Outcomes of a cystic fibrosis carrier testing clinic for couples

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ABSTRACT

Objective: To review the outcomes of offering carrier testing for cystic fibrosis (CF) to couples considering pregnancy, and to women in early pregnancy and their partners.

Methods: An after-hours clinic was established in Newcastle for discussion of issues related to prenatal testing. Couples were offered CF carrier testing by extracting DNA from a mouthwash sample. An expanded one-step model was used with both partners being tested initially for the p.F508del cystic fibrosis transmembrane conductance regulator gene (*CFTR*) mutation. If one partner was a p.F508del carrier, the other partner was tested for an additional 28 *CFTR* mutations.

Results: Of 1000 individuals who were offered CF carrier testing, none declined. No re-collections of mouthwash samples were required, and results were available within 14 days. There were 730 individuals who had no family history of CF (73%); 27 were carriers (4%; 95% CI, 2.4%–5.3%), and there were two high-risk couples where both partners were carriers of p.F508del. There were 270 individuals who had an affected family member with CF or a child identified as a CF carrier through newborn screening; 126 were carriers (46%; 95% CI, 40.6%–52.8%), and there were two high-risk couples — one couple where both partners were carriers of p.F508del, and another couple where the woman was homozygous for p.F508del and the man was a p.F508del carrier. The information on carrier status led the four high-risk couples to change their reproductive decisions to avoid having a child with CF.

Conclusion: CF carrier testing for couples using an expanded one-step model will detect about 80% of high-risk couples and enables various reproductive choices. We believe that all couples considering pregnancy, and women in early pregnancy and their partners, should be offered CF carrier testing.

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Cystic fibrosis (CF) is a severe autosomal recessive condition associated with recurrent pulmonary infections, pancreatic exocrine insufficiency and reduced life expectancy. In Australia and New Zealand, one in 3000 babies are identified as having CF by newborn screening.[1] CF carrier frequency varies in different ethnic groups, with people of European ancestry having the highest carrier frequency of one in 25. The commonest cystic fibrosis transmembrane conductance regulator gene (*CFTR*) mutation is p.F508del (deletion of the codon for phenylalanine at position 508),[2] which accounts for 75% of all mutations, but more than 1500 *CFTR* alterations have been identified.[3] Ten mutations are associated with serious disease, and each of these have a frequency of more than 1% in the CF population. In couples where both partners are carriers of a *CFTR* mutation, there is a one in four chance of having a child with CF.

When the *CFTR* gene was identified in 1989, the possibility of population screening for carriers was raised. In 1997, the United States National Institutes of Health recommended that pregnant women and their partners, and couples planning a pregnancy, regardless of ethnicity, should all be offered CF carrier testing.[4] No recommendations have been made at a state or federal level in Australia, and there is no Medicare Benefits Schedule item number for CF carrier testing. An article in the Journal on this subject has raised the question: "Why are we waiting?"[5]

In a large prenatal study in Edinburgh, CF carrier testing was offered in antenatal clinics. This model treated each couple as a unit, whereby couples received their results as low risk if neither or one was a carrier, or high risk if both were carriers. The incidence of CF in the area was reduced by 50%. The rationale for not informing individuals of their carrier status was to reduce anxiety, costs and counselling time.[6]

Pilot studies in New South Wales have offered CF and Tay–Sachs disease carrier testing to Jewish high school students, but no such screening programs have been incorporated into health department policies.[7,8] In 1996, a rural community was offered CF testing by hair-root analysis, recruiting through high schools, general practices and the local radio station. This study identified 20% of the estimated number of carriers in the community.[9]

In a pilot study conducted in 1998, CF carrier testing by mouthwash was offered to pregnant couples attending the John Hunter Hospital for nuchal translucency ultrasound screening for Down syndrome. The uptake was 84%, with 23 carriers and no carrier–carrier couples identified. Carriers had some initial but no long-term anxiety. After delivery, all carriers said that they would, in retrospect, have carrier testing again.[10] As a result, CF carrier testing was then offered to couples at the prepregnancy and early pregnancy counselling clinic at Hunter Genetics.

We aimed to assess the outcomes of a CF carrier testing service that was offered to couples considering pregnancy, and to women in early pregnancy and their partners.

METHODS

General practitioners and obstetricians in the Hunter New England Area Health Service were notified by letter about the availability of CF carrier testing at an after-hours clinic that had been established in Newcastle for discussion of issues related to prenatal testing. A locally designed Microsoft Access (Microsoft, Redmond, Wash, USA) database was used to store data on clinic attendance, clinical information and outcomes from 1 January 2003. Data for the 5-year period ending 31 December 2007 were retrospectively extracted. The uptake of testing, the number of identified carriers and the reproductive decisions made by couples with a one in four or higher risk of having a child with CF were assessed.

