Cystic Fibrosis and Fragile X Syndrome: The Arguments for Antenatal Screening

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Abstract: The ultimate public health aim of genetic screening is prevention. This can be achieved by reducing birth prevalence through primary or secondary methods such as pre-conceptional or antenatal screening. Tertiary prevention by neonatal screening is also an option where there is direct unbiased evidence for a substantial improvement in prognosis. In addition to this, the information provided during screening is also of value, enabling individuals to make choices that otherwise would not have been available. Having elucidated the natural histories and genetic defects underlying two common, serious genetic disorders, cystic fibrosis and fragile X syndrome, considerable efforts have been channelled into ascertaining the most efficacious method of prevention. To date there is only indirect evidence to suggest that neonatal screening improves prognosis in cystic fibrosis. Similarly, treatment for fragile X syndrome is limited and therefore early identification of the disorder by neonatal screening is unlikely to improve long term outlook. Thus the focus of this review is on primary and secondary preventive methods.

CYSTIC FIBROSIS

Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasians affecting one in 2,400 births in the UK. It is associated with considerable morbidity and premature mortality caused by progressive lung disease and eventual respiratory failure. Median life expectancy for a person with CF is 25 years, however regression analysis predicts that for children born in the 1990s, it may over 40 years [1]. Improvements in the general standard of living and the overall management of CF including treatment, early diagnosis and the provision of specialist care units are thought to be collectively responsible for increasing life expectancy.

CF is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which is situated on chromosome 7. Spanning over 250 kb of genomic DNA and encompassing 27 exons, it encodes for a protein product which functions as a membrane bound cyclic-AMP regulated chloride channel. To date, over 800 mutations within the CFTR gene have been identified. However, most of these are extremely rare such that over 77% of mutated chromosomes world-wide can be accounted for by only 24 mutations [2].

CF is inherited as a simple Mendelian recessive disorder. Non carriers have two normal CFTR genes, asymptomatic carriers possess one normal and one mutated gene and in affected individuals both CFTR genes are mutated. In the UK, one person in 24 is a carrier for CF and for one couple in every 600 both partners will be carriers. These couples have a one in four risk of CF in every pregnancy. If they have two pregnancies, they have a one in two chance that at least one child will have CF [1-(3/4*3/4)] and for three pregnancies it will be 2 in 3 [1-(3/4*3/4*3/4)].

Screening Technology

The laboratory techniques for detecting the most common mutations are straightforward and

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can be performed on blood, mouthwash, buccal smear or even urine. In general, the polymerase chain reaction (PCR) is used to test for a single mutation such as delta F508 or a number of common mutations simultaneously (multiplexing). There are currently 3 commercially available assay kits which can each detect 8, 12 and 31 of the most common mutations, accounting for 82%, 83% and 85% of UK carriers. Thus for a kit that detects 85% of carriers, 72% of carrier couples would be found (85%*85%).

Screening Options

The main objective of genetic screening for CF is to reduce the birth prevalence by identifying couples with a high risk of an affected pregnancy. A number of preventive options are available to such couples including avoiding pregnancy, changing partners, artificial insemination using donor sperm or egg, pre-implantation genetic diagnosis or prenatal diagnosis with selective termination during pregnancy. There are three possible strategies by which carrier couples could be systematically identified. An indirect approach would be to screen individuals of reproductive age such as school graduates, company employees or general practice attendees. The main difficulty with this strategy however is that a carrier's individual risk of a child with CF will differ substantially from their ultimate risk, this being determined by the carrier status of their future reproductive partner. Thus a carrier individual with a one in 96 risk [1/4 (1 in 24)] of CF may eventually have a one in 4 or a one in 620 risk (Table 1).

Another strategy is to systematically test family members of affected individuals (cascade screening). Following identification of an index case, clinical genetic services generally provide counselling and testing within the nuclear family. The option is then available for family members to inform more distant relatives and this may eventually lead to the identification of other carrier couples. The efficacy of this process could be improved by directly approaching family members to offer them testing. As an overall method of reducing birth prevalence however, cascade screening is unlikely to have a large impact. Mathematical models predict that only 8% to 24% of all carrier couples would be detected [3].

