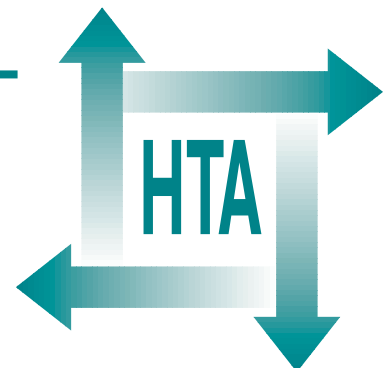# An assessment of screening strategies for fragile **X** syndrome in the UK

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**Health Technology Assessment
NHS R&D HTA Programme**

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An assessment of screening strategies for fragile X syndrome in the UK

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Competing interests: B Carmichael is a member of the Fragile X Society; she has relatives who are affected by fragile X syndrome.

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The NHS R&D Health Technology Assessment (HTA) Programme was set up in 1993 to ensure that high-quality research information on the costs, effectiveness and broader impact of health technologies is produced in the most efficient way for those who use, manage and provide care in the NHS.

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The views expressed in this publication are those of the authors and not necessarily those of the HTA Programme or the Department of Health. The editors wish to emphasise that funding and publication of this research by the NHS should not be taken as implicit support for the recommendations for policy contained herein. In particular, policy options in the area of screening will be considered by the National Screening Committee. This Committee, chaired by the Chief Medical Officer, will take into account the views expressed here, further available evidence and other relevant considerations.

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List of abbreviations

ACGT   UK Advisory Committee on Genetic Testing

AGG   a nucleotide triplet of adenine–guanine–guanine

ALSPAC   Avon Longitudinal Study of Pregnancy and Childhood (now Avon Longitudinal Study of Parents and Children)

CF   cystic fibrosis

CGG   a nucleotide triplet of cytosine–guanine–guanine

CI   confidence interval

CVS   chorionic villus sampling

ESHG   European Society of Human Genetics

FMR1   symbol for 'fragile X syndrome' gene (*Fragile Mental Retardation 1*)

FMR2   symbol for gene associated with FRAXE (*Fragile Mental Retardation 2*)

FMRP   protein from *FMR1* gene

FRAXA   chromosomal fragile site at Xq27.3 that corresponds to the CGG repeat expansion of the *FMR1* gene

FRAXD   chromosomal fragile site in the Xq27.3–28 region

FRAXE   chromosomal fragile site at Xq28 that corresponds to the expansion in the *FMR2* gene

FRAXF   chromosomal fragile site in the Xq27.3–28 region

GP   general practitioner

HNPCC   hereditary non-polyposis colorectal cancer

MIP   maternally-inherited premutation

MMR   mismatch repair

MR   mental retardation

mRNA   messenger ribonucleic acid

NSC   National Screening Committee [UK]

NTM   normal transmitting male

PCR   polymerase chain reaction

PIP   paternally-inherited premutation

POF   premature ovarian failure

WJ 1968   Wilson and Jungner criteria 1968

Executive summary

Background

Fragile X syndrome is an inherited form of learning disability that was defined in the late 1970s by cytogenetic detection of an associated fragile site on the X chromosome (Xq27.3). Cytogenetic estimates of the prevalence of fragile X syndrome were as high as 1 in 1039 males but have since been revised downwards. Fragile X syndrome is associated with few medical problems and the subtle physical features make clinical diagnosis difficult. The unusual pattern of inheritance, delineated in the 1980s, was explained once the fragile X syndrome gene (*FMR1*) had been identified in 1991. This gene contains a highly variable repeat of the nucleotide triplet, cytosine–guanine–guanine (CGG). Fragile X syndrome is caused by a large expansion of this CGG repeat (full mutation) that leads to silencing of the *FMR1* gene so no gene product (FMRP) is made. This is the ultimate cause of the learning disability that, in males, is sufficient to preclude independent living.

Family studies show that all individuals with a full mutation inherit it from a female (usually unaffected) who carries either a full mutation or a premutation, a smaller repeat expansion (approximately 55–200 repeats) that is unstable on **female** transmission. The chance of a premutation expanding to a full mutation is positively associated with the size of the repeat (approximately 95% by 90 repeats) but only for female transmissions. When a man transmits a premutation, it remains a premutation; his children are, therefore, unaffected by overt learning difficulties. The potential for population screening or systematic case-finding and extended family testing exists because every unaffected mother of an affected child has a detectable CGG repeat expansion. Reliable prenatal diagnosis is possible in males.

Objectives

To assess the feasibility and acceptability of population screening by addressing the following questions in the context of existing services for families with fragile X syndrome.

- Is there a suitable test for all fragile X genotypes?
- What are the UK population distribution of *FMR1* repeat sizes, and the prevalence of full and premutations in both sexes?
- What reliable information, in terms of the chance of an affected child, is available to women with premutations between 55 and 200 repeats?
- What is the effect of a premutation on the person who carries it?
- What information is available to women with intermediate alleles of 41 to 54–60 repeats?
- How many affected people are diagnosed?
- Given the practice of offering extended family testing (cascade testing), what is the population prevalence of 'as-yet-undiagnosed' female carriers of a full or premutation? What proportion of women at risk can be reached by cascade testing?
- What are the costs of fragile X syndrome to an affected person and their family and to the NHS and society?
- What is the attitude of families to the benefits and costs of a diagnosis of fragile X syndrome, and to the prospect of population screening?
- What data are available from existing population screening programmes?
- What alternatives to population screening exist and are these feasible?

Methods

A key aspect of the review process was to assemble a team with extensive first-hand experience of all aspects of fragile X syndrome, including affected families and the services they use, and a wide knowledge of the relevant literature. They had followed the critical discussions at all the biennial international workshops on fragile X syndrome, including a special session at the 7th International Workshop in 1995 at which an earlier (and substantially different) draft of this report was discussed.

The biomedical literature review of 2429 papers was based on MEDLINE searches, extending to PsycINFO and BIDS for the psychological aspects of [fragile X syndrome] screening. Questionnaire-

based information was obtained from the UK Fragile X Society and data were collected directly from all the regional clinical genetics centres in 1995 and 1998.

Results

Unlike cytogenetic approaches, DNA analysis can reliably determine the *FMR1* CGG repeat number and detect full mutations; however, a combination of polymerase chain reaction and Southern blotting tests is required, which limits high throughput. There are UK population-based data on *FMR1* repeat sizes of up to 60 repeats but insufficient to provide a reliable estimate of the prevalence of premutations (approximately 60–200 repeats). The few data and estimates in the literature of women carriers of the premutation range from 1 in 246 to 1 in 550. Two UK DNA-based estimates of the prevalence of males with the full mutation are 1 in 4090 (Coventry) and 1 in 5530 (Wessex). There are reasonable family-based data for the risk of expansion to a full mutation for the larger premutations but in the 50–69 repeat range the estimates are less secure. This is particularly true of the general population, in which limited screening data (approximately 60 transmissions) produced no full mutation. Women with premutations have about a 16% chance of menopause before 40 years of age compared with approximately 1% in the general population. It was suggested by one study that, in boys with special educational needs, those with an intermediate allele (41–60 repeats) are over-represented.

Probably less than half of those with fragile X syndrome are currently known to UK regional genetics centres. Systematic case-finding, as in New South Wales, Australia, can increase this figure markedly and, coupled with family cascade counselling, can lead to both an increase in reproductive confidence and a 60% reduction in prevalence. Simulations indicate, however, that case-finding and cascade counselling can only reach about half of premutation carriers, although these individuals would include most of those at the highest risk.

The costs of fragile X syndrome are as much social as financial and affected families are generally supportive of the idea of screening. Systematic case-finding and cascade testing are a partial alternative to population screening but require more staff, together with laboratory and other consumables, at regional genetics centres to be feasible.

Conclusions

Programmes of systematic case-finding and cascade testing could achieve benefits for those women most at risk. A trial of systematic case-finding and cascade testing to evaluate the benefits and costs of such an approach would be based on reasonably secure risk figures for counselling. The same is not true for a trial of population screening. The uncertainty about the risks for women from the general population with 55–65 repeats can only be resolved with more research. Ongoing research should clarify a possible link between intermediate alleles and learning difficulties.

Chapter 1

Fragile X syndrome in the context of genetics services

The challenging climate in which this assessment exercise is set

The request by the NHS Executive for an assessment of possible screening strategies, to identify individuals with the fragile X syndrome or those at risk of having an affected child, is both timely and challenging. It goes to the heart of the current debate on the goals of genetics services and how these can best be achieved. In putting together the team to undertake this assessment, the authors were aware of the importance of being able to provide information to health planners on how any proposed screening procedure would integrate with the definitive clinical genetics services for families with fragile X syndrome. Any new programme aimed at identifying and helping at-risk families would also need to win the support of both the affected families and the general public, if it was to be effective in practice. The need to be sensitive to public perception of what the NHS Executive and the UK National Screening Committee (NSC) are considering with respect to the fragile X syndrome was highlighted by the adverse publicity in the *Sunday Times*, *Daily Mail* and *Daily Express* on 9/10 June 1996, with headlines such as 'Mass screening for 'delinquency' gene planned' and 'Searching for the mean gene'. The importance that the UK Government attaches to these broader consider-ations in formulating health policy was shown by the creation of the Advisory Committee on Genetic Testing (ACGT) and the Human Genetics Advisory Commission (whose terms of reference included "To advise on ways to build public confidence in, and understanding of, the new genetics"[1]). The new Human Genetics Commission into which these two bodies have been subsumed, has a similar remit.

In the few years since the bulk of this report was put together, it has become increasingly clear that our assessment would fail if it was not set in a somewhat broader context than that originally envisaged. Judging by recent debate[2,3] and, indeed, significant differences between this report and the earlier report on fragile X syndrome commissioned by the NHS Executive,[4] there still appears to be no consensus on matters as fundamental as the goal of medical genetics services. The combined clinical experience of the authors of this report (see appendix 1) made them particularly well suited to consider the broader context and to temper any recommendations with first-hand knowledge of both developing and delivering genetics services to families with fragile X syndrome.

The goals of genetics services

A primary consideration in any evaluation of service developments is the overall goal of such services. In the end it is this that must determine priorities in development and be the yardstick against which success or failure is measured. Until fairly recently, a widely held view was that the goal of medical genetics was to reduce the birth prevalence of babies with, or destined to develop, genetic disease, as illustrated by the following statement: "... The long-term aim of genetic counselling is to see that as few children as possible are born with serious genetically determined or part genetically determined handicaps...".[5] Although it would be wrong to imply that this reflects the public health approach today, this view is still prevalent in the literature on genetic screening. Murray and colleagues[4] asserted that "There would be two distinct pur-poses of [fragile X syndrome] screening – (a) to reduce birth prevalence and (b) to bring forward a clin-ical diagnosis". Haddow[2] argued that the term 'CF [cystic fibrosis] carrier screening' should be dropped in favour of 'CF screening', since the success of a CF screening programme is to be measured in the number of affected CF fetuses identified. Such views, however, sit uneasily with the desire to avoid any societal or state pressure on a woman to abort an affected fetus or remain childless. This issue has long been recognised[6] and led to the promotion of a 'non-directive' stance in genetic counselling (reviewed by Kessler)[7] but the development of genetic screening in a public health context and the desire to demonstrate cost-effectiveness has tended to swing the argument the other way.

