ORIGINAL ARTICLE

Non-invasive prenatal diagnosis for cystic fibrosis: detection of paternal mutations, exploration of patient preferences and cost analysis

Melissa Hill[1,2†], Philip Twiss[1†], Talitha I. Verhoef[3†], Suzanne Drury[1], Fiona McKay[1], Sarah Mason[1], Lucy Jenkins[1], Stephen Morris[3] and Lyn S. Chitty[1,2*]

[1]North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[2]Genetics and Genomic Medicine, UCL Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust, London, UK
[3]Research Department of Applied Health Research, University College London, London, UK
*Correspondence to: Lyn Chitty. E-mail: l.chitty@ucl.ac.uk
†These authors contributed equally to this work.

ABSTRACT

Objectives We aim to develop non-invasive prenatal diagnosis (NIPD) for cystic fibrosis (CF) and determine costs and implications for implementation.

Methods A next-generation sequencing assay was developed to detect ten common CF mutations for exclusion of the paternal mutation in maternal plasma. Using uptake data from a study exploring views on NIPD for CF, total test-related costs were estimated for the current care pathway and compared with those incorporating NIPD.

Results The assay reliably predicted mutation status in all control and maternal plasma samples. Of carrier or affected adults with CF ($n = 142$) surveyed, only 43.5% reported willingness to have invasive testing for CF with 94.4% saying they would have NIPD. Using these potential uptake data, the incremental costs of NIPD over invasive testing per 100 pregnancies at risk of CF are £9025 for paternal mutation exclusion, and £26 510 for direct diagnosis.

Conclusions We have developed NIPD for risk stratification in around a third of CF families. There are economic implications due to potential increased test demand to inform postnatal management rather than to inform decisions around termination of an affected pregnancy. © 2015 The Authors. *Prenatal Diagnosis* published by John Wiley & Sons, Ltd.

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INTRODUCTION

Cystic fibrosis (CF) is a severe, autosomal recessive, multi-system condition, predominantly affecting the respiratory and digestive systems. CF is caused by mutations, of which there are more than 1900, in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The prevalence of CF is 1 in 2500 to 1 in 3500 live births, and for people with Northern European ancestry, the carrier frequency is 1 in 25.[1] Advances in multi-disciplinary care have improved long-term outcomes for people with CF. In the UK, median survival is now 43 years.[2,3]

Prenatal diagnosis of CF currently requires an invasive test to obtain fetal genetic material and so carries a small risk of miscarriage[4] but is an option that is valued by carrier couples as it allows them to either plan and prepare for the birth of an affected child or make decisions about termination of pregnancy.[5] Non-invasive prenatal diagnosis (NIPD) based on analysis of cell-free fetal DNA (cffDNA) in maternal plasma has been reported to exclude the paternal mutation in couples carrying different CF mutations.[6–8] However, these reports described analyses developed for individual families, a labour-intensive approach for implementation into routine clinical practice. Here, we describe the development of a next-generation sequencing (NGS) assay designed to detect or exclude ten of the most common CF mutations, for use when each parent carries a different *CFTR* mutation and the paternal mutation is one of the ten included in this panel. To inform implementation strategies, we also report a cost analysis of NIPD for CF, which was informed by an exploration of stakeholder preferences through a questionnaire-based survey of potential service users and providers.[9]

METHODS

Ethical approval

National Health Service Research Ethics Committee approval was obtained for the collection of maternal and paternal blood samples for NIPD test development (01/0095) and for the questionnaire study (10/H0714/3).

Development of the NIPD assay

Sample collection

Normal and heterozygous genomic DNA (gDNA) control samples with known *CFTR* mutations were ascertained from our Regional Genetics Laboratory records and used to assess test performance, before testing on maternal plasma samples collected, as part of a larger programme designed to develop standards for NIPD (RAPID RP-PG-0707-10107), from women undergoing invasive diagnostic prenatal testing because of a risk of CF. Outcome of invasive testing was known in all cases.