Couples presenting to the clinic who were considering pregnancy, as well as women in early pregnancy (< 14 weeks' gestation) and their partners, were offered CF carrier testing and were counselled by a genetic counsellor about CF, carrier testing, folate supplements and testing options for aneuploidy. Couples were informed that the testing cannot identify all known *CFTR* mutations, and individuals of non-European ancestry were counselled about their lower risk of being a carrier. A medical family history was also obtained.

An expanded one-step model of carrier testing was used. For couples who underwent

1 *CFTR* mutations tested for in individuals whose partner was a carrier of p.F508del*

p.F508del	p.F316leufsX
p.I507del	p.R347P
p.G542X	p.S1251N
p.G551D	p.E60X
p.N1303K	p.W1282X
c.1585-1G>A	p.D1152H
p.R553X	c.2988+1G>A
c.489+G>T	c.2657+5G>A
p.R117H	c.1766+1G>A
p.R1162X	c.579+1G>A
c.3717+10kbC>T	p.G85E
p.R334W	p.K684fs
p.A455E	p.I148T
p.K684fs	p.R560T
p.T1176fs	

CFTR = gene encoding cystic fibrosis transmembrane conductance regulator.
*Nomenclature used is according to the Human Genome Variation Society guidelines for the description of sequence variations. ◆

carrier testing, each individual provided a mouthwash sample. Women who attended without a partner were offered a take-home mouthwash kit for their partner. DNA was extracted from samples and was initially tested for p.F508del. If one individual in a couple was a carrier of p.F508del, the other partner was tested for an extended panel of 28 *CFTR* mutations.

DNA was extracted from mouthwash samples by selective binding to glass fibres, followed by low-salt elution (High Pure PCR Template Preparation Kit, Roche Diagnostics, Sydney, NSW). The p.F508del mutation was detected by fragment sizing on a denaturing urea polyacrylamide gel using exon 10 internal primers, with one primer fluorescently end-labelled. Elucigene CF29 (Tepnel, Oxfordshire, UK), a multiplex-ARMS PCR (multiplex amplification refractory mutation system polymerase chain reaction) assay with a sensitivity of 82% for individuals with United Kingdom ancestry,[2] was used to detect an additional 28 *CFTR* mutations (Box 1). Testing costs of $50 for p.F508del and $250 for the other 28 mutations were covered by Hunter Genetics.

Results of carrier testing were available within 14 days. Couples were notified of their results by telephone, and a letter containing this information was sent to each couple and their referring doctor. The residual risks of being a carrier and having a child with CF were explained, and identified carriers were offered cascade family testing. Individuals negative for p.F508del have a one in 100 residual risk of being a carrier. For those negative for p.F508del and the other 28 *CFTR* mutations, the risk is reduced to one in 150. Couples who are both negative for p.F508del have a one in 40 000 residual risk of having a child with CF. Couples with one carrier and the other negative by extended panel testing have a one in 600 residual risk of having a child with CF. Couples with a one in four or higher risk of having a child with CF were offered an urgent appointment with a geneticist and genetic counsellor to discuss their options and facilitate their choices.

RESULTS

Of 1062 individuals who attended the clinic, 1000 were offered CF carrier testing (94%), of whom none declined the offer. The remaining 62 were not offered testing as the women were past 14 weeks' gestation or presented for other reasons. Of the individuals tested, 83% presented as couples, and no recollections of mouthwash samples were required. The couples were divided into two groups: those without a family history of CF, and those with a family history of CF. Couples with a family history of CF included those who had a family member with CF and those with a child identified as a CF carrier through newborn screening.

In the group without a family history of CF, 730 individuals were tested (73%); 27 were carriers of p.F508del (4%; 95% CI, 2.4%–5.3%), representing a carrier frequency of one in 27. Of the 27 carriers, 11 (41%) were pregnant women and 16 (59%) were individuals from couples who were planning a pregnancy (seven of whom had fertility problems). In two couples, both partners were found to carry p.F508del and consequently altered their reproductive decisions (Box 2).

In the group with a family history of CF, 270 individuals were tested (27%); 126 carriers were identified (46%; 95% CI, 40.6%–52.8%), representing a carrier frequency of one in two. Two couples were identified as being at high risk of having a child with CF. In one of these couples, both individuals were carriers of p.F508del. In the other couple, the woman had CF and was homozygous for p.F508del and the man was a carrier of p.F508del. Both of these couples consequently altered their reproductive decisions (Box 2).