Pembrey[8,9] argued that a public health objective that does not accord with the aspirations of the very families it aims to help is likely to be viewed as state-inspired eugenics. He claimed that the aim should **not** be to reduce the birth prevalence of genetic disease, which is also one of the conclusions of the report by the Committee on Assessing Genetic Risks, from the US Institute of Medicine.[10] A reduction in birth incidence of a genetic disorder may be the **consequence** of genetic counselling and prenatal diagnostic services, as this report documents, but it depends on what parents choose to do. This distinction between the objective and consequence of genetics services is not just playing with words. It impacts directly on the formulation of health policy, on how health authorities might measure the success or failure of genetics services and on what outcomes are used in cost–benefit analysis.[11] Without making this distinction, it is easy to slip into the nonsense of calling a positive prenatal diagnosis and subsequent abortion a 'benefit' (to the public health), when it is clearly a terrible 'cost' to the woman. Adopting 'reduction of the birth prevalence' as the goal makes it inevitable that the success of a genetic screening programme will be largely judged by the proportion of the target population that take up the screening offer. It is then only a small step to setting primary health teams a target percentage uptake, perhaps even backed by financial incentives, as with immunisations. In considering the possibility that genetic screening might be offered through public health services, the American National Institutes of Health–Department of Energy Task Force on Genetic Testing[12] stated that "It might be difficult for public health personnel to appreciate that someone who refuses genetic screening is not jeopardising the health of the public". The uptake of genetic screening, particularly in the context of reproductive decisions, will be appropriately influenced by many social and cultural factors. It follows that any given population has a level of screening uptake that is 'right' for that population, reflecting their particular genetic information needs. It would be remarkable indeed if this were consistently 100%.

What then should be the overall goal of medical genetics services and a guiding principle for this assessment of screening for the fragile X syndrome? Pembrey and Anionwu[13] defined the aim of medical genetics as being "**to help those families with a genetic disadvantage live and reproduce as normally as possible**". This had its origins in a 1985 WHO definition[14] but has been considerably modified. It implies that medical

genetics services are concerned to restore normal biosocial function, which begs the question of what is normal. However, by expressing the goal in this way, it does allow that 'to live and reproduce normally' is not a universally agreed behaviour but one that can have various interpretations, which are culturally dependent.

Disease-specific or generic clinical genetics services

Since genetic analysis pervades much of medicine these days, a distinction needs to be drawn between genetics in medicine generally and a specifically clinical/medical genetics service led (usually) by clinical geneticists. No clear consensus on this has yet had time to emerge but there is one pragmatic distinction. If a clinician, faced with a specific diagnosis, family history or genetic test result, considers that he/she is professionally obliged to try to forewarn the patient's relatives of their genetic risk or to discuss prenatal diagnosis, then this is the province of medical genetics. Otherwise it is not. Fragile X syndrome clearly falls within medical genetics by this or any similar definition. One issue to be considered in this review is whether all genetics services for fragile X syndrome families, including any 'screening' activity that might be recommended, should fall within the remit of medical genetics services.

There has been little discussion, let alone analysis, of how and why a few local or regional **disease-specific** genetics services have developed outside general clinical genetics services in the UK but this issue is relevant (this is not a reference to neonatal screening services that just happened to begin as a phenylketonuria-specific service). If there are cogent reasons for enhancing fragile X syndrome services specifically, should these additional services be within or alongside the established national network of regional genetics services? The most striking examples of disease-specific services that include genetic testing, and which operate outside regional genetics services, are the haemophilia and haemoglobinopathy services. The most likely general explanation is simply one of timing. The basic genetic defects in haemophilia A and B, and in sickle cell disease and β-thalassaemia (and with this knowledge, the development of tests for both the affected and carrier states), were known well before regional clinical genetics centres were established in the 1970s. Once firmly established, integration with the developing regional clinical genetics

centres did not come naturally. Indeed, for a condition like haemophilia, in which clinical management is complex, ongoing and costly, a case can be made for having special disease-specific centres and, for families, there is a certain 'neatness' and convenience in the staff of such centres providing the genetics services as well. On the other hand, DNA banking, family tracing, coordination of prenatal diagnosis and interpretation of complex genetic test results for patients (and the health professionals who care for them) – just some of the expertise available at regional clinical genetics centres – are costly to recreate in numerous disease-specific clinics and centres. There is no particular case for any clinical management provided for people with fragile X syndrome and their families to come from special 'fragile X syndrome centres'. It is expected that genetics services to identified fragile X syndrome families will continue to develop within regional genetics services. This is not to say that any screening programme that might be established would be within regional genetics services, only that close links with such services would be essential if the clinical benefit is to be realised.

The emergence of disease-specific genetic screening

There are various ways of defining 'screening'. In their first report in 1998 the NSC defined it as "the systematic application of a test or inquiry, to identify individuals at sufficient risk of a specific disorder to warrant further investigation or direct preventive action, amongst persons who have not sought medical attention on account of symptoms of that disorder".[15] For the present purpose, genetic screening needs to be distinguished from genetic testing. In genetic testing it is the family member(s) who sets the initial agenda with respect to the genetic consultation. Typically this is sought, or advised by their doctor, because of the diagnosis of a genetic or possibly genetic disorder in a family member. By contrast, in genetic screening, it is the health professionals who set the initial agenda. An approach is made to healthy members of the general population or a section of the population. It should be noted that such individuals may have a family history of a disorder such as fragile X syndrome, which only comes to light as a result of the screening process. The term screening is also often used when health professionals systematically approach relatives of individuals with a known specific genetic disorder, an activity that, incidentally,

falls within the NSC definition of screening. Most genetics services activity is triggered by a recent diagnosis of what is, or might be, a genetic disorder in the family or by someone responding to their family history at a particularly relevant time such as a (planned) pregnancy. It was families needing help and counselling in just these situations that led to the development of genetics clinics in the first place. In the UK, these early medical genetics clinics slowly developed into a national network of regional genetics centres in the 1970s as advances in cytogenetics, biochemical genetics and, eventually, molecular genetics were able to enhance the precision of patient diagnosis, prenatal testing and carrier detection. In general, these services were concerned with the **recurrence** of a genetic condition in the family. With disorders inherited in an autosomal dominant or X-linked fashion, identifying the family at risk through the diagnosis of an affected individual allows genetics services to be focused on at-risk relatives. These at-risk family members constitute a significant proportion of all at-risk individuals in the population; in other words, a significant proportion of all affected individuals represented **recurrences** in families. This is not true for autosomal recessive conditions, such as the haemoglobinopathies and CF, or largely sporadic birth defects such as Down's syndrome or neural tube defects. The only way to identify, and therefore forewarn, the majority of couples at risk of conceiving a baby with an autosomal recessive condition, or to alert a woman carrying a baby affected with a 'sporadic' birth defect, is through some screening procedure. It was this fact more than anything else that triggered the development of the currently established genetic screening programmes in the UK. A move to consider screening for fragile X syndrome, with its extended affected families, cannot be regarded as just 'more of the same'. The inheritance pattern puts it in a different recurrence–risk category from established screening programmes, so any review will need to go back to first principles.

Why select screening for fragile X syndrome for review?

Despite the recent revision of the prevalence, which reduced the estimate to no more than 1 in 4000 males, fragile X syndrome probably remains the commonest single **inherited** cause of significant learning difficulties. The clinical features are subtle, which mitigates against early clinical diagnosis and, in turn, timely

genetic counselling to the extended family. By contrast molecular genetic diagnosis **within affected families** has proved effective. The unusual inheritance results in all first-affected members of families having unaffected mothers whose at-risk genotype is detectable by DNA analysis and who therefore could, in theory, have been forewarned before the pregnancy. Encouraging results from systematic case-finding among people with learning difficulties, followed by family screening, has been reported.[16,17] Despite this background of scientific and clinical advance, the experience of affected families and health professionals alike, suggested that clinical genetics services for fragile X syndrome families in the UK were far from satisfactory. The call by the NHS Executive in 1994 for an assessment of screening for fragile X syndrome was seen by the authors as a timely opportunity to start putting the matter right.

Key messages

- All genetics services relating to fragile X syndrome need to be developed within an overall goal and policy framework that is acceptable to the families they are intended to help.
- On the basis of the clinical features and pattern of inheritance of fragile X syndrome, there is no case for the development of stand-alone disease-specific centres like haemophilia centres.
- Unlike Down's syndrome and the haemoglobinopathies, for which the case for population screening is fairly well established, a significant proportion of those born with fragile X syndrome represent **recurrences** in 'known' families. This raises the possibility of a rather different approach to meeting the needs of those at risk of an affected child.

Chapter 2
General clinical features of
fragile X syndrome

Early recognition of fragile X syndrome

An excess of mentally handicapped males over females in the population was recognised and documented by Penrose in 1938.[18] He did not attribute this to X-linked conditions but to constitutional and social differences. The possibility that a significant proportion of this excess was accounted for by X-linked traits was suggested by Priest and colleagues[19] and Turner and Turner,[20] who both showed that there were many more pairs of brothers both affected with mental handicap than sisters. The hypothesis was developed by Lehrke[21] who commented not only on the excess of mentally handicapped males but also on the influence of the intelligence of a mother on that of her offspring, and on the large number of pedigrees published documenting X-linked inheritance of mental handicap.[22–28]

One of those pedigrees was that from Martin and Bell,[22] which has now been shown to be affected with fragile X syndrome by demonstrating both the fragile site on cytogenetic analysis and the expansion mutation in the fragile X gene (*FMR1*) on DNA analysis.[29] Martin and Bell[22] described 11 handicapped males in two generations. Two females within the family were also mildly affected. The affected males were descended from a sibship of two **normal** brothers and their sister, thus demonstrating the unusual features of the inheritance of fragile X syndrome; this is discussed in more detail below.

The chromosomal abnormality in fragile X syndrome was first documented by Lubs in 1969.[30] Standard chromosome preparations were used and an unusual secondary constriction was noted at the end of the long arm of the X chromosome in four affected males and two normal females. Other authors found similar appearances but reports were relatively few.[31,32] Sutherland[33] showed that this chromosomal appearance was seen only when certain folate-depleted culture media were used, explaining why it had not been consistently seen before in other pedigrees.

At this time an X-linked syndrome of macro-orchidism and mental handicap was becoming recognised[34–37] but it was not until Sutherland and Ashforth presented data from 13 males with mental handicap, macro-orchidism and an X chromosomal fragile site that the main features of fragile X syndrome were delineated.[38]

Pattern of inheritance

The simple account below illustrates the challenge faced by the counsellor who seeks to explain the genetics in a meaningful way to people being offered genetic testing or to health professionals involved with other family members. Fragile X syndrome exhibits some unusual features in its inheritance that are not usually seen in X-linked traits. Even in early pedigrees, males who were apparently clinically normal were shown to transmit the condition;[22,24,26,39–41] however, since there was often little objective information on their phenotype, this paradoxical inheritance was initially difficult to confirm. As more pedigrees were published, the normal transmitting male (NTM) became a well-recognised phenomenon and the normal intelligence, external phenotype and chromosomes of such individuals were well documented.[42–46]

The occurrence of heterozygous females, who demonstrated some of the clinical features of fragile X syndrome, was documented in the earliest pedigrees.[22,24–26] Although clinically-manifesting carrier females were known to occur in other X-linked conditions, their existence in fragile X syndrome pedigrees where there were NTMs appeared paradoxical. Once the fragile site was associated with the syndrome, the variation in the range of expression in affected and asymptomatic heterozygous females was rapidly established.[47–52] About half of female obligate gene carriers do not express the fragile site.[53] Females who do express the site are more likely to be intellectually impaired but the correlation between these variables is not close.[53,54] About one-third of female obligate gene carriers are intellectually impaired, although the extent of this is very

variable.[54,55] A similar proportion of female gene carriers show some of the facial features seen in affected males, most commonly when they are also intellectually affected.[54,55]

In explaining what were incompatible features for regular X-linked inheritance, an important clue was the observation that the daughters of NTMs (who must have inherited their father's X chromosome) were almost never intellectually impaired.[56,57] Formal segregation analysis of fragile X syndrome families, assuming regular X-linked inheritance, showed that there was a 'deficit' of about 20% of affected males.[46,53] Sherman and colleagues[46,53] were able to show that sibships with NTMs contained fewer affected individuals than expected and that the mothers and daughters of such men were virtually always unaffected. The chance of a child being affected with fragile X syndrome depended on the parent from whom the gene was inherited and on whether they were themselves affected. Progeny of NTMs were far less likely to be affected than progeny of normal carrier women. Progeny of normal carrier women were less likely to be affected than progeny of affected carrier women. These observations that the risk of mental impairment depended on position in the pedigree became known as the Sherman paradox.