Sample processing and DNA extraction

Maternal plasma samples were separated from 20 mL of blood within 48 h of blood draw and processed for storage at −80 °C as previously described.[10] The cell free DNA (cfDNA) was extracted from 4 mL of maternal plasma using the QIAsymphony into a final volume of 60 μL EB buffer (Qiagen). The gDNA was extracted from 2 mL of blood with the Quickgene-610L Nucleic Acid Isolation System (Fujifilm) into a final volume of 200 μL elution buffer.

Next-generation DNA sequencing

Ten *CFTR* mutations commonly seen in our regional genetics service, including those on the UK neonatal screening panel, were selected. A targeted amplicon approach was used. PCR primers were designed, using Primer 3 software, to amplify five amplicons of *CFTR* covering these ten mutations (Table 1) and the *ZFX/ZFY* and *HLA-B* genes. Each amplicon was individually optimised. Mutation targets for each patient DNA sample were given a common molecular barcode and were amplified in individual single-plex PCRs. PCR was carried out using 10 μL of Phusion High-Fidelity PCR Master Mix (NEB), 500 nM of each primer in a final reaction volume of 20 μL. Cycling conditions were 98 °C for 1 min, followed by 42 cycles of 98 °C for 10 s, 64 °C for 30 s and 72 °C for 30 s, followed by 72 °C for 10 min. Amplicons were pooled and purified using a MinElute PCR Purification Kit (Qiagen), quantified using a Qubit dsDNA BR Assay Kit (Invitrogen) and amplicon quality assessed using a Bioanalyzer (Agilent). Purified PCR products were diluted to 2 nM in Elution Buffer (Qiagen), and samples were pooled to give a single 2 nM library. This pooled library was denatured using 2 M sodium hydroxide, diluted to 8 pM and mixed with an 8 pM PhiX control to give a 20% PhiX spike, providing sequence diversity. The library was sequenced with the Illumina MiSeq using a single-end 100-cycle protocol. After de-multiplexing, the number of reads containing the wild-type and mutant alleles was counted for each target site. The presence of fetal DNA was confirmed by the detection of paternal *CFTR* sequences, *ZFY* or paternal *HLA*-type sequences in the maternal plasma.

Table 1 **Mutations in the cystic fibrosis transmembrane conductance regulator gene that have been included in the next-generation sequencing panel**

cDNA	Protein
c.489 + 1G > T	
c.1521_1523delCTT	p.(Phe508del)
c.1519_1521delATC	p.(Ile507del)
c.1624G > T	p.(Gly542*)
c.1646G > A	p.(Ser549Asn)
c.1647T > G	p.(Ser549Arg)
c.1652G > A	p.(Gly551Asp)
c.1657C > T	p.(Arg553*)
c.1679G > C	p.(Arg560Thr)
c.3846G > A	p.(Trp1282*)

Estimation of assay applicability

The ten mutations included in the NGS assay have a combined allele frequency of 77%.[11] We estimated how many couples would be eligible to use this assay using the reported allele frequency[11] for each mutation on the panel to determine the probability that the father has the mutation and the mother does not have the mutation (Table 2).

Health economics

To assess the economic consequences of implementing NIPD for prenatal diagnosis of CF, we estimated the total test-related costs of three different clinical pathways: the current invasive testing only pathway, NIPD for the paternal CF mutation and

Table 2 **Probability that the father carries one of the ten common mutations and the mother carries a different mutation to the father**

CFTR mutation	Probability father has the mutation	Probability mother does not have the mutation	Probability father has the mutation and mother has a different mutation
c.489 + 1G > T	0.009	0.991	0.008919
p.Phe508del	0.686	0.314	0.215404
p.Ile507del	0.003	0.997	0.002991
p.Gly542*	0.024	0.976	0.023424
p.Ser549Asn	0.001	0.999	0.000999
p.Ser549Arg[a]	—	—	—
p.Gly551Asp	0.021	0.979	0.020559
p.Arg553*	0.009	0.991	0.008919
p.Arg560Thr	0.002	0.998	0.001996
p.Trp1282*	0.014	0.986	0.013804
Total			0.297015

Allele frequencies obtained from Bobadilla *et al.*[11]

CFTR, cystic fibrosis transmembrane conductance regulator.

[a]Allele frequency not reported.