The third approach is aimed specifically at identifying carrier couples during pregnancy or whilst it is being planned. Although the latter option would allow more reproductive choice, the absence of an organised infrastructure for pre-conceptional screening means that this method is likely to prove difficult to administer. Screening during pregnancy offers a more practical approach and could easily be incorporated into routine antenatal care.

Two screening methods have been proposed to identify carrier couples during pregnancy. Sequential screening involves testing the expectant mother first and only obtaining a sample from the father if she is found to be a carrier. There are

Table 1. Risk of CF Pregnancy According to Parents' Screening Results and Proportion of Detectable Mutations

Mutations Detectable (%)	Parents screening results			
	+/-	-/NT	+/- or -/NT	-/-
70	1 in 310	1 in 7,500	1 in 4,500	1 in 24,000
75	1 in 370	1 in 8,900	1 in 5,300	1 in 35,000
80	1 in 460	1 in 11,000	1 in 6,400	1 in 54,000
85	1 in 620	1 in 15,000	1 in 8,300	1 in 95,000
90	1 in 920	1 in 22,000	1 in 12,000	1 in 210,000

./.=result for each parent; +=mutation detected; -=none detected; NT=not tested

three possible outcomes: the woman is not found to be a carrier and her partner is not tested, only the woman is identified as a carrier and both are carriers. Table 1 shows that the couple risk of CF for each situation, based on a screening test with an 85% carrier detection rate, is one in 15,000, one in 620 and one in four for the three options respectively. Approximately 96% of couples will fall into the first category with a further 3% being discordant for carrier status and the remaining 1% as carrier couples. The other method is couple screening where samples are obtained from both partners at the same time. Testing is carried out in the same way as in sequential screening however carrier females are not informed of their results until the partner's becomes available. The results can either be disclosed to reveal each partner's carrier status or non-disclosed where only the couple's final risk of CF is provided. With the latter approach, only carrier couples would know their individual carrier status. Reporting results in this way aims to avoid anxiety experienced by discordant couples with an intermediate risk of CF. Disclosed reporting has the advantage of providing personal information, which can be used in the event of changing reproductive partners.

Table 2 shows the results from 11 antenatal screening studies: 5 in the UK, 2 in mainland Europe and 4 in the United States. Of almost 53,000 women offered screening, 74% accepted and of 704 carriers, 92% of partners were also tested. There were 57 carrier couples, 51 (89%) of whom underwent prenatal diagnosis. Eighteen CF pregnancies were confirmed and all, but one, were terminated. Overall therefore these results show that antenatal screening is acceptable and feasible.

Financial Costs

The cost effectiveness of CF antenatal screening has been estimated under a variety of conditions [5]. The four components of the screening process namely, information giving, DNA testing, genetic counselling and prenatal diagnosis, were costed individually from both the Leeds antenatal screening study and from the literature. The cost of the particular screening

Table 2. Antenatal Screening for CF: Results from 11 Studies* (Numerator in Parentheses)

Study*	Strategy	Accepted screening	Partners of carriers tested	Prenatal diagnosis
Aberdeen	Sequential Couple	91% (1,487) 89% (321)	98% (47) NA	100% (2)
Edinburgh	Sequential Couple	83% (4,978) 76% (12,566)	99% (189) NA	92% (33)
Leeds	Sequential	62% (3,773)	98% (127)	33% (1)
Manchester	Mixed	85% (529)	100% (10)	100% (1)
Oxford	Couple	67% (543)	NA	(0)
Copenhagen	Sequential	80% (2,443)	94% (NK)	(0)
Berlin	Sequential	99% (637)	100% (20)	100% (1)
L Angeles	Sequential	67% (3,192)	85% (47)	100% (1)
Maine	Couple	NK (1,682)	NA	100% (1)
Rochester	Sequential	59% (3,334)	88% (96)	80% (4)
San Jose	Sequential	78% (5,161)	86% (116)	100% (7)
TOTAL		74% (38,964)	92% (651)	89% (51)

NA=not applicable NK=not known.
* For more details of each study and references see [4]

programme was then obtained by summing the proportion of carriers detected by the DNA test and the uptake of screening. Various assumptions were made about the proportion of couples with missing carrier status information from previous pregnancies (20%), the proportion changing partners between first and second pregnancies (20%) and the uptake of prenatal diagnosis (100%). With a multiple mutation test that detects 85% of carriers and an uptake rate of 75%, the estimated cost of sequential screening was £78,000 per affected pregnancy detected compared to £90,000 for non-disclosed couple screening. These costs are much smaller than the estimated lifetime treatment costs, which range from £164,000 to US$800,000 [6,7].