A number of theories were proposed to explain the unusual features in the inheritance of fragile X syndrome.[56-65] With what is now known of the mutational mechanism, the two-stage model put forward by Pembrey and colleagues[56,57] and Nussbaum and colleagues[63] has been shown to be the one that fitted most closely. The molecular genetics are discussed later but, in essence, we are dealing with a DNA sequence in the fragile X gene that can expand over generations to a threshold beyond which the gene becomes silenced.

Clinical features

Fragile X syndrome is an important cause of mental handicap in males. The degree of impairment can vary from profound handicap through to isolated learning problems but most affected males have a severe to moderate degree of impairment, with IQs in the range of 35–49.[66] At this level of handicap, affected males are unable to live independently. Although developmental problems are present, they may not become apparent until after school entry.[67] There is evidence of a gradual decline in intellectual function in affected males with increasing age.[67-74]

In addition to their mental handicap, fragile X syndrome males display behavioural abnormalities. These are not necessarily specific to fragile X syndrome but are seen in this condition at greater frequency than in similarly intellectually handicapped peers. Such problems include marked avoidance of gaze, repetitive mannerism and obsessional traits. Hyperactivity is common.[75,76] Repetitive speech patterns are characteristic, with unusual rhythm and perseverative phraseology.[77,78]

In contrast, medical problems are uncommon. Clinical features, in addition to their behaviour, that can aid diagnosis include affected males tending to have large heads for their age (greater than the 50th centile), large ears and a 'long' face.[66] Final adult height does not seem to vary from the general population but there may be accelerated growth in childhood.[66] Macro-orchidism is found in about 80% of adult males but is much less frequent (about 22%) in the prepubertal child.[66]

Epilepsy occurs in about 20% of affected males; seizure type is not specific and standard therapy is usually prescribed.[79,80] Joint laxity is common but is rarely a problem.[81,82] Mitral valve prolapse is said to occur in between 22% and 80% of affected males.[83,84] There are a number of other features (reviewed by Hagerman[85] and Merenstein and colleagues[86]) that are seen at greater frequency than in the general population, including strabismus, myopia, cleft lip, scoliosis and sleep apnoea in infancy.

A small group of affected males have physical features similar to those of the Prader–Willi syndrome.[87] Individuals are short in stature, obese and have small hands and feet. However, the feeding problems in infancy and characteristic behaviour seen in Prader–Willi syndrome are absent.[88]

As already discussed, about a third of female gene carriers are intellectually impaired but usually less severely than males. In a group of intellectually impaired females, about half were thought to have only borderline features.[54] Affected girls also have behavioural problems, with shyness and social isolation being particularly common. Psychiatric abnormalities have been noted at a greater frequency than in the general population, even for women matched for their relationship to mentally handicapped males.[89]

Both males and females can transmit the fragile X syndrome without themselves having

any overt clinical effect from it. These males and most of the females would be expected to carry what is known as a premutation (see page 5). There are, as yet, no reports on the behaviour and cognition of such individuals when they have been ascertained from the general population and, until this type of study is undertaken, minor effects of the premutation cannot be excluded. In studies with secondary ascertainment of subjects through affected family members, female premutation carriers who are clinically unaffected have normal intelligence with no discrepancies demonstrated on detailed analysis of the various component subtests in IQ assessments.[90] Evidence is accumulating, however, that such women have a tendency to premature ovarian failure (POF). There was initially also a suggestion of an excess of twins in their offspring, raising the possibility of an underlying abnormality of endocrine function,[91,92] but the association with twinning awaits replication. A recent study in England[93] showed a clear excess of premutations in familial idiopathic POF. Of 147 index women with idiopathic POF, six (four familial, two sporadic) had premutations. POF does not appear to be associated with the full fragile X mutation.[94] A worldwide collaborative study provided convincing evidence that, within fragile X syndrome families, POF is only associated with premutation carriers and not with full mutation or non-carrier relatives.[95] This study[95] showed that a remarkable 16% (63/395) of premutation carriers had experienced the menopause before the age of 40 years compared with 0% of 128 women with the full mutation and 0.4% (1/237) of non-carrier relatives. Not only do these observations raise the question of offering fragile X syndrome testing to women with POF but they establish the fact that a premutation can have a detrimental biological effect. Recently, Hundscheid and colleagues[96] presented evidence for a parent-of-origin effect in the development of POF in premutation carriers, with paternally-inherited premutations (PIP) being more likely to give rise to POF than maternally-inherited premutations (MIP). However, both Murray and colleagues[97] and Vianna-Morgante and Costa[98] were unable to demonstrate a difference in the percentage of POF in PIP and MIP carriers. Sherman[99] discussed the possible explanation for these differing results, including bias introduced by potentially reduced fertility in female premutation carriers with POF. However, she concluded that more data are needed, both to resolve the parent-of-origin issue and also to explain why only some premutation carriers have POF.

Beyond POF it remains uncertain whether or not there are phenotypic differences between premutation carriers and properly matched controls. Such information will be important in the context of any proposed population screening, because truly informed consent will depend upon it.

Other fragile sites on the X chromosome

As understanding of the molecular mechanism underlying fragile X syndrome developed, it became apparent that there was a group of families in whom a fragile site at the distal end of the X chromosome (Xq) had been identified but in whom the mutation seen in fragile X syndrome was absent.[100–102] Detailed cytogenetic studies led to a number of distinct fragile sites being recognised that had previously been considered a single entity. The fragile site at Xq27.3 showed the expansion mutation in *FMR1* that is associated with fragile X syndrome is known as FRAXA. Sutherland and Baker[103] described FRAXE (the chromosomal fragile site at Xq28 that corresponds to the expansion in the *FMR2* gene) and Hirst and colleagues[104] described FRAXF (the chromosomal site in the Xq27.3–28 region). (In fact, one of the pedigrees reported by Sutherland and Baker has subsequently been shown to have FRAXF). The *FMR2* gene has been identified[105] and its expression studied in FRAXE families.[106] For both FRAXE and FRAXF, the families were originally ascertained because of mentally handicapped members and there has been a debate about whether either is associated with a clinical phenotype. Both have been shown to have similar mutational mechanisms to that seen in FRAXA.[107] Evidence is accumulating that FRAXE is associated with a mild mental impairment,[108–114] although some males with absent *FMR2* expression may not be intellectually impaired.[106] FRAXF is less likely to be associated with a clinical phenotype, because the correlation between fragility and mental impairment has been poor in all published pedigrees. A different diagnosis for the mental handicap segregating in the original FRAXF pedigree has been made (AJB: personal communication, 1998). A further common fragile site, FRAXD 1998 (that is not sensitive to folate depletion in culture media) has been identified proximal to FRAXA (in the Xq27.3–28 region) in about 5% of the population. Its expression is always at a low level and should not lead to cytogenetic confusion with FRAXA.[115,116] About 10% of families diag-nosed as having fragile X syndrome before the identification of the expansion mutation by DNA analysis lack

the molecular changes at FRAXA, and a proportion of these are likely to be affected by either FRAXE or FRAXF.[117]

Importance of other X-linked causes of mental handicap

Turner and Turner[20,118] estimated that about 20% of severe mental handicap in males is due to X-linked genetic mutations. Fragile X syndrome accounts for about one-third of X-linked mental handicap.[49,119–121] There are well over a hundred conditions in which mental handicap is associated with genes on the X chromosome.

Some of these are well-recognised diseases with other clinical manifestations or dysmorphic features or with associated biochemical abnormality. These conditions are usually diagnosed because of other clinical findings, e.g. Hunter's syndrome. Many other conditions are based on dysmorphic clinical signs that may be subtle, making diagnosis difficult, particularly in older patients. The majority of these X-linked conditions are called non-specific mental retardation (MR), MRX or primary MR – meaning that the only problem relates to the ability to learn. Progress is being made in mapping and identifying the mutant genes responsible.[122–125] Mapping of the gene may allow for relatively accurate carrier delineation and prenatal diagnosis for those identified as carriers but can only be carried out if the family pedigree is large, preferably with mental handicap in three generations. Once the gene is known, more reliable mutation detection becomes possible.

The prevalence of each of the syndrome and biochemically defined causes of mental handicap is low, with the possible exceptions of α-thalassaemia MR and X-linked hydrocephalus. However, the prevalence of the heterogeneous group of non-specific, or primary, MR is greater than that of the fragile X syndrome.

Key messages

- Fragile X syndrome is an inherited form of learning disability only delineated in the last 15–20 years.
- The clinical features are subtle, and there is commonly a delay in making a specific diagnosis.
- Although X-linked (and associated with a chromosomal fragile site at Xq27.3), it demonstrates an unusual and complex pattern of inheritance – both males and females can be unaffected carriers. Affected males and (more mildly) affected females can inherit it through a common unaffected grandfather – termed an NTM.
- The daughters of an NTM will inherit his X chromosome and the so-called 'premutation' but are never affected, because a 'premutation' only changes to a full mutation (causing the child to be affected) on transmission by a female.
- The level of learning disability in affected males is such that they are unable to live independently, although serious medical complications are uncommon.
- Female premutation carriers (but not full mutation carriers) are predisposed to POF.
- There are other chromosomal fragile sites at Xq27/8 that can be confused with fragile X syndrome on cytogenetic analysis.
- Fragile X syndrome accounts for about 25–30% of X-linked mental handicap.

Chapter 3

Understanding the biology of fragile X syndrome

Outline of key molecular genetic features

The gene that is not functioning in fragile X syndrome is the *FMR1* gene located in the Xq27.3 region of the long arm of the X chromosome. The *FMR1* gene has a variable (polymorphic) nucleotide triplet, cytosine–guanine– guanine (CGG) sequence repeat in the first transcribed part of the gene (first 5' exon). This repeat sequence is untranslated and does not code for part of the *FMR1* protein (FMRP). The key molecular genetic feature is that the CGG repeat can sometimes increase in size and become un-stable so that it expands in size over generations. When the expansion reaches a critical size (over 200 CGG repeats), it triggers a molecular silencing of the *FMR1* gene. An important question from the screening point of view is whether an unstable CGG repeat can be recognised – the premutation – that is likely to expand in one or two generations and cause fragile X syndrome.

