lead to diagnostic errors, especially for PD made following a diagnosis of echogenic bowel. Therefore, in order to avoid false results, it is essential in such cases to study both the parents and the foetus.

Despite progress in PD strategy over recent decades, this test is still based on invasive methods which are not devoid of risk. The risk of miscarriage after a PD has been estimated to be 2% for chorionic villus sampling and from 0.5 to 1% for amniocentesis (Wilson, 2000). The recent development of a non-invasive method based on foetal cells circulating in maternal blood seems to be promising, but it is not used as a routine test at this time (Saker *et al*., 2006).

The availability of PD of CF with the possibility of a pregnancy termination for affected foetuses may have repercussions on disease incidence. Combining the data of the present study with those of our NBS programme, we were able to confirm that PD led to a modification in the incidence rate close to 30% over the study period (Scotet *et al*., 2007). The recent development of pre-implantation genetic diagnosis will also, in the future, affect the incidence of CF in the countries in which it is applied (Sermon *et al*., 2007).

REFERENCES

Audrézet MP, Chen JM, Raguénès O, *et al*. 2004. Genomic rearrangements in the *CFTR* gene: extensive allelic heterogeneity and diverse mutational mechanisms. *Hum Mutat* **23**: 343–357.

Baranov VS, Gorbunova VN, Ivaschenko TE, *et al*. 1992. Five years' experience of prenatal diagnosis of cystic fibrosis in the former U.S.S.R. *Prenat Diagn* **12**: 575–586.

Beaudet AL, Spence JE, Montes M, *et al*. 1988. Experience with new DNA markers for the diagnosis of cystic fibrosis. *N Engl J Med* **318**: 50–51.

Beaudet AL, Feldman GL, Fernbach SD, *et al*. 1989. Linkage disequilibrium, cystic fibrosis, and genetic counseling. *Am J Hum Genet* **44**: 319–326.

Boué A, Muller F, Nezelof C, *et al*. 1986. Prenatal diagnosis in 200 pregnancies with a 1-in-4 risk of cystic fibrosis. *Hum Genet* **74**: 288–297.

Brambati B, Tului L, Fattore S. 1993. First-trimester fetal screening of cystic fibrosis in low-risk population. *Lancet* **342**: 624.

Brock DJ. 1983. Amniotic fluid alkaline phosphatase isoenzymes in early prenatal diagnosis of cystic fibrosis. *Lancet* **2**: 941–943.

Brock DJ. 1984. Amniotic fluid alkaline phosphatase isoenzymes in early prenatal diagnosis of cystic fibrosis. *Lancet* **1**: 102.

Casals T, Gimenez J, Ramos MD, *et al*. 1996. Prenatal diagnosis of cystic fibrosis in a highly heterogeneous population. *Prenat Diagn* **16**: 215–222.

De Braekeleer M, Rault G, Bellis G. 2004. Reproductive attitudes of couples having a child with cystic fibrosis in Brittany (France). *J Hum Genet* **49**: 285–289.

Dicke JM, Crane JP. 1992. Sonographically detected hyperechoic fetal bowel: significance and implications for pregnancy management. *Obstet Gynecol* **80**: 778–782.

Farrall M, Law HY, Rodeck CH, *et al*. 1986. First-trimester prenatal diagnosis of cystic fibrosis with linked DNA probes. *Lancet* **1**: 1402–1405.

Férec C, Verlingue C, Audrézet MP, *et al*. 1993. Prenatal diagnosis of cystic fibrosis in different European populations: application of denaturing gradient gel electrophoresis. *Fetal Diagn Ther* **8**: 341–350.

Jedlicka-Köhler I, Götz M, Eichler I. 1994. Utilization of prenatal diagnosis for cystic fibrosis over the past seven years. *Pediatrics* **94**: 13–16.

Le Caignec C, Isidor B, De Pontbriand U, *et al*. 2007. Third case of paternal isodisomy for chromosome 7 with cystic fibrosis: a new patient presenting with normal growth. *Am J Med Genet A* **143**: 2696–2699.

Le Maréchal C, Audrézet MP, Quéré I, *et al*. 2001. Complete and rapid scanning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene by denaturing high-performance liquid chromatography (D-HPLC): major implications for genetic counselling. *Hum Genet* **108**: 290–298.

McIntosh I, Raeburn JA, Curtis A, *et al*. 1989. First-trimester prenatal diagnosis of cystic fibrosis by direct gene probing. *Lancet* **2**: 972–973.

Mennie ME, Gilfillan A, Compton M, *et al*. 1992. Prenatal screening for cystic fibrosis. *Lancet* **340**: 214–216.

Mischler EH, Wilfond BS, Fost N, *et al*. 1998. Cystic fibrosis newborn screening: impact on reproductive behavior and implications for genetic counseling. *Pediatrics* **102**: 44–52.

Novelli G, Sangiuolo F, Dallapiccola B, *et al*. 1990. Delta F508 gene deletion and prenatal diagnosis of cystic fibrosis in Italian and Spanish families. *Prenat Diagn* **10**: 413–414.

Saker A, Benachi A, Bonnefont JP, *et al*. 2006. Genetic characterisation of circulating fetal cells allows non-invasive prenatal diagnosis of cystic fibrosis. *Prenat Diagn* **26**: 906–916.

Sawyer SM, Cerritelli B, Carter LS, *et al*. 2006. Changing their minds with time: a comparison of hypothetical and actual reproductive behaviors in parents of children with cystic fibrosis. *Pediatrics* **118**: e649–e656.

Scotet V, De Braekeleer M, Roussey M, *et al*. 2000. Neonatal screening for cystic fibrosis in Brittany, France: assessment of 10 years' experience and impact on prenatal diagnosis. *Lancet* **356**: 789–794.

Scotet V, De Braekeleer M, Audrézet MP, *et al*. 2002a. Prenatal detection of cystic fibrosis by ultrasonography: a retrospective study of more than 346,000 pregnancies. *J Med Genet* **39**: 443–448.

Scotet V, Gillet D, Duguépéroux I, *et al*. 2002b. Spatial and temporal distribution of cystic fibrosis and of its mutations in Brittany, France: a retrospective study from 1960. *Hum Genet* **111**: 247–254.

Scotet V, Assael BM, Duguépéroux I, *et al*. 2007. Time trends in birth incidence of cystic fibrosis in two European areas: Data from newborn screening programmes. *J Pediatr* **152**: 25–32.

Sermon KD, Michiels A, Harton G, *et al*. 2007. ESHRE PGD Consortium data collection VI: cycles from January to December 2003 with pregnancy follow-up to October 2004. *Hum Reprod* **22**: 323–336.

Southern KW, Munck A, Pollitt R, *et al*. 2007. A survey of newborn screening for cystic fibrosis in Europe. *J Cyst Fibros* **6**: 57–65.

Super M, Schwarz MJ, Malone G, *et al*. 1994. Active cascade testing for carriers of cystic fibrosis gene. *BMJ* **308**: 1462–1467.

Tsui LC. 2008. Cystic Fibrosis Mutation Database. http://www.genet.sickkids.on.ca/.

Welsh MJ, Ramsey BW, Accurso FJ, Cutting GR. 2001. Cystic fibrosis. In *The Metabolic and Molecular Basis of Inherited Disease* (8th edn), Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Vogelstein B (eds). McGraw Hill: New York; 5121–5188.

Wilson RD. 2000. Amniocentesis and chorionic villus sampling. *Curr Opin Obstet Gynecol* **12**: 81–86.