The cost per affected birth avoided by antenatal screening, estimated in non-UK studies and converted into sterling using purchasing power parity range from £205,000 to £1,043,000 [8,9]. The principle reasons for the higher costs are the unit cost of the DNA tests and the fact that only one pregnancy was considered, whereas screening in the first pregnancy and using information in later pregnancies almost halves the cost.

FRAGILE X SYNDROME

Fragile X syndrome is the most common known cause of inherited learning disability. In Caucasians, it affects approximately one in 4,000 males and one in 8,000 females [10]. Males tend to be moderately to severely learning disabled whereas in females the condition is often mild. Physical features of fragile X include facial atypia, ocular and musculo-skeletal abnormalities and in adult males, macro-orchidism. Behavioural problems can also be present, manifested as poor eye contact, autistic-like mannerisms and attention deficit disorders. In children, the characteristic physical features are often absent and developmental delay masked by apparent disruptive behaviour. Consequently, pinpointing the potential causes and achieving a diagnosis may take several years. Currently there is no cure for fragile X syndrome. However, educational, psychological and social interventions help with behavioural problems.

In 1991 the gene responsible for fragile X syndrome, the fragile X mental retardation (FMR-1) gene, was cloned [11]. Located at Xq27.3, the FMR1 gene is characterised by a trinucleotide CGG (cytosine-guanine-guanine) repeat sequence. In the general population the number of repeats ranges from 5 to approximately 54, with 30 being the most common. This number remains fairly constant between generations so that only small increases or decreases in repeat size occur during transmission from parent to offspring. In fragile X families, the mutated FMR-1 gene has more than 54 repeats and is termed either a premutation (PM), with 55-199 repeats or a full mutation (FM), with greater than 200. Individuals with a PM are asymptomatic carriers. In males the PM behaves similarly to the normal FMR-1 gene, in that meiotic transmission does not result in great alterations in CGG repeat size. However, when transmitted by a female, the PM may undergo hyper-expansion, resulting in a child with a FM. All males and 50% of females with a FM have clinical fragile X syndrome. The risk of hyper-expansion is determined by the size of the PM such that a larger repeat size is more likely to hyper-expand. In studies of fragile X families, the hyper-expansion risk in a PM of 60 repeats is 10% to 18% compared to 90% to 93% in a PM of 100 repeats. The overall risk is 68% to 78% [12]. The smallest premutation reported to have resulted in FM offspring is 59 repeats.

Table 3 shows the prevalence of PMs in low risk females estimated in a variety of ethnic groups. Although studies used different cut-off points to define a PM, it is apparent that there are intrinsic differences between populations. As a consequence it is unfeasible to combine the studies to provide an overall estimate. In the absence of sufficient data by which the risk of hyper-expansion in the general population can be estimated, a model must be used. This assumes that the prevalence of fragile X syndrome remains constant and that there is random transmission of normal and mutated chromosomes. The model predicts that for any given prevalence of fragile X syndrome, a proportion of FM offspring must arise from PM as opposed to FM carrier females. The risk of hyper-expansion is simply that which

Table 3. Frequency of Fragile X Premutations in Females with no Known Family History of Fragile X Syndrome from 11 Studies*

Study	No. of female samples**	Smallest PM***	PMs	
			No.	Freq. (1 in)
1. USA	197	57	1	197
2. Japan	227	-	0	>227
3. USA	561	75	1	561
4. Canada	10,624	55	41	259
5. Canada	735	55	2	368
6. USA	745	60	3	248
7. Finland [13]	1,477	60	6	246
8. Canada [14]	378	-	0	>378
9. Israel [15]	9,426	52	101	93
10. Israel [16]	10,587	60	67	158
11. Israel [17]	8,426	55	58	145

* For more details on studies 1 to 6 and references see [12].