Importantly, family studies have not been able to trace back to the point where the premutation emerged from a 'normal' repeat size. This means that even third-degree relatives of an affected individual have a high risk, as shown in *Figure 1*.

The fragile X gene, *FMR1*, and its product, FMRP

The phenotype of the fragile X syndrome is likely to be the direct consequence of the absence of the *FMR1* gene product, a 70–80 kDa protein (FMRP).[126,127] In unaffected people this protein can be identified, using a labelled antibody, in a variety of cells including white blood cells. It is reduced or absent in affected males.[102,126,128,129] It is normally present in many types of cell in the body but is found in high concentrations in nearly all neuronal cells of the brain, in the testes in adult life,[130] and in both fetal ovaries and testes in the early months of life.[131] Under-standing of the function of FMRP is limited but its structure indicates that it is involved in binding RNA.[132–135] There is evidence that it may play a role in ribosomal function and
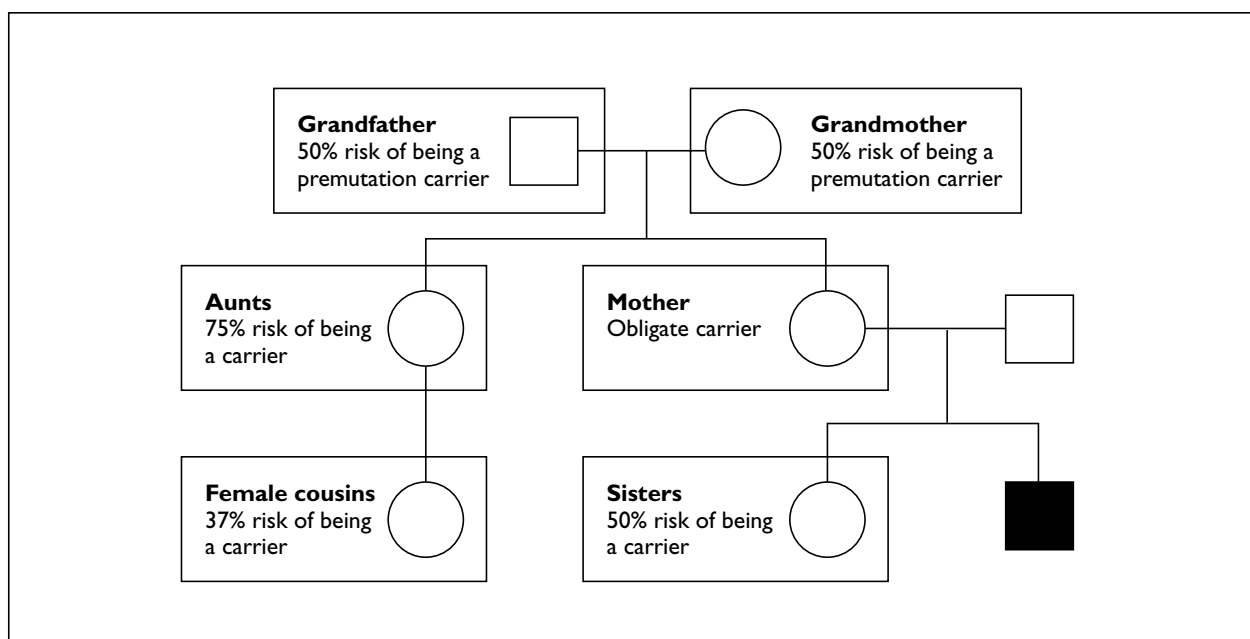
FIGURE 1 *Risks to female relatives. A small proportion of male relatives will be identified as premutation carriers*

translation of certain proteins.[136–140] Although predominantly cytoplasmic in localisation, FMRP may also act as a shuttle molecule exporting messenger (m)RNA(s) to the cytoplasm from the nucleus.[141] To add to the complexity, alternative splicing in different tissues results in 24 distinct transcripts, although whether these all lead to functional proteins is unclear.[127,142,143] The likely functions of FMRP at the molecular and cellular levels have recently been reviewed.[144]

The *FMR1* gene, comprising 17 exons spanning 39 kb, is located at the FRAXA fragile site at Xq27.[145] The change in the *FMR1* gene that causes fragile X syndrome has been termed a dynamic mutation and was the first example of this kind of disease-causing mutation in man. The novel feature of this type of mutation is its instability when transmitted between generations; this instability lies behind the unusual pattern of genetics discussed earlier. Similar mutations have been described in other genetic disorders, nearly all of which have phenotypes with CNS dysfunction.

Variation and expansion mutations in the CGG repeat of the *FMR1* gene

Normal variation

With rare exceptions (see below), the mutation associated with the fragile X syndrome is in a CGG trinucleotide repeat array within the untranslated region of the first exon of the *FMR1* gene. This array can contain a variable number of CGG repeats, commonly interspersed with two adenine–guanine–guanine (AGG) triplets,[146–151] and in genetic terms each repeat size can be regarded as a *FMR1* allele (sometimes referred to as a FRAXA allele). The lowest reported CGG repeat number is 5 but where the traditional 'upper limit of normal' lies is complicated by the fact that it is not just the repeat number *per se* but the transgenerational (in)stability that determines if there is any risk to offspring. A recent review[152] illustrates this difficulty of only being able to definitely classify alleles in the 'grey area' once there is information on their stability, by stating (our emphasis) that
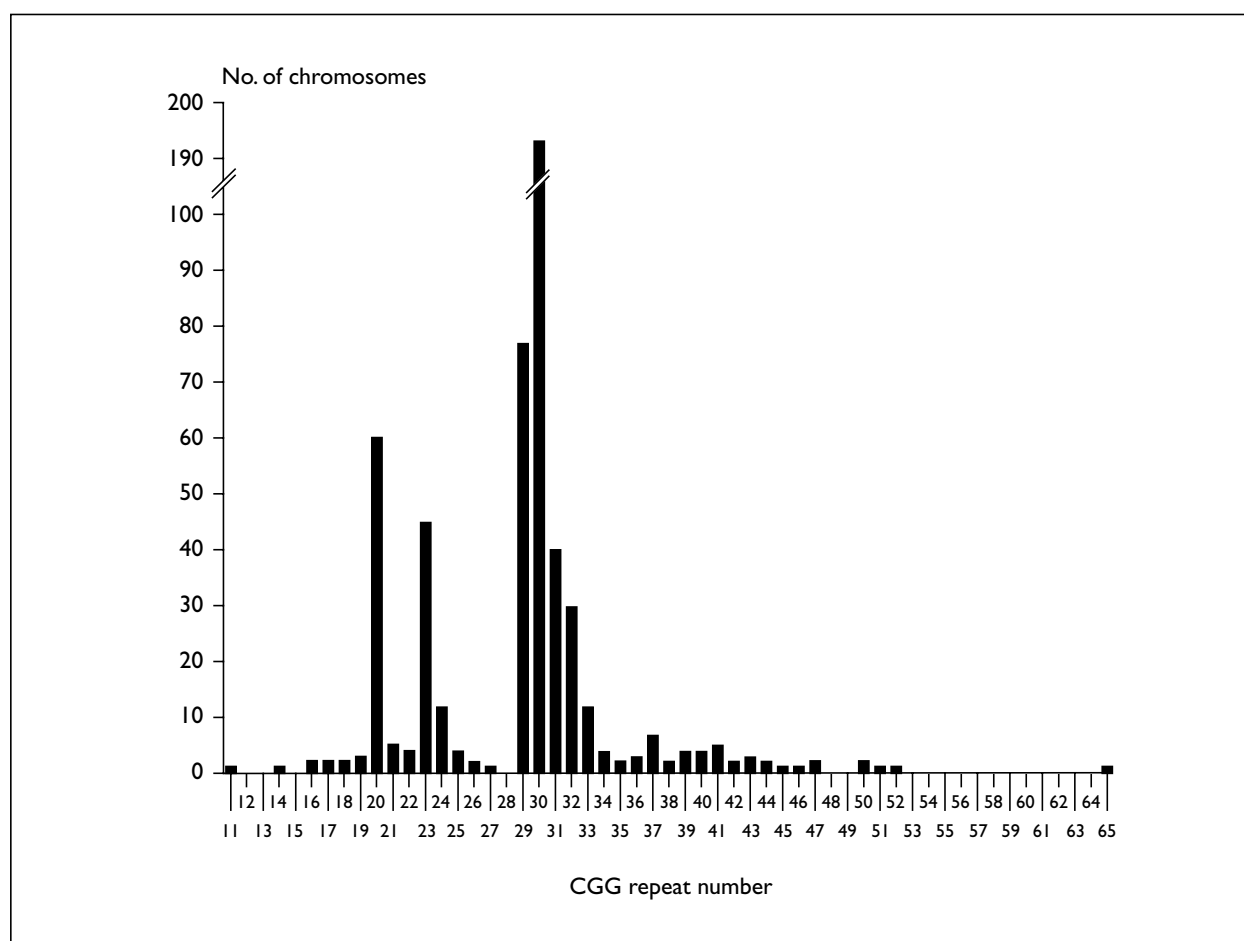
FIGURE 2 *The distribution of alleles in 543 boys from a contemporary unselected general population sample (ALSPAC)*

"**stable** alleles with a CGG repeat < 55 units are defined as normal and **unstable** alleles ranging from 43 to 200 repeat units are regarded as pre-mutation alleles". The selection of a lower cut-off point of 43 for classifying an unstable allele as a premutation in clinical practice is not widely accepted. In the normal general population, there is a characteristic distribution of alleles with a peak at about 30 repeat units, the commonest allele, and two smaller peaks occurring at 20 and 23 repeat units. *Figure 2* shows the distribution of alleles in 543 boys from a contemporary unselected general population sample from around Bristol, UK (Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC); Jacobs PA, *et al.*, Wessex Regional Genetics Laboratories, Salisbury: personal communication, 1998). In general, this roughly matches the composite distribution of 6052 alleles from 'unaffected' populations around the world compiled by Murray and colleagues.[4] Of great relevance to any screening activity, however, is the proportion of alleles in the upper tail of the distribution as it merges with the premutation range. A 'control' distribution of 726 alleles in women in a study in Wessex, UK,[153] found rather fewer alleles in the range 40–60 repeats (1.9%) compared with the ALSPAC study of boys (3.7%) shown in *Figure 2* but the same as in 1477 women in Finland (1.86% of alleles).[154] In the Wessex study, all boys with unexplained learning diffi-culties in the defined general population were eligible for inclusion and their mothers' **untrans-mitted** X chromosomes were used as the control allele. Whether this approach to 'controls' or the use of buccal cells rather than blood introduces a bias against the larger alleles is unclear. It is possible that intermediate alleles are under-represented in fertile women. The rather small ALSPAC study, which is to be extended to more boys, represents as unselected a general population male sample as is likely to be achieved beyond anonymous Guthrie card testing.