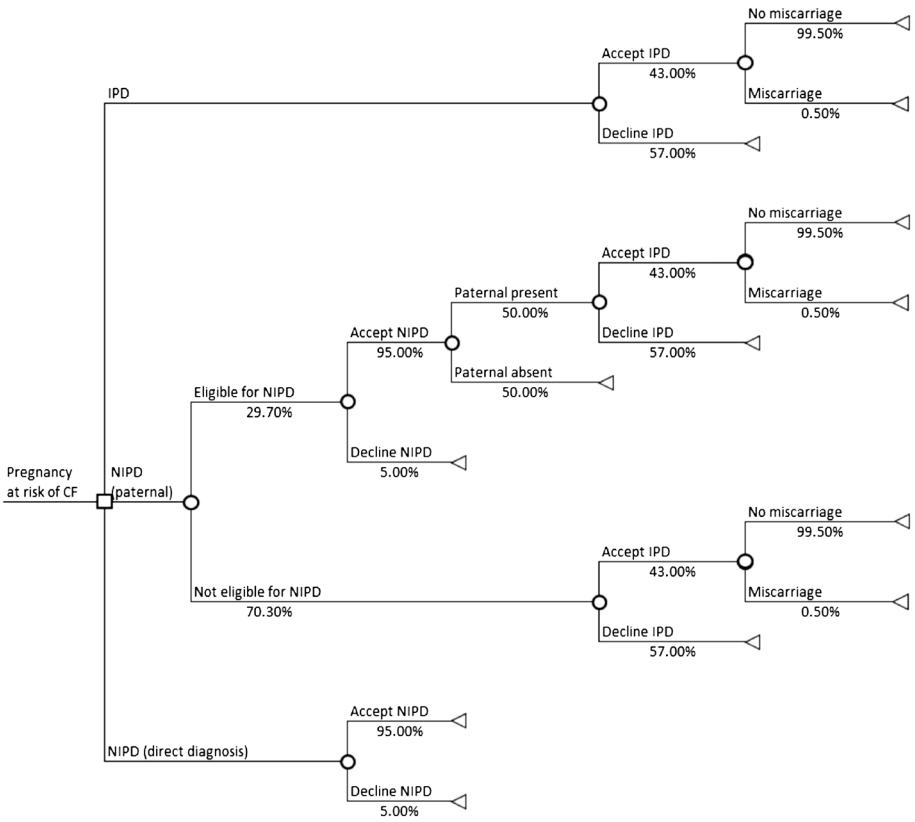

Figure 1 Decision tree depicting the clinical pathways of current invasive testing only, non-invasive prenatal diagnosis (NIPD) for the paternal cystic fibrosis (CF) mutation and NIPD for direct diagnosis

NIPD for direct diagnosis (Figure 1). A questionnaire-based study of stakeholder views and preferences was undertaken to estimate the uptake of invasive testing and NIPD with detailed results published elsewhere.[9] In brief, adult patients with CF and carriers of CF attending one children's and one adult NHS regional specialist CF centre serving large geographical areas were invited to complete a questionnaire while waiting to see the clinician, or to complete it at home and return it via reply paid envelope. The questionnaire addressed views on key attributes of NIPD, assessed attitudes towards cffDNA testing for CF and asked whether they had had, or would have, an invasive test for CF, if they would have NIPD if available and what their reasons for testing are. The current price of prenatal molecular testing for CF (£370) and NIPD for paternal exclusion (£550) in our Regional Genetics laboratory were used. An estimated price of £750 for the NIPD test currently under development for direct diagnosis was used. Costs of sending in the NIPD sample (£5) and cost of performing the invasive test (£750), which includes chorionic villus sampling, counselling, quantitative fluorescent PCR (QF-PCR) and karyotyping, were obtained from our local fetal medicine unit. NHS reference costs were used to determine the phlebotomy costs, and the cost of feeding back the NIPD results was estimated from Unit Costs of Health and Social Care.[12] The number of women undergoing each test and the total costs of the tests were calculated per 100 pregnancies at risk of CF. As the proportion of carrier parents eligible for NIPD for paternal exclusion is approximately 29.7%, in this pathway, we assumed that the remaining parents were offered invasive testing only. Lastly, we calculated the total number of procedure-related miscarriages for each pathway. Input parameters for the economic analysis are shown in Table 3. In view of the uncertainty around the number of eligible parents for NIPD for parental exclusion, the uptake of NIPD and IPD and the costs of NIPD, we varied these parameters over a wide range in a sensitivity analysis on the incremental costs of each NIPD pathway compared with the current pathway.