** Samples were obtained from the following sources: general outpatients, Guthrie cards, blood donors, women requesting DNA tests unrelated to learning disability, egg donors, women attending clinics for pre-conceptional advice antenatal care.

*** Most studies used a cut-off repeat size of 54 to define a premutation. For studies using 52 repeats to define a PM, where possible a higher cut-off is taken to allow for comparison.

would be required to arrive at the expected frequency of FM offspring from a particular female PM frequency. Table 4 shows the predicted risk of hyper-expansion according to the prevalence of PMs in females and of fragile X syndrome in males. Whilst one in 4,000 is thought to be the best estimate of fragile X syndrome in males, representing complete ascertainment, two further studies have suggested that, at least in some countries, it may be as many as one in 2,000 or as low as one in 5,530 [18,19]. Hyper-expansion risks have therefore been calculated for each estimate of fragile X syndrome prevalence. The highest risk predicted by the model is 45% which is obtained from the lowest PM frequency and the highest fragile X syndrome prevalence. This is still much lower than that observed in fragile X families. Since this discrepancy cannot be explained by the PM size distribution, which is naturally lower in the general population than in affected families or by increasing the prevalence of fragile X syndrome, the only conclusion is that in the general population the PM does not undergo mutation as rapidly.

Screening Technology

The vast majority of fragile X mutations detected in low risk populations will be PMs. Polymerase Chain Reaction (PCR) is a rapid, inexpensive and reliable method by which normal alleles and PMs can be sized. It can be performed on mouthwash or blood samples. For heterozygous alleles, i.e., CGG repeats of differing lengths, PCR will be informative, presenting both alleles as two bands on the electrophoretic gel. However approximately one third of female FMR-1 alleles are homozygous (i.e., the same size) or differ in size by one base pair, thus producing one band on the gel instead of two. As PCR is only able to amplify smaller alleles, the same pattern will also be obtained if one of the alleles is a FM. In order to distinguish between FMs and homozygous alleles, Southern Blotting (using a blood sample) is required. Therefore any large-scale screening programme must make a provision for both techniques. The performance of the screening test will be dependent on the chosen PM and fragile X syndrome prevalence estimates.

Table 4. Estimated Risk of Full Mutation (FM) Offspring According to Premutation (PM) Frequency in the General Female Population and Prevalence of Fragile X Syndrome in Males

	Fragile X syndrome (males)		
PM frequency (1 in)	**1 in 5,530 [18]**	**1 in 4,000 [9]**	**1 in 2,000 [17]**
50	1%	2%	4%
100	3%	4%	8%
150	4%	6%	11%
200	5%	8%	15%
250	7%	9%	19%
300	8%	11%	23%
350	10%	13%	26%
400	11%	15%	30%
450	12%	17%	34%
500	14%	19%	38%
550	15%	21%	41%
600	16%	23%	45%

Screening Options

As with cystic fibrosis, a reduction in birth prevalence could be achieved by identifying those at high risk of an affected pregnancy. In this situation however, it is the female alone whose carrier status dictates the risk of fragile X syndrome. Given that the burden of responsibility rests solely on one individual, screening programmes should aim to minimise the length of time between testing and childbearing. In doing so any anxiety caused by the knowledge of carrier status could be concentrated into a short period of time when it is most relevant and useful. For this reason general population screening for fragile X syndrome is inappropriate. Another option for identifying PM and FM females is to perform cascade screening within affected families. As a means of informing reproductive choice however, this method is limited. This is partly because diagnosing the index case often takes years by which time the family may already be complete with further affected births having occurred. Even once the diagnosis has been made, evidence shows that communication between family members often breaks down due to increased tensions, stigma and anxiety [20,21]. Models also predict that even if extended to third degree relatives, cascade screening would only detect 12% of females at risk of having an affected child [22].