Females, having two X chromosomes, have two *FMR1* alleles that may have repeat arrays of the same size (i.e. homozygotes) or have different repeat sizes (i.e. heterozygotes). Results from five studies, including a total of 1518 normal females and collated by Murray and colleagues,[4] showed an average of 29% (range 18–33%) to be homozygous. One contribution to the wide range may be population differences but it also reflects the resolution of the analytic method used and the confidence of distinguishing two alleles that differ by only one repeat. A recent report of screening 1477 pregnant women in Kuopio, Finland, gave a homozygosity rate of less than

15%.[154] The Wessex research studies[153] (Murray A, *et al.*, Wessex Regional Genetics Laboratories, Salisbury: personal communication, 1998) of 2521 females gave a total homozygosity rate of 16.8%. This is close to the theoretical figure expected from the individual allele frequencies. However, in clinical practice (where the fear is that homozygosity may reflect one normal allele and one expanded mutant one), there may be less confidence in accepting that two alleles differing by a single repeat are definitely present without further study; hence the higher homozygosity rate quoted. Wildhagen and colleagues,[155] in their analysis of fragile X syndrome screening, used a figure of 40% for the polymerase chain reaction (PCR) test 'showing only one band', and therefore needing further molecular analysis. If two different alleles can be resolved by DNA analysis, then that female's genotype can be unambiguously defined. If only one allele repeat size is found and an expansion ruled out, then they are presumed to be homozygous.

In discussing allele sizes, there is some merit in using the classification of repeat sizes adopted by Jacobs and colleagues[153,156] because of the un-certain significance of alleles in the 41–60 repeat range, especially when found in someone with no relative affected with fragile X syndrome. The classes (with the expected frequencies in the general unaffected population) are as follows:

- minimal < 11 repeats (rare)
- common 11–40 repeats (approximately 98%)
- intermediate 41–60 (approximately 2%)
- premutation 61–200
- full mutation > 200.

As discussed later, there is a separate issue of what cut-off to use between intermediate and premutation in clinical practice. The suggestions range from 43 repeats to 61 repeats. A fairly common cut-off cited in the literature is 54/55 repeats.

Expansion mutations and the silencing of the *FMR1* gene

Progression from premutation to full mutation is considered below but, when the increase in size of the repeat array is more than 200 repeats (i.e. full mutation), there is usually hypermethylation of the CGG repeat and its flanking regions within exon 1 of the *FMR1* gene. This region includes a CpG island that is thought to act as a promoter for the *FMR1* gene and hypermethylation results in a shutdown of transcription and the absence of FMRP.[126,128,129,157] The latter causes the MR. In

support of the view that the *FMR1* gene 'silencing' is due to hypermethylation is the finding that, for *in vitro* cells with full mutations in the 300–800 repeat range, transcription can be reactivated by the demethylator 5-azadeoxycytidine.[158] All males with a hypermethylated full mutation are mentally retarded, as are the majority of females but to a lesser degree.[102,159–162] The expansion size does not have an influence on the severity of the clinical phenotype in the males,[102,163] presumably because hypermethylation of the *FMR1* gene at a particular repeat-size threshold produces an 'all or none' effect. This effect occurs, of course, at the level of individual cells. However, an individual with fragile X syndrome is essentially a mosaic of genetically different cells.

Mosaics

The mitotic instability of the repeat of the full mutation in somatic cells in early embryogenesis causes longer and shorter expansions.[164,165] This results in all fragile X syndrome patients being somatic mosaics in one sense. This is of little consequence for the clinical molecular classification of patients if all the expansions are in the full mutation range. However, there are two special subclasses of mosaicism based on size and methylation status.

Size mosaics occur in those patients with both a full mutation and premutation, a pattern that is detected in about 25% of male and less than 10% of female patients.[159,166] Murray and colleagues[4] summarised seven studies, finding a range of 15–41% of size mosaics in affected males. It is likely that the differences are a matter of technical sensitivity. There were also a few cases reported in which a male had cells with a full mutation and cells with a deletion involving the *FMR1* gene.[163,167–170]

Methylation mosaics are those with variations between cells in the methylation status of a full mutation. The proportion of leucocytes with an unmethylated full mutation may vary from low (approximately 10%) to 100%. Sixteen intellectually normal males with a high proportion (> 60%) of leucocytes with an unmethylated full mutation have been reported.[171–178] Such males are probably rare.

Genotype/phenotype correlation

The extent to which the genotype predicts the presence or degree of learning difficulty is clearly of crucial importance when it comes to prenatal or newborn screening where no direct assessment of IQ is possible.

Males
Males represent the simplest situation, because they have a single X chromosome, even though they may manifest cellular mosaicism (see above). Although patients with fragile X syndrome range from profound to borderline MR, the presence of a methylated full mutation is regularly associated with learning difficulties. No correlation is observed between the degree of MR and the size of the full mutation.[163] Furthermore, the degree of learning difficulty does not seem to be influenced by the presence of premutation alleles in a proportion of cells in addition to the full mutation.[163]

Females
For females, the genotypic prediction of learning difficulties is relatively poor, with the normal process of X inactivation being an additional factor. De Vries and colleagues[162] compared their study with five earlier studies,[159,160,179–181] and came to the conclusion that 52–71% of females with the full mutation had IQ scores of < 85. In their study,[162] 50% of females with a full mutation had an IQ below 70 points. Like several previous studies, although not all, they found a "positive association between the performance IQ and the proportion of active X chromosomes with the normal as opposed to the full mutation allele". However, this observation does little to help a pregnant mother faced with the prospect of having a girl with a full mutation. There is no way of predicting if, and to what degree, the girl will have learning difficulties.

Transgenerational instability of the CGG repeat

Limited data from the general population to support inferences from affected families
There are few data on transgenerational (in)stablity of CGG repeats of various lengths other than through the family studies of patients with fragile X syndrome. Murray and colleagues[156] provided some data from a population-based study of boys with learning difficulties and their mothers, and Ryynänen and colleagues[154] obtained limited transmission data in a screening feasibility study of 1477 low-risk pregnant women. Recently, Drasinover and colleagues[182] reported on the transmission in 108 pregnancies from 107 women (with 53–135 CGG repeats), who

were among 10,587 healthy women from the general Israeli population who had accepted fragile X screening prior to pregnancy. This impressive dataset provided the best **population-based** information to date of the transmission (in)stability of intermediate alleles between 51–60 repeats and of premutations. It is still, however, a relatively small sample from which to assess the reliability of inferences from the study of transmissions in families ascertained through patients with fragile X syndrome.

The study of fragile X syndrome pedigrees can do little to illuminate the early stages of progression, because no family study has detected the full progression from a 'common' class of repeat (11–40 repeats) in an ancestor to a premutation (55 or 61–200 repeats). Classically, the retrospective analysis of ancestors reveals progression from a premutation to the full mutation of the affected index case (through which the family was ascertained) in one or two generations. However, the study of branches of extended pedigrees can show the premutation to be stably transmitted over many generations,[183,184] an observation that ties in with the observed difference in prevalence of premutations and full mutations described earlier. It is important to recognise that what is described below is largely inferred from family studies. In particular, such studies provide little information on the **rate** or **change of rate** of expansion over the generations from the 'common' class of alleles, through the 'intermediate' class (41–55 or 61 repeats) to the premutation.

Common and intermediate alleles

Over time, of course, some common alleles must progress towards larger CGG repeat sizes but it has been observed rarely. For practical purposes relevant to screening, 'common'-sized repeat arrays (11–40) do seem to be transmitted in a stable manner from parent to child. In the Wessex studies, no instances of instability in the minimal and common ranges in 726 transmissions were found in one dataset[153] and, in another dataset,[156] only one unstable transmission was found in 88 male and 254 female meioses. This particular case, of a paternal expansion from 29 to 39 repeats, was associated with instability at more than one locus and is discussed below. To our knowledge, only two other cases of change within the common range (< 41) have been reported; a 34-repeat allele that was both stably and unstably transmitted in two separate transmissions[146] and a 29-repeat allele that reduced in size to 21 repeats.[185]

Intermediate-sized alleles (40–60) seem to be less stable than 'common' alleles. Murray and colleagues[156] found 4/84 (4.8%) female transmissions and 0/21 male transmissions to be unstable. Two were below the cut-off of 54–55 repeats commonly used by other groups to define premutations, being increases from 50 to 51 and 53 to 54 repeats. In the other two, the parental allele was higher (and would be classified by most as a premutation[152]), and the expansions were from 55 to 56 and 60 to 68 repeats. In the latter case, the repeat went on in the family to expand to a full mutation. An unpublished update of the Wessex study (Murray A, *et al.*, Wessex Regional Genetics Laboratories, Salisbury: personal communication, 1998) gave the following results. A change of one or two repeats up or down occurred in 8.1% of 123 transmissions of alleles in the 41–50 range, and in 7/17 (41%) transmissions of alleles in the 51–60 range. Drasinover and colleagues[182] found that 11/48 (23%) transmissions from mother to fetus in this range were associated with increases in the repeat number, with the proportion being higher (6/14, 43%) in those women with 56–60 repeats compared with those with 51–55 repeats (5/34, 15%). The increases may be more than one or two repeats (see *Table 1*), with two of the five women in the 53–55 range having babies with increases of 8 and 10 repeats.

Mogk and colleagues[186] reported a family in which one brother with 59 repeats had an affected grandson (> 200 repeats), while his half-brother had 47 repeats that became 48 in his daughter and 49 in his granddaughter. These observations illustrate the difficulty of defining meaningful classes of alleles in the upper tail of the distribution of the unaffected population. Because an expansion from a 'common' allele to a full mutation always passes through two or more generations as an asymptomatic premutation, this presents an important opportunity for forewarning couples of their risk before they have an affected child. It is how best to realise this potential for alerting couples that is the big question. The difficulty of assessing the (in)stability of 'intermediate' alleles in the 41–60 repeat range in the individual case certainly poses a challenge for any proposed screening programme.

Premutations

As indicated earlier, the term premutation implies instability on transmission and this is not just determined by repeat size. Once in the premutation range of 55 or 61–200 repeats, the

TABLE I *Rate of expansion of intermediate alleles from mother to fetus (adapted from Drasinover, et al.[182])*

Number of CGG repeats in mother	Total number of pregnancies with transmission of abnormal allele	Cases with increased number of CGG repeats in fetus	
		Mother	**Fetus**
51–55	34	53	54
		54	55
		55	55
		62	64
		56	56
56–60	14	56	56
		56	57
		59	59
		61	57
		58	63
		61	60

picture is a little clearer, although population-based data are still limited, with the best coming from the Israeli study.[182] The transgenerational instability is dependent on the sex of the transmitting parent and the size of the repeat. Only women with a premutation have a risk of having an affected child with a full mutation. Fathers with premutations (who are NTMs) must transmit their X chromosome to their daughters but the premutation remains. Although there is an occasional further expansion within the premutation range, their daughters are not at risk of a full mutation. Regressions in premutations sizes can also occur and occasional regressions back to normal (< 40 repeats) within one generation have been recorded.[187,188]

It is not clear why the expansion from premutation to full mutation occurs only when transmitted by the mother. It is likely to be due in part to selection against sperm carrying full mutations. In keeping with this is the observation that only premutations were found in the sperm of four males with a full mutation in their somatic cells.[189] These findings initially suggested that the time of the transition from premutation to full mutation is after conception in the early embryo (and sparing the germline) but there was conflicting evidence from the study of female fetuses, in which the ovary showed full mutations and no evidence of premutations.[190] Simulation studies likewise supported preconceptional expansion from premutation to full mutation.[191] It is therefore possible that, in a male with the full mutation, a few germ cells regress to a premutation and it is these that survive and proliferate.