RESULTS

NGS assay development

All eight mutations in the gDNA samples from CF carriers were reliably detected at an allele frequency of 50%. Three normal control samples gave either no or extremely low numbers of mutation reads (zero to 0.09% of wild-type reads) across all amplicons (Table 4). Sequencing of three controls, using spiked mutant gDNA with three *CFTR* mutations with an expected mutant allele frequency of 10%, gave percentage mutant allele reads of 10.4%, 8.8% and 4.4% thus demonstrating detection of low level mutant allele targets (Table 4).

In all four maternal plasma cell-free DNA samples, it was possible to accurately determine inheritance of the paternal mutant allele (Table 5), and the results of NGS panel testing were concordant with mutation status determined by

Table 3 Input parameters for the economic analysis

Parameter	Value	Source
Proportion of carrier parents eligible for paternal mutation NIPD	29.7%	Table 1 – supplementary information
Uptake invasive testing	43.0%	Questionnaire results
Uptake NIPD	95.0%	Questionnaire results
Cost of invasive molecular testing for CF	£370	Regional Genetics laboratory
Cost of counselling, invasive test and cytogenetics	£750	Local fetal medicine unit
Cost of NIPD to exclude paternal CF mutation	£550	Regional Genetics laboratory
Cost of NIPD to directly diagnose CF	£750	Estimation from the Regional Genetics laboratory
Cost of phlebotomy	£4	NHS reference costs[14]
Cost of sending in NIPT sample	£5	Local fetal medicine unit
Cost of feedback NIPT results	£27	Unit Costs of Health and Social Care[15]
Risk of procedure-related miscarriage with invasive testing	0.5%	Tabor *et al.*[4]

Total costs of invasive testing were £370 + £750 = £1120. Total costs of NIPD were £550 + £4 + £5 + £27 = £586 for paternal exclusion and £750 + £4 + £5 + £27 = £786 for direct diagnosis. Costs of pretest genetic counselling and ultrasound for dating and exclusion of multiple pregnancies are not included as it applies equally to all scenarios.
NIPD, non-invasive prenatal diagnosis; CF, cystic fibrosis.

traditional testing in all cases. In families 2 and 3, very low (0.02%) or absence of paternal mutation counts in maternal plasma indicated transmission of the wild-type paternal allele. This is consistent with an unaffected fetus at 50% risk of having inherited the maternal mutation, which would be compatible with carrier status. In families 1 and 4, low but significant paternal mutation counts in the maternal plasma (15.9% and 5.9% respectively), which were not seen (family 1) or seen in very low level (0.02%) in the maternal gDNA (family 4), indicated that the fetus had inherited the paternal mutation and was at least a carrier of CF; further testing would be required to determine if the maternal mutation had been inherited.

Applicability of the NGS assay using the ten mutations

An estimated 11.9% of carrier parents in the UK will be heterozygous for a mutation in this NGS panel. As the maternal mutation does not need to be on the panel, we estimate that 29.7% of CF carrier parents in the UK could be offered testing (Table 2).