Alternative options for identifying females at high risk of an affected pregnancy include pre-conceptional and antenatal screening. For the same reasons as those provided for CF, pre-conceptional screening is not practicable. For antenatal screening, two tier screening methods have been suggested in which individuals are tested where there is evidence of either mild learning disability in the women herself or a family history of either fragile X syndrome or unexplained learning disability [23]. There are strong arguments against these [24]. Firstly, screening women with learning disabilities is very unlikely to identify any PMs and only a proportion of FMs (only 50% of FM females have learning disability). Secondly, in order to screen women with a family history of fragile X syndrome, there must be an affected relative and the diagnosis has to have been made. Finally, women may not be aware of their family history and even if there is knowledge of unexplained learning disability, there are practical problems associated with establishing its

Table 5. Antenatal Screening for Fragile X Syndrome: Practical Experience in 4 Studies

Study	Women screened	FMs		PMs		Prenatal diagnoses	FM pregnancies	
		No.	Rate (1 in)	No.	Rate (1 in)		No.	Terminated
Finland [13]	1,738	0	>1700	6	290	6 (100%)	2	0 (0%)
Israel I [15]	9,830	4	2458	115	85	119 (100%)	NS	NS
Israel II [16]	10,587	NS	NS	138	77	110 (80%)	NS	NS
Israel III [17]	9,459	4	2365	130	73	108 (87%)	9	9 (100%)

The Israeli studies used a cut-off of 52 repeats to define a PM whereas in Finland 60 repeats was used. The Israel III study includes pre-conceptional patients; numbers are not available. The total number of carriers detected in this study was 134, 10 of whom were not pregnant. Uptake of prenatal diagnosis is therefore based on 124 pregnancies, 7 of which were repeat pregnancies. Three pregnancies were twin.
NS=not specified

relevance. Screening all pregnant women of reproductive age from apparently low risk populations would overcome these impracticalities and avoid stigmatising specific subgroups.

The preliminary results from five large-scale population-based antenatal screening programmes have now being reported (Table 5). Information on the uptake of screening was only available for the Finnish study where it was 85%. All studies reported the uptake of prenatal diagnosis in the carriers where the overall rate was 87%. Only two of the studies have reported the outcome of pregnancy for cases where a fetal FM was found. Nine of the eleven were terminated, all four males and five of seven females. These are too few data to draw conclusions about the acceptability of termination in such circumstances. However, the results of prenatal diagnosis performed because of a family history of fragile X syndrome suggest that it is acceptable. Murray and colleagues reviewed seven studies, which reported the outcome of 60 prenatally diagnosed FM pregnancies [12]. Data on a further 53 have been made available through personal communication from Dr Sarah Nolin from the USA-based Institute for Basic Research. In total, there are 113 FM pregnancies for which outcome is known; 88 (78%) were terminated; 46 (88%) of 52 males and 42 (69%) of 61 females.

Financial Costs

The cost-effectiveness of antenatal screening has been estimated in the UK to be £93,000 per case detected. This was based on a fragile X syndrome prevalence of one in 4,000 and of one in 270 for the premutation in females [10,12]. Unit costs were the same as those used in the cost-effectiveness study for antenatal cystic fibrosis screening [5]. In a more detailed economic analysis from The Netherlands, the cost per carrier detected was found to be US$46,400 [25]. The prevalence of fragile X syndrome was assumed to be one in 4,000 with a female PM frequency of one in 435. The cost of screening is much lower than the lifetime costs of care, which were estimated to be US$957,734 and US$533,673 for males and females respectively.

CONCLUSION

Many developed countries already provide routine antenatal screening for common serious abnormalities, such as Down's syndrome and neural tube defects. In spite of the vast amount of evidence showing that antenatal screening for cystic fibrosis is cost-effective, feasible and acceptable, it has yet to be incorporated into routine obstetric care. One obvious deterrent to its introduction is that in comparison to Down's syndrome, it appears expensive, costing 3 times as much to detect one CF case. Also the fact that quality of life and life expectancy are improving is leading to ethical dilemmas about the perceived seriousness of the disorder. As for fragile X syndrome, the limited amount of experience with antenatal screening suggests that it is also acceptable and feasible. However as shown in this review there are still many unknowns which ultimately leave it a few years behind CF in terms

of its likely introduction into routine obstetric care. Until more large-scale pilot screening studies are performed, many of the questions pertaining to the risk of fragile X syndrome in the general population will remain unanswered.

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