Possible mechanism and influences on CGG expansion
Loss of interspersed AGG triplets
There is some evidence that the instability in both meiosis (leading to germ cells) and mitosis critically depends on the length of **pure** CGG tracts within the 3' end of the array.[146,148] The instability threshold in other triplet-repeat disorders is about 34 pure repeats and this also seems to be the case for fragile X syndrome if just pure CGG tracts are taken into account.[148,192,193] The majority of *FMR1* alleles have the CGG tract interrupted by regularly interspersed AGG triplets, every 10th, 11th or 12th CGG repeat unit.[146–148,192,194,195] Most *FMR1* alleles are likely to be stable because either the total repeat length is less than 34 or there are sufficient interspersed AGGs to the keep the pure 3' CGG tract below the 34 threshold. *In vitro* studies[196] have shown that AGGs within a CGG tract can prevent the formation of the stable hairpin structures implicated in the replication slippage that is probably the immediate cause of the expansion.[196–198] Although 68% of common alleles have two interspersed AGGs, 63% of premutations have no AGG, and a further 37% have only one, bringing the 3' pure CGG tract up to the instability threshold.[194] Clearly the loss of one or more AGG could contribute to the incipient instability that will drive common alleles into the intermediate and premutation range. The meiotically unstable allele described earlier, in which the repeat increased from 47, to 48, to 49 over three generations, did not have any intervening AGG sequences.[186] Eichler and colleagues[199] suggested that an individual allele could develop a long tract of pure CGG repeats by at least two mechanisms: gradual increase

in the repeat number from the 3' end of the repeat, or sudden loss of an AGG interrupting triplet, giving more rapid progression to an unstable allele. However, in most of the alleles in the populations studied,[149,192,194] the loss of one AGG triplet would not raise the uninterrupted 3' CGG tract above the instability threshold. Other factors must also play a role in promoting incremental expansion. In an analysis of 4613 unrelated chromosomes (4596 from the Wessex studies[153]), haplotyping suggested two distinct lineages that are prone to expansion. The interspersion pattern within the CGG repeat is a strong determinant of instability, the best predictor of which is a function of the number of AGG interspersions and total length, which in turn is highly correlated with the largest uninterrupted CGG repeat length (Shipley F, *et al.*, Department of Human Genetics, University of Southampton: personal communication, 1998). There may be other, as yet undefined, determinants of instability at the *FMR1* locus. The potential clinical value of using the length of the pure 3' CGG array for assessing the risk of an expansion to a full mutation on transmission still requires evaluation.[152]

Mismatch repair defects

Finding the rare family, as mentioned above, in whom there is instability at other loci as well as the fragile X repeat raised the possibility that coincidental inheritance of genes affecting general genome stability might lead to (rapid) expansion.[200] One such mechanism could be defects in the DNA mismatch repair (MMR) genes and this has been explored in people known to have such a genetic mutation.[201] The cancer predisposition in most individuals with hereditary non-polyposis colorectal cancer (HNPCC) is attributable to mutations in any one of the five MMR genes. One consequence of MMR deficiency is somatic instability of microsatellite repeat sequences. Fulchignoni-Lataud and colleagues[201] found some indication of increased somatic instability at the *FMR1* CGG repeat in HNPCC patients compared with a control group. Although this study only analysed **somatic** instability, there is evidence that instability at the *FMR1* locus is well correlated in both somatic and germinal cells.[202] However, others did not find that mutations in the two MMR genes, *hMSH1* and *hMLH1*, resulted in *FMR1* CGG instability.[203] It is a reasonable hypothesis to propose that some concomitant MMR deficiency in an ancestor might have contributed to an episode of expansion of the *FMR1* CGG repeat.

Further studies along these lines are needed before any firm conclusions can be drawn. However, the hypothesis illustrates both the way that research might go and just how complex assessing instability could be, let alone the opportunities created by research in this area for public misunderstanding of the links between fragile X syndrome and cancer!

Empirical risks of expansion (that can be used in genetic counselling)

It will be a long time before the factors influencing expansion of the *FMR1* CGG repeat are fully understood. Furthermore, there is no guarantee that such understanding will translate into the ability to classify every person into a clinically meaningful risk group with respect to having an affected child, as can be done with regular Mendelian disorders. In practice, clinicians will have to depend on empirical risk figures to either reassure potential parents or trigger their further action with respect their own reproductive decisions or family studies.

As expected at the two extremes of the allele distribution in the unaffected population, the matter is relatively straightforward. For repeat sizes up to 40 triplets, the Wessex researchers[153,156] reported one expansion in 1068 transmissions (updated figures are comparable). Although further population studies may modify this figure, 1/1000 fits with the rarity of reported observed expansions with this allele range. General clinical genetics experience indicates that such a figure would be very reassuring to the approximately 96% of women who only carried these 'entirely normal' alleles.

The interesting modelling of the dynamics of 'premutation' alleles by Murray and colleagues,[4] based on five studies with family data on the risk of expansion to a full mutation,[204–208] showed that with a premutation of 90 repeats or more there is at least an 80% chance of expansion to a full mutation on transmission of that X chromosome. With a figure this high, nearly all women would wish to take account of the risk in their reproductive decision-making and there would be an unequivocal clinical obligation to inform the woman and offer family genetic counselling.

In practical terms, the counselling challenge for the health professional is specifying the risks to offspring associated with a CGG repeat of 41–90. It is important to appreciate that, with the

inheritance pattern of fragile X syndrome, a woman may be concerned not only for her future child but also for the children of her relatives for whom she feels a family responsibility. There are two types of risk to consider: the risk of her own child having a full mutation and being affected; and the chance that her child will have a premutation that could progress to a full mutation in one or two generations.

Expansion to a full mutation

In affected family studies, the smallest premutation that has been reported to progress to a full mutation in one generation is 56,[204] although this number is being reassessed and the emerging consensus is that the lowest is 58–59 (Nolin SL, Institute for Basic Research, Staten Island, New York, USA: personal communication, 2000). It clear from the collation of the published family data, as undertaken by Murray and colleagues,[4] that there is an increasing chance of expansion to a full mutation on transmission, the greater the size of the maternal premutation. What is less clear is the actual level of the absolute prior chance of expansion to a full mutation for any given size of maternal premutation – just the information a woman will want to incorporate into her reproductive decision-making. To date, the only prospective general population data comes from the screening trials in Finland[154] and Israel.[182] This experience amounts to 25 pregnancies in women

with > 60–135 repeats and 60 pregnancies in which the mother has > 50–60 repeats. At the time, in the absence of even this population-based data, Murray and colleagues[4] attempted an elegant indirect estimate of the expected expansion risk for the known premutation size distribution in the general population. Their approach aimed to overcome some of the bias towards an exaggerated expansion risk inherent in affected family studies. *Figure 3* is reproduced from their paper; applying these regression curves to the premutation size frequency distribution in 48 females (derived from six published studies[185,193,207,209–211]) they obtained an average expansion risk of between 26% and 37%. This is less than half the average expansion risk of 68–78% seen in the affected families from five reported studies.[204–208] The authors noted, however, that this lower rate is still too great to be consistent with the difference in the estimated prevalence of premutations and full mutations in the population. Working backwards from the observed population prevalence of 1 in 4000 clinically affected males (who therefore have the full mutation), they used the following argument, assuming a steady state for the full mutation prevalence from one generation to the next. Because affected (full mutation) individuals only come from a maternal transmission (in which the mother has either a full mutation or a premutation) with an equal chance of passing into a male or female, the prevalence
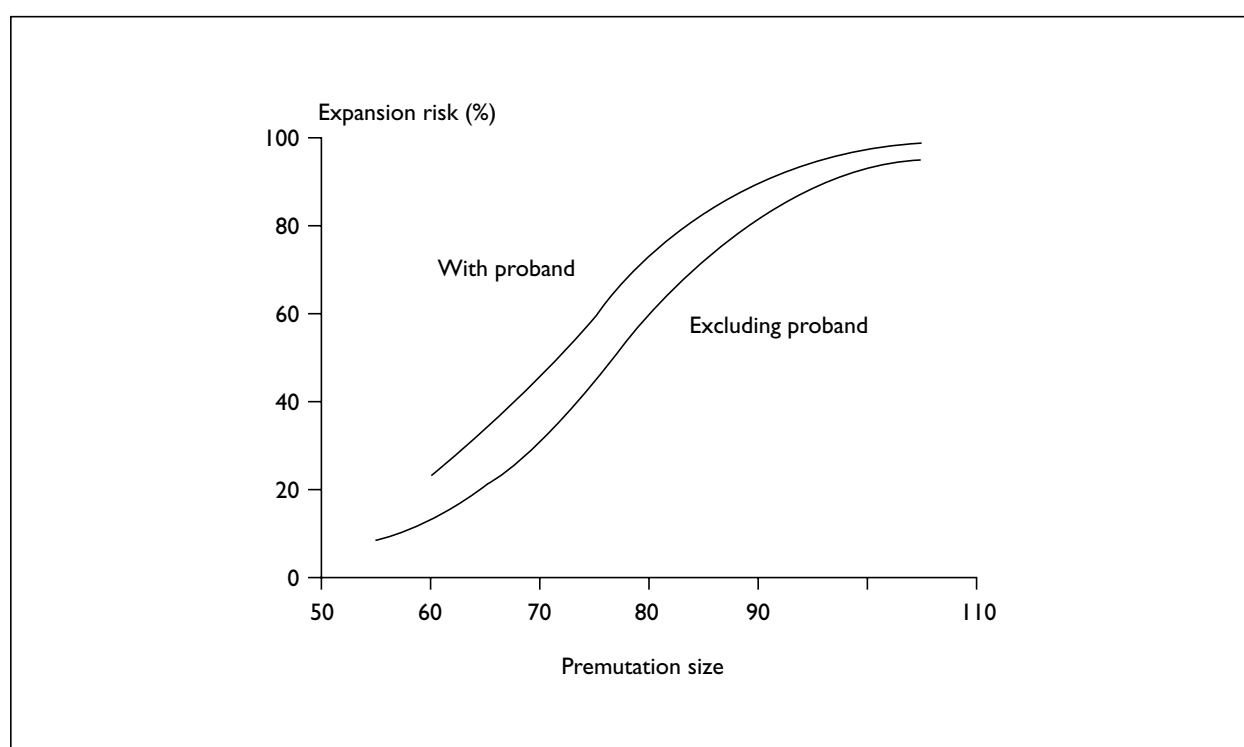
FIGURE 3 *Risk of expansion from premutation to full mutation in one female generation[4] (reproduced with permission)*

of full mutation females will also be 1 in 4000. Assuming full mutation carriers have a reproductive fitness of 50% (and remembering that a female has an equal chance of transmitting her normal X chromosome), 1 in 16,000 in the next generation will have received a full mutation from a full mutation mother. All the others making up the 1 in 4000 prevalence will be the result of an expansion of a maternal premutation to a full mutation. This figure of 1 in 5333 (1 in 4000 minus 1 in 16,000) is generated from a pool of 1 in 273 premutation carrier mothers – the estimated prevalence of the premutation in females used in this study.[4] The above implies that the average expansion from premutation to full mutation is 10% (1 in 5333 divided by half

of 1 in 273). The figure rises to 18.75% (rather than 10%) if the prevalence of full mutations is 1 in 4000 and the prevalence of the premutation in females is 1 in 500, but only rises to 12.5% if the prevalence figures are 1 in 6000 and 1 in 500, respectively, as indicated by rather conservative interpretation of recent observations. In the recently published Israeli experience[182] (*Table 2*), no expansion to full mutation was found in nine pregnancies with 56–65 repeats but three of the five women with repeats of ≥ 70 had a fetus with a full mutation. The combined figure of 3 in 14 (21.4%) lies between the range estimates from the two indirect calculations by Murray and colleagues.[4] The more limited Finnish data (*Figure 4*) is in keeping with the Israeli experience.