Stakeholder views and preferences

One hundred and forty-two potential service users responded to the questionnaire (88.9%). For those affected with CF ($n = 92$) 45.7% were female, while for carriers of CF ($n = 50$), the majority were female (72%) and 91.7% had a child affected with CF. The group was well educated, 42.8% having college or other training and 21.7% a degree or equivalent.[9] Of these 142 service users, 131 answered the questions regarding uptake of invasive or NIPD testing. More participants said they would decline (74/131) than would accept (57/131) invasive diagnostic testing for CF. The most common reasons for wanting prenatal diagnosis were 'to prepare for the possible birth of a baby with CF' ($n = 33$; 62.3%) and to 'help make a decision about whether or not to continue the pregnancy' ($n = 17$; 32.1%). Most participants ($n = 130$; 94.9%) said that they would choose NIPD for CF and 90% would be prepared to pay for it, with 49.2% prepared to pay up to £50, 39.0% prepared to pay £100–200 and 10.3% prepared to pay more than £200. One hundred and fourteen participants described potential benefits of NIPD. These fell into five main categories: no miscarriage risk ($n = 90$), simpler and less stressful test ($n = 22$), preparation for the birth of an affected child ($n = 20$), informing decisions around termination of pregnancy ($n = 6$), earlier testing ($n = 6$) and more people likely to accept a blood test ($n = 9$). The 115 responses to questions regarding concerns fell into three main categories: no concerns ($n = 101$), increase in terminations ($n = 13$) and increased pressure to terminate ($n = 2$). No significant differences were seen between responses of carriers and those affected with CF.

Health economics

Total costs were £586 for NIPD for paternal exclusion, £786 for direct diagnosis and £1120 for invasive testing. From the questionnaire responses, we estimated 43% of women at risk of having a baby with CF would have IPD at a total cost of £48 160 per 100 pregnancies at risk, and 28/100 would undergo NIPD for paternal exclusion if available with a total of 36/100 who would undergo invasive testing (six after NIPD, 30 without NIPD). The total cost for this pathway per 100 women was £57 185. We further estimated that 95 women would undergo NIPD for direct diagnosis of CF if available, and none would require invasive testing, costing in total £74 670. NIPD to exclude the paternal CF mutation would therefore increase the costs by £9025, while the incremental costs of NIPD to directly diagnose CF would be £26 510 if compared with the current pathway (Table 6). The rate of miscarriages per 100 pregnancies was low, 0.22 in the current pathway, and implementation of paternal mutation NIPD could lower this to 0.18. No procedure-related miscarriages were expected for NIPD to directly diagnose CF. The sensitivity analysis (Table 7) shows that the incremental costs of each NIPD pathway compared with the current pathway were higher than the base case if more parents were eligible for NIPD (for paternal exclusion pathway only) or if the uptake of NIPD or the costs of NIPD were higher. If the uptake of invasive testing was higher, the incremental costs of both NIPD pathways were lower than the base case value and vice versa. The NIPD pathways were more costly than the current pathway in each scenario.

DISCUSSION

Here, we report the successful development of NIPD for the detection or exclusion of a range of paternal CF mutations. This will reduce the need for invasive diagnostic testing, which will only be required if the paternal mutation is present, as if it is absent, the fetus cannot be affected with

Table 4 **The ten cystic fibrosis transmembrane conductance regulator mutations used on the next-generation sequencing panel showing the validation of the assay**

		Target	Control samples													
	Amplicon	wt	wt	wt	c.489 + 1G > T	c.489 + 1G > T[a]	p. Phe508del	p. Phe508del	p. Phe508del[a]	p. Ile507del	p. Gly542*	p. Gly542*[a]	p.Ser549Arg; p.Arg553*	p. Arg553*	p. Arg560Thr	p. Trp1282*
1	c.489 + 1G > T															
	wt	63 507	80 847	87 904	3790	117 281	-	-	-	-	-	-	-	-	-	-
	Mutation	48	29	45	986	13 624	-	-	-	-	-	-	-	-	-	-
2	p.Phe508del															
	wt	39 505	65 137	53 881	-	-	29 065	16 391	94 324	-	-	-	-	-	-	-
	Mutation	0	0	0	-	-	38 038	24 215	9114	-	-	-	-	-	-	-
2	p.Ile507del															
	wt	39 505	65 137	53 881	-	-	-	-	-	102 390	-	-	-	-	-	-
	Mutation	6	13	0	-	-	-	-	-	73 954	-	-	-	-	-	-
3	p.Gly542*															
	wt	35 070	76 550	61 765	-	-	-	-	-	-	11 479	67 557	-	-	-	-
	Mutation	8	15	5	-	-	-	-	-	-	9323	3093	-	-	-	-
4	p.Ser549Asn															
	wt	43 652	63 265	62 241	-	-	-	-	-	-	-	-	-	-	-	-
	Mutation	9	9	6	-	-	-	-	-	-	-	-	-	-	-	-
4	p.Ser549Arg															
	wt	43 652	63 265	62 241	-	-	-	-	-	-	-	-	6382	-	-	-
	Mutation	25	41	55	-	-	-	-	-	-	-	-	117 728	-	-	-
4	p.Gly551Asp															
	wt	43 652	63 265	62 241	-	-	-	-	-	-	-	-	-	-	-	-
	Mutation	12	7	12	-	-	-	-	-	-	-	-	-	-	-	-
4	p.Arg553*															
	wt	43 652	63 265	6 224 127	-	-	-	-	-	-	-	-	6382	7814	-	-
	Mutation	20	32		-	-	-	-	-	-	-	-	134 550	5825	-	-
4	p.Arg560Thr															
	wt	43 652	63 265	62 241	-	-	-	-	-	-	-	-	-	-	84 779	-
	Mutation	1	0	1	-	-	-	-	-	-	-	-	-	-	99 571	-
5	p.Trp1282*															