Of the couples from both groups where one partner was a carrier of p.F508del and the other partner was negative for p.F508del, no other mutations were identified by extended panel testing.

DISCUSSION

The most significant finding in our study was that couples with a high risk of having a child with CF made decisions that altered their reproductive plans to avoid having a child with CF. Most women who have a child with CF, diagnosed by newborn screening, choose to have prenatal diagnosis in subsequent pregnancies and to terminate affected pregnancies.[11] In a large study of pregnant women in France who underwent prenatal diagnosis because of a one in four risk of having a child with CF, 96% decided to terminate an affected pregnancy.[12] In Massachusetts, since preconception and prenatal testing were introduced in 2001, there has been a significant decrease in incidence of severe CF (ie, births of babies homozygous for p.F508del).[13]

The primary aim of couple testing during pregnancy is to offer reproductive options before the birth of a child with CF. However, prepregnancy testing is preferable as it expands reproductive options to include in-vitro fertilisation and pre-implantation genetic diagnosis, use of donor gametes, adoption, and not having children. These options may be preferred by some couples as they remove the issue of terminating an affected pregnancy. But many pregnancies are unplanned and the only reproductive options during pregnancy are prenatal diagnosis or no testing.

The high uptake of testing in our study may have been influenced by specific referral of couples for testing and the arrangement whereby couples did not incur the costs of testing. However, studies have shown that most couples find CF carrier testing acceptable.[6,10] A study of population carrier screening for CF in Western Australia has shown that people planning children were 90% more likely to have testing than those with no reproductive plans.[14]

Concurrent testing in the expanded one-step model that we used saves time and anxiety by eliminating the need to recall the partner of a carrier for testing. It also provides high-risk couples with more time for reproductive decision making. In contrast to the model used in Edinburgh, each individual was informed of their results. This provides each individual with information on their carrier status, and accurate residual risks of

2 Reproductive decisions and outcomes of couples with a high risk of having a child with cystic fibrosis (CF)

Pregnancy status at time of testing	Results of *CFTR* mutation testing	Reproductive decisions	Outcomes	Comments
Couples without a family history of CF				
Not pregnant	Both individuals were carriers of p.F508del	IVF and PGD	On IVF program; no pregnancy achieved at time of study completion	The woman had recurrent abdominal pain suggestive of chronic pancreatitis. Further investigations revealed that she carried both p.F508del and p.R117H. She had an abnormal sweat chloride level but normal lung function, with a phenotype of atypical CF.
Pregnant (6 weeks' gestation)	Both individuals were carriers of p.F508del	Chorionic villus sampling	Fetus was an unaffected female (p.F508del carrier)	The couple stated that the process was stressful but that they preferred to know their carrier status to enable the option of prenatal diagnosis and termination of an affected pregnancy. They plan to have IVF and PGD for their next pregnancy.
Couples with a family history of CF				
Not pregnant	Both individuals were carriers of p.F508del	Chorionic villus sampling	Woman became pregnant 6 months after carrier testing; fetus was affected (homozygous for p.F508del) and couple decided to terminate pregnancy	The couple's teenage niece had a late diagnosis of CF, and the couple was identified as a result of cascade family testing.
Not pregnant	The woman had CF and was homozygous for p.F508del; her partner was a carrier of p.F508del	IVF and PGD when ready to have children	Not pregnant, and not on IVF program	The couple were concerned about the high risk (one in two chance) of having a child with CF. The man, aged 19 years, was also concerned about his readiness to become a parent.

CFTR = gene encoding cystic fibrosis transmembrane conductance regulator. IVF = in-vitro fertilisation. PGD = pre-implantation genetic diagnosis. ◆

being a carrier and having a child with CF. Most couples prefer concurrent testing with full disclosure as they want to know the results for themselves, to inform relatives, and to use in case they consider pregnancy with a new partner in the future.[15]

In our study, the number of couples tested was small when compared to the 8000 births per year in the Hunter New England Area Health Service. More people may be tested if CF carrier testing was offered in routine antenatal care. GPs could provide this service when couples present for early pregnancy care, and identified high-risk couples could be referred to local genetic services for counselling. The cost of testing could be incurred by the couple and supported by a Medicare rebate, which would require federal funding.

CF is a common and severe disease that places considerable emotional and financial burden on affected individuals, their families and health services. We believe that all couples considering pregnancy, as well as women in early pregnancy and their partners, should be offered CF carrier testing to enable informed reproductive decisions.

COMPETING INTERESTS

None identified.

AUTHOR DETAILS

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