TABLE 2 *Rate of expansion of premutations from mother to fetus (adapted from Drasinover, et al.[182])*

Number of CGG repeats in mother	Total number of pregnancies with transmission of abnormal allele	Cases with increased number of CGG repeats in fetus		Fetuses with full mutations (%)
56–60	14	6 (42.9%)		0
		Mother	*Fetus*	
61–65	9	61	62	0
		64	66	
		65	100	
66–70	7	70	160	14.3
		70	Full mutation (300)	
≥ 71	3	80	135	66.7
		80	Full mutation	
		135	Full mutation	
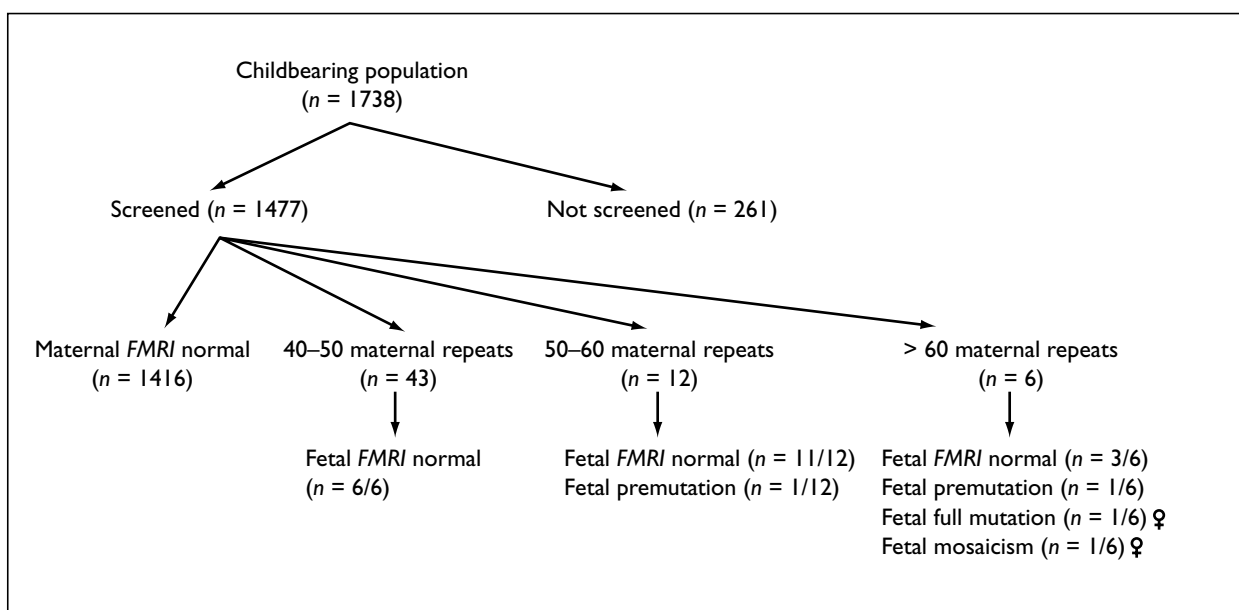
FIGURE 4 *The outcome of offering fragile X syndrome screening in early pregnancy to a general low-risk population in Kuopio, Finland[154] (reproduced with permission)*

What are we to make of this discordance between the expansion risks estimated by Murray and colleagues[4] (an average expansion risk of 26–37% for premutations in the general population) and the 10–19% risk derived from the difference in the estimated prevalences of the premutation and full mutation? Murray and colleagues[4] rightly concluded that either one of the prevalence estimates was wrong, or the curve in *Figure 3* applied only to affected families, or there is a tendency for the normal X chromosome to be transmitted to the conceptus rather than the mutated one (to which one could add differential implantation, embryonic survival, for example). Data published since this analysis[4] continue to support the big differences in the prevalence of fragile X syndrome patients with a full mutation and premutation carriers, so resolution of the paradox is unlikely to come with more accurate prevalence figures alone. However, it has to be remembered that the premutations in these population studies are defined solely on the basis of CGG repeat size, with no direct indication of repeat instability. As regards distorted segregation ratios in families in which the premutation or full mutation is being transmitted, it is possible to look at this directly. Sherman and colleagues[212,213] have analysed the outcome of prospectively ascertained pregnancies and transmission of the (pre)mutant X chromosome is roughly what the Mendelian ratios would predict. The segregation ratio of the premutation allele to the total transmissions was 0.47 and for full mutation carriers the ratio was 0.52. However, in these studies on affected families, 97% of the carriers had more than 60 CGG repeats.

The recent report from Drasinover and colleagues[182] on an Israeli population study included 68 mothers with 51–60 CGG repeats with intriguing and statistically significant segregation results. In the maternal 51–55 repeat range, the 49 fetuses segregated into 15 normal:34 abnormal alleles, giving a segregation ratio of 0.69, while in the 56–60 repeat range, the segregation was 5:14 (ratio 0.74). This transmission ratio distortion was not seen in pregnancies in which the mother had 61–65 repeats (7:9, ratio 0.56), 66–70 repeats (8:7, ratio 0.47) or ≥ 71 repeats (6:3, ratio 0.33); however, in these ranges the absolute numbers became small. This finding of preferential transmission of alleles in the 51–60 repeat range may be one factor maintaining the high population prevalence of premutations but does not explain the difference in the two indirect estimates of the risk of expansion of a premutation to a full mutation calculated by Murray and colleagues.[4]

The idea that the risks of expansion shown in *Figure 3* apply only to affected families and not to premutation carriers ascertained from the general population is a very real possibility, and highly relevant to any plans to introduce population screening. From what is known of the molecular biology, it is likely that this ascertainment difference in expansion risk will be most marked in the 51–65 range. The lower risk curve (probands excluded) shown in *Figure 3* starts at an 8% risk of expansion to full mutation for maternal alleles of 55 repeats, rises to a 12% risk at 60 repeats and to 20% at maternal alleles of 65 repeats. The pooled Israeli[182] and Finnish[154] experience of general population screening showed that there was not a single change to full mutation with 62 transmissions of maternal alleles in the 50–65 range. Exact numbers were not obtainable from the grouped data from these two studies but the 62 transmissions definitely included 16 transmissions in the 55–65 range and the estimated total is more than 20. Thus there is some evidence that the modelling from affected family-based studies overestimates the risk in the low premutation range.

There were a number of assumptions in the analysis by Murray and colleagues,[4] which they acknowledged, that could also contribute to the discrepancies between the general population model and expansion risks deduced from family studies. For example, their population model assumed normal reproductive fitness in women with premutations. In view of the growing evidence that the premutation is associated with POF,[93,99] such women could have reduced fertility even before their premature menopause. The above discussion highlights how reliant we are on the limited population data from Israel and Finland to provide the empirical risk figures needed to counsel women with CGG repeats in the (low) premutation range (51–65), with respect to learning difficulties in their offspring. There is even more uncertainty about what it means to have an allele in the intermediate range (40–54 or 60).

Expansions within the intermediate range

As highlighted above, the nature of fragile X syndrome inheritance means that any genotype result that implies an unstable allele will immediately trigger requests for information on risks to future family members, even if they are one or two steps removed from the person who has been tested. There has been little systematic analysis of transmission (in)stability in the intermediate range. As discussed earlier, the most useful

information comes from the Israeli population screening study[182] and the Wessex study.[156] In an update of the latter study (Murray A, *et al.,* Wessex Regional Genetics Laboratories, Salisbury personal communication, 1998), maternal alleles in the 41–50 range had an 8% chance of changing by one or two repeats on transmission, and alleles in the 51–60 range had a 40% rate of unstable transmission. As indicated earlier (*Table 1*), in the Israeli study a 23% rate of unstable transmission from maternal alleles was found in the 51–55 range and 43% in the 56–60 repeat range, suggesting that throughout the intermediate allele range of 41–60 repeats, there is a positive association between the number of repeats and the possibility of expansion on transmission. Ryynänen and colleagues[154] reported (*Figure 4*) that during screening of 1477 low-risk pregnancies, 12 women had alleles in the 51–60 range and one of their fetuses had an expansion to 76 repeats, although the authors did not break the figures down into how many of the 12 women transmitted the abnormal allele to their fetus. The segregation ratio results of Drasinover and colleagues[182] indicate that this figure is unlikely to be the Mendelian 50:50.

In practice, a woman carrying an intermediate allele will want to know the chance that her child will have of a further expanded allele. Assuming there is not a complicated link between the transmission ratio distortion and allele instability (which Drasinover and colleagues[182] did not analyse), a 70%, not 50%, chance of passing this intermediate allele on to her child can be assumed for a woman carrying an allele in the 51–60 repeat range. Thus, using the Israeli figures, for a woman with an allele in the 51–55 range, the chance of a further expanded allele in her child is about 16% and for a carrier of an allele in the 56–60 range, this figure rises to about 30%.

From the counselling point of view, is a change of one or a few repeats a forerunner of an expansion to a full mutation in the next generation or two? In particular, a decision has to be made on what repeat size (with or without evidence of instability on transmission of that allele within the family) justifies the offer of systematic family follow-up. The Wessex clinical genetics service has chosen a repeat size of 51 or greater as an indication to offer register-based systematic family follow-up. Instability data are not normally available but the grounds for extending clinical follow-up to families in whom there was an instance of minor transmission instability in the 41–50 range are less clear. The Rotterdam team argued that an allele

of 43 repeats or above that has shown instability on transmission should be treated as a premutation[152] but this view is not widely accepted.

Mutations in the *FMR1* gene other than CGG expansion

There have been a number of reports of mutations in the *FMR1* gene other than the easily detected CGG expansion, all resulting in a clinical phenotype compatible with fragile X syndrome. These have been summarised by de Vries and colleagues,[152] and include partial or complete deletions of the *FMR1* gene as well as base pair changes in various exons. There have been few systematic searches for such mutations but they are considered to make up less (possibly much less) than 5% of clinical cases of fragile X syndrome. Gronskov and colleagues[214] screened the *FMR1* gene for mutations in 118 mentally retarded males suspected of fragile X syndrome but with no expansion. No pathogenic *FMR1* mutations were found. The typical clinical features and absent FMRP in these deletions and point mutations have confirmed that it is the absence of FMRP that underlies the fragile X phenotype.

Relationship of molecular changes to cytogenetic findings

The fragile site is seen at Xq27.3 where the *FMR1* gene is located. The fragile site becomes apparent when DNA replication is impaired, for example, when chromosomes are studied after growth in folate-depleted medium. Clear cytogenetic evidence of the fragile site is only seen when there is a full expansion. Premutation carriers have a normal karyotype. The mechanism by which the full expansion of the CGG repeat causes a fragile site is not fully understood but it alters chromatin structure[215,216] and presumably prevents full replication before chromosome condensation at metaphase.

Key messages

- The fragile X syndrome is caused by molecular silencing of the *FMR1* gene and consequent lack of FMRP, which seems to be needed for RNA processing in the brain.
- The gene silencing is caused by intergenerational expansion of a CGG trinucleotide repeat DNA sequence in the *FMR1* gene beyond a critical length (> 200 repeats).