(Continues)

wt	68 398	55 077	72 883	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12 500
Mutation	9	13	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11 719
Sex chr marker																			
ZFX	29 051	76 122	33 139	-	152 529	75 726	42 498	103 476	97 466	-	-	154 579	-	234 960	25 651				
ZFY	34 994	53	36 324	-	2	16	51 726	10 674	105 767	-	-	187 497	-	5	0				

- Mutation not tested.
[a]10% spiked genomic DNA sample.

CF. This approach to NIPD for CF has been approved by the UK Genetic Testing Network, the regulatory body responsible for evaluating new genetic tests and recommending their use within the NHS.[13]

Our data clearly indicate the need to optimise PCR conditions for each mutation target and analyse maternal gDNA alongside the plasma sample to assess the level of background in any given sequencing run. In our spiking experiment, one sample showed a significant deviation from the expected level of 10%, and in others, the ratio of wild-type to mutant counts for some heterozygous control samples deviated from the expected level of 50%. It is unclear whether this is due to the quality of individual DNA samples or an intrinsic amplification bias of specific primer pairs. Furthermore, very low 'background' counts were sometimes observed for mutation targets not present in the DNA sample being tested (Table 2, cases 2 and 4). It is likely that these are sequencing or PCR-related errors which have occurred during the early stages of amplification that are well documented in the literature.[14] In cases where no paternal mutation is detected in maternal plasma, the presence of cffDNA should be confirmed by analysis of *ZFX/ZFY* and *HLA-B* sequences to control against false-negative results occurring because of very low levels of cffDNA in the maternal plasma.[15]

To date, the paternal exclusion assays for CF reported in the literature have been developed on a case-by-case basis, usually using PCR-based methodologies, which cannot be applied to multiple different mutations simultaneously in one assay.[6–8] This approach cannot be readily implemented into a service laboratory where significant throughput of assays testing multiple cases or multiple mutations in a single run are required to optimise turnaround times and minimise costs. We have addressed this by developing an NGS assay that incorporates a panel of mutations, which also allows for expansion of the number of mutations tested for and therefore would be of use to more families. This assay can be used to simultaneously test different families for different mutations as well as testing other samples for different conditions. However, the high frequency of the *CFTR* mutation p.Phe508del means an estimated 47% of carrier parents will both carry this mutation and will not be eligible to utilise this test. NIPD for recessive disorders where the parental mutations are the same has been demonstrated using relative mutation dosage.[10,16] These assays are reliant on precise single-molecule counting techniques and accurate assessment of the fetal fraction of cfDNA. The practical limitations of estimating fetal fraction, the number of repeat tests required and the need for a high proportion of cffDNA in the sample have hindered translation into clinical practice. Other approaches to NIPD for direct diagnoses of recessive conditions have been described, including the analysis of single-nucleotide polymorphisms by whole genome sequencing and relative haplotype dosage analysis[17] and targeted NGS.[18] However, costs and time requirements for testing with these methods currently inhibit widespread routine clinical implementation. An approach for offering direct diagnosis when parents carry the same mutation would be to sequence the *CFTR* mutations alongside several informative single-nucleotide polymorphisms

Table 5 Numbers of reads for the wild-type and mutant sequences with the next-generation sequencing assay for ten common cystic fibrosis transmembrane conductance regulator mutations in maternal plasma samples

Family	Sample	Confirmed genotype	p.Phe508del		p.Gly551Asp		c.489 + 1G > T		p.(Arg553*)		Sex chromosome marker	
			wt	Mutant	wt	Mutant	wt	Mutant	wt	Mutant	ZFX	ZFY
1	Maternal gDNA	p.Gly551Asp/wt	106 052	0	60 873	31 429	-	-	-	-	163 337	0
1	Paternal gDNA (not available)	p.Phe508del/wt	-	-	-	-	-	-	-	-	-	-
1	Maternal plasma cfDNA	p.Phe508del/wt	108 237	9364	84 808	62 779	-	-	-	-	120 054	11 060
2	Maternal gDNA	p.Phe508del/wt	58 949	88 064	-	-	-	-	-	-	137 041	11
2	Paternal gDNA	c.489 + 1G > T	-	-	-	-	131 471	68 657	-	-	114 436	145 574
2	Maternal plasma cfDNA	p.Phe508del/wt	54 003	68 117	-	-	108 880	21	-	-	26 298	0
3	Maternal gDNA(not available)	p.(Arg553*)/wt	-	-	-	-	-	-	-	-	-	-
3	Paternal gDNA	p.Phe508del/wt	12 813	21 653	-	-	-	-	23 012	335	17 693	23 163
3	Maternal plasma cfDNA	p.(Arg553*)/wt	45 550	0	-	-	-	-	13 427	13 878	34 688	2398
4	Maternal gDNA	p.Phe508del/wt	20 724	32 876	25 320	6	-	-	-	-	68 069	1
4	Paternal gDNA (not available)	p.Gly551Asp/wt	-	-	-	-	-	-	-	-	-	-
4	Maternal plasma cfDNA	p.Phe508del/p.Gly551Asp	28 076	26 776	22 616	1339	-	-	-	-	8700	29

- Mutation not tested.
gDNA, genomic DNA.

around the *CFTR* locus; this could facilitate calculation of fetal fraction in the maternal plasma[19] and relative mutation dosage could be used to determine inheritance.[18] This approach is under development in our laboratory, and we have used the predicted costs of this assay in the economic analysis to estimate the cost of direct diagnosis of CF using NIPD.

Ideally, implementation of new technologies should be accompanied by stakeholder views and evaluation of costs. Our survey of potential service users showed that interest in NIPD was high, largely due to the improved safety, with over 90% of potential service users reporting they would have NIPD if it was available, compared with 43.5% who would currently consider invasive testing. However, while many stated results would guide decisions about termination, a large proportion would have the test to prepare for the birth of a baby affected with CF. This is compatible with other studies exploring the reproductive choices of couples at risk of CF.[5,20–24] Consequently, it is likely that the high potential uptake of NIPD includes many couples who would currently decline invasive testing and who would want testing for information only, rather than to guide decisions around termination of pregnancy. These findings have clear consequences for the cost of prenatal testing for CF in the NHS as the likely increase in uptake means that the cost of a prenatal diagnostic service based on NIPD, whether for the exclusion of the paternal allele or for direct diagnosis, will be significantly higher than the current care pathway based on invasive testing, even if we take into account that hypothetical uptake of genetic tests can differ from actual uptake.[25–27] When discussing prenatal testing for CF, professionals should be careful to include the fact that early postnatal diagnosis is now available as screening for CF now forms a routine part of neonatal screening in the UK and some other countries, as this may influence decisions of parents committed to continuing the pregnancy. The findings of this cost analysis are quite distinct to a study costing NIPD for fetal sex determination, which found that NIPD was cost neutral for the NHS as the cost of NIPD was offset by the reduction in invasive testing,[28] the key difference being that current rates of invasive testing and termination for serious sex-linked disorders are high by comparison with those for CF.

The use of NIPD for CF for information only raises ethical questions around whether, in a state-funded health care system, directing resources to a test that would not change pregnancy management can be justified.[29] In the UK, NIPD for fetal sex determination is used to direct invasive testing in pregnancies at risk of serious sex-linked conditions. However, this test has not been approved for use in women who are carriers of haemophilia, where termination of an affected pregnancy is increasingly rare and fetal gender could be determined by ultrasound at 20 weeks. Accordingly, some clinical services offer NIPD only when it is likely to change clinical management as the additional costs are not balanced by clinical benefits.[30] The definition of clinical benefit is crucial in such policy decisions as there is evidence that there are psychological benefits to early testing and having information to plan and prepare.[31,32] In addition, decisions may vary from the couple's initial intention once they receive a result.

Table 6 **Results of the economic analysis of prenatal diagnosis for cystic fibrosis per 100 pregnancies**

Pathway	Number of women undergoing NIPD	Total costs of NIPD	Number of women undergoing IPD	Total costs of IPD	Total costs (NIPD + IPD)	Number of procedure-related miscarriages
Current (invasive testing only)	0.00	£0	43.00	£48 160	£48 160	0.22
NIPD (paternal exclusion)	28.22	£16 534	36.30	£40 651	£57 185	0.18
Difference (paternal exclusion) compared with current	+28.22	+£16 534	−6.70	−£7 509	+£9 025	−0.03
NIPD (direct diagnosis)	95.00	£74 670	0.00	£0	£74 670	0.00
Difference (direct diagnosis) compared with current	+95.00	+£74 670	−43.00	−£48 160	+£26 510	−0.22

NIPD, non-invasive prenatal diagnosis; IPD, invasive prenatal diagnosis.

Table 7 **Sensitivity analysis describing the incremental costs of NIPD for paternal exclusion and NIPD for direct diagnosis compared with the current pathway (invasive testing only) at different levels of test uptake and costs**

Scenario	Incremental costs (paternal exclusion)	Incremental costs (direct diagnosis)
Base case scenario	£9025	£26 510
20% eligible for paternal NIPD	£6077	
40% eligible for paternal NIPD	£12 154	
80% Uptake of NIPD	£5341	£14 720
100% Uptake of NIPD	£10 252	£30 440
30% Uptake of IPD	£11 295	£41 070
60% Uptake of IPD	£6056	£7470
Paternal NIPD test costs £300	£1971	
Paternal NIPD test costs £800	£16 078	
Direct NIPD test costs £500		£2760
Direct NIPD test costs £1000		£50 260

NIPD, non-invasive prenatal diagnosis; IPD, invasive prenatal diagnosis.

Notably, most respondents raised no concerns about NIPD for CF, with a very few worried about the potential for NIPD to increase the number of terminations for CF. These findings are in line with those from qualitative interviews with carriers of other single-gene disorders.[31]

Study limitations

The numbers of clinical cases tested are small, and further cases are needed for ongoing validation. The general applicability of the questionnaire study findings may be limited as the study was conducted in only two regional centres and some groups of potential service users are not well represented, including carrier fathers and carriers without children or whose child with CF has died. Furthermore, responses may vary from those made in real life.

CONCLUSIONS

We have successfully developed an NGS assay to allow NIPD to be used for risk stratification in a significant proportion of CF families. Consideration of stakeholders' views and cost-effectiveness alongside test development indicates that introduction of NIPD for CF would be welcomed and uptake is likely to be high. However, as many would use NIPD for CF to inform postnatal management rather than decisions around termination of an affected pregnancy, there are potential economic implications, and further work is required to resolve ethical issues that might arise. These findings may have implications for NIPD for other conditions and highlight the need for prospective consideration of the ethical and economic issues that may arise as more tests are developed.

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WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Non-invasive prenatal diagnosis of some single-gene disorders, including CF, is possible by analysis of cell-free fetal DNA in maternal plasma.

WHAT DOES THIS STUDY ADD?

- Use of a next-generation sequencing panel to detect ten common cystic fibrosis (CF) mutations could provide a flexible approach to non-invasive prenatal diagnosis in around 30% of parents who are carriers of CF mutations.
- Potential service users would welcome the introduction of non-invasive prenatal diagnosis for CF.
- As uptake is likely to be high, increased costs for prenatal testing must be considered by policy makers.

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