- Within the population, there is variation in the CGG repeat length and its stability on transmission, with the variant *FMR1* genes (alleles) classed as follows.
  - About 96% of the population have only **common** alleles (11–40 repeats) or the rare **minimal** alleles (< 11 repeats), and these are essentially stable on transmission.
  - Nearly all the remainder have an **intermediate** allele (41–60 repeats) that, as a group, showed minor changes on transmission in an increasing proportion related to the repeat size (< 10% in the 41–50 range, about 23% in the 51–55 range and about 43% in the 56–60 range).
  - Many authors, while recognising a 'grey zone', placed the cut-off between 'normal' and 'premutation' at 55 repeats or above. **Premutation** alleles (51, or 55, or 60–200 repeats, depending on definition) become increasingly unstable with repeat size, such that a 90-repeat allele has at least an 80% chance of expansion to a **full mutation** (> 200 repeats) on female transmission.

- Limited general population data suggest studies based on families ascertained through an individual with fragile X syndrome over-estimate the risk of expansion of a small premutation (55–65 repeats) to full mutation on female transmission.
- A premutation does not expand to a full mutation on male transmission.
- Only the full mutation results in a cyto-genetically detectable fragile site in some cells.
- In studies of affected families, usually only those with a **full mutation** have significant mental handicap but adequate general population studies of the cognition and behaviour of people with premutations or intermediate alleles have yet to be done. One study found an over-representation of intermediate alleles in boys with unexplained learning difficulties, but this has not been replicated.
- It is not possible currently to provide a clinically helpful estimate of the risk of expansion to a full mutation (and associated MR) in a child or grandchild of an individual with a CGG repeat number in the 50–60 range.

Chapter 4

The prevalence of fragile X syndrome

Determining the prevalence of fragile X syndrome

Before the expansion mutation in the *FMR1* gene was identified, determining prevalence in the population even of those with MR caused by fragile X syndrome was difficult. The cytogenetic test to detect the fragile site expression at Xq27.3 was labour intensive and its sensitivity and specificity were considerably less than 100%. The majority of early prevalence studies examined mentally handicapped populations of males for fragile sites around Xq27.3 and extrapolated from these figures to the total population from which these groups were drawn to produce a prevalence estimate. The resulting data are now known to be over-estimates, as they included individuals with other fragile sites in the same region (Xq 27–28), namely FRAXD, E and F. Up until the early 1990s, the prevalence of fragile X syndrome was widely quoted as 1 in 1250 males based on these data. However, no cases of fragile X syndrome were found in cyto-genetic screening of 2439 neonates[217] and several authors expressed concern that rather fewer-than-expected cases were being ascertained based on this prevalence.[218–221] The discovery of the expansion mutation and development of a DNA-based diagnostic test has allowed some of the earlier samples to be revisited and prevalence studies to be extended to premutations as well as full mutations.

Prevalence based on DNA analysis, including re-testing of existing population samples

The UK study of Coventry schoolchildren[220,222] and the Australian New South Wales case-finding programme[16,223] have been reassessed using DNA analysis,[224] and very similar population prevalence estimates have been obtained for fragile X syndrome of about 1 in 4000 males (*Table 3*).

This prevalence is similar to the estimate of 1 in 5530 (95% confidence interval (CI), 1/4007 to 1/8922) males derived from the Wessex population-based study of boys with unexplained learning difficulties,[153,225] and also to an estimate of 1 in 4400[226] in a Finnish population. A recent study[227] in The Netherlands gave a figure of 1 in 6045 (95% CI, 1/9981 to 1/3851). The figure of 1 in 4000 males was adopted in calculations by two recent assessments of fragile X syndrome screening[4,155] and is the estimated prevalence used for calculations in this report, although where critical to the conclusions the effect of varying the prevalence is indicated.

The prevalence of the full mutation in females can also be expected to be 1 in 4000. This is because those with the full mutation only come from a maternal transmission (in which the mother has either a full mutation or a premutation) with an equal chance of passing it to a son or daughter. The assumptions made in this type of argument were discussed earlier (page 16). About 50% of females with the full mutation have some degree of learning difficulties,[159,160,206,228] so about 1 in 8000 may be expected to have fragile X syndrome.

DNA-based fragile X screening studies at institutes and schools for the mentally retarded

One approach to case-finding is to screen those attending schools and adult training centres. Many such studies were carried out by cytogenetic analysis in the past (see the review by Sabaratnam, *et al.*[229]) and now similar studies are being undertaken using DNA analysis. This also permits an assessment of the frequency of alleles in the premutation and inter-mediate range in those attending special schools/institutions. De Vries and colleagues[152] have collated some recent studies (see *Table 4*).

Fragile X syndrome in different ethnic groups

There is relatively little published on DNA-based fragile X syndrome screening in different ethnic groups. As indicated in *Table 5*, clinical and cytogenetics studies in the 1980s established that fragile X syndrome is found in many populations throughout the world.[230–238] DNA-based studies have confirmed this widespread occur-rence. Goldman and colleagues found fragile X syndrome in 6.1% of 148 mentally retarded, black South African males,[239] and in a reanalysis of five Chinese patients with fragile X syndrome using

TABLE 3 *Reassessment of prevalence of fragile X syndrome after molecular testing (Turner et al.[224])*

Intellectual handicap	Webb, et al.,[222] Coventry, England (total: 28,611 boys)		Turner, et al.,[223] Sydney, Australia (total: 58,094 boys)	
	Moderate/severe	**Mild**	**Moderate/severe**	**Mild**
Number karyotyped	60	159	149	323a
Number fragile X positive	6	10	9	5
Prevalence	1 in 952 or 10.5/10,000		1 in 2610 or 3.8/10,000	
Number DNA tested	6	10	9	5
Number DNA positive	4	2	8	2
Prevalence	1 in 4090 or 2.4/10,000		1 in 4350 or 2.3/10,000b	

a Only those 8–12 years old were screened
b Not corrected for those who refused permission; if this is done, prevalence becomes 3.0/10,000

TABLE 4 *Overview of DNA screening programmes among institutes and schools for the mentally retarded (from de Vries, et al.[152])*

Location	Number studied			Number with grey zone allele			Number with premutation	Number with full mutation			Characteristics of population studied
	M	**F**	**M + F**	**M**	**F**	**M + F (CGG range)**	**M + F**	**M**	**F**	**M + F**	
England Webb, et al., 1986[222] Turner, et al., 1996[224]	219	–	219	–	–	–	–	6	–	6 (2.7%)	Mentally retarded children in schools/institutions
Australia Turner, et al., 1986[223] Turner, et al., 1996[224]	472	–	472	–	–	–	–	10	–	10 (2.1%)	Mentally retarded children in special schools
England Jacobs, et al., 1992[312]	180	74	254	–	–	11 (41–49)	0	4	0	4 (1.6%)	Children with special educational needs
USA Hagerman, et al., 1994[313]	299	140	439	–	–	–	1 (M)	1	3	4 (0.9%)	Retarded/learning disabled children
England Slaney, et al., 1995[314]	103	51	154	–	–	–	0	4	0	4 (2.5%)	Schoolchildren with learning difficulties
England Murray, et al., 1996[153]	1013	–	1013	35 (41–60)	–	35	1 (M)	5	–	5 (0.5%)	Boys with learning difficulties
USA Meadows, et al., 1996[315]	888	391	1279	3	7	10 (50–60)	0	1	1	2 (0.2%)	Children in special needs education programmes
The Netherlands de Vries, et al., 1997[227]	870	661	1531	10	9	19 (43–60)	0	9	2	11 (0.7%)	Mentally retarded children/adults in institutions, special schools
Subtotals[†]	3353	1317	4670			75 (1.8%)	2 (0.04%)	24 (0.7%)	6 (0.5%)	30 (0.6%)	

† Without (reassessed) studies of Webb, et al., 1986,[222] and Turner, et al., 1986[223]

TABLE 5 *Reports of fragile X syndrome in different ethnic groups*

Ethnic group	Reports
Southern European	Sordo, *et al.*, 1983[230] Sanfilippo, *et al.*, 1986[231]
Black American	Howard-Peebles & Stoddard, 1980[232] Carpenter, *et al.*, 1982[233]
North African	Mattei, *et al.*, 1981[234]
Asian	Bundey, *et al.*, 1985[235] Gardner, *et al.*, 1983[236]
Japanese	Arinami, *et al.*, 1986[237]
Filipino	Rhoads, 1984[238]

DNA-based methods the diagnosis was confirmed.[240] There is always the possibility that founder effects, in terms of the founding group having an over-representation of relatively unstable alleles in the intermediate or premutation range, could result in some populations having a higher prevalence of fragile X syndrome.

Prevalence of the premutation

As indicated earlier (see page 10), the term premutation implies instability on transmission and this is not just determined by the size of the trinucleotide repeat. However, in practice, instability cannot easily be assessed so repeat size is usually used. Most studies designed to assess the population prevalence of premutations define these as 54 repeats or above. This was the case in the nine studies collated by Murray and colleagues.[4] By combining six studies in females,[185,193,207,209–211] there were 48 premutations in a total of 26,178 X chromosomes examined, which, given that females have two X chromosomes, makes a premutation carrier rate of 1 in 273. The study by Rousseau and colleagues[209] of French Canadians provided data on 21,248 of these X chromosomes and it has been questioned[241] whether the high prevalence of premutation carriers in this population is caused by a founder effect and is not typical of populations elsewhere. However, smaller studies gave comparable results. Recently, the results of population screening in 10,587 healthy Israeli females (with no overt family history of MR) have been reported.[182] Unfortunately, the figure for 54 repeats or above is not provided but 1 in 271 had 61–135 repeats on one of their X-chromosomes. There may, however, be biases in this figure since it is not reported whether it includes female relatives of those found to have a premutation. The combined results, collated by

Murray J and colleagues,[4] of seven studies in 13,592 males[185,192,193,207,242–244] gave a premutation prevalence of 1 in 800. The figure would be expected to be about half the carrier rate in women, that is, about 1 in 550. In a recent analysis of 543 boys from the general population around Bristol, ALSPAC found one premutation of 65 repeats and two 'borderline' alleles of 51 and 52 repeats (Murray A, *et al.*, Wessex Regional Genetics Laboratories, Salisbury: personal communication, 1998). It is premature to conclude that there is a real difference between the sexes. In their cost–benefit analysis of fragile X screening, Wildhagen and colleagues[155] used a premutation prevalence of 1 in 435 for females and half this figure (1 in 870) for males. They reasoned that there may well be a founder effect in the French Canadian study and sought to correct for this. Rousseau and colleagues[209] had calculated a full mutation prevalence of 1 in 2381, that is 1.68 times the estimate of Turner and colleagues[224] (see *Table 3*); so, assuming a simple relationship between the frequency of pre- and full mutations, they divided Rousseau and colleagues' premutation prevalence of 1 in 259 by 1.68 to give a figure of 1 in 435. It is possible that the 1 in 271 figure from Israel is also increased because of a founder effect. However, as indicated earlier, the report by Drasinover and colleagues[182] provides very little information on the ascertainment of the 10,587 individuals who accepted the screening offer on a self-pay basis. Although they did not have an overt family history of MR, it is not known if these individuals, particularly the 138 with ≥ 51 repeats, were related or not. If a woman was told that she had an increased number of repeats (and could be offered a prenatal test in a future pregnancy), she may well have encouraged her sisters (who would have a high chance of a similar expanded repeat) to undergo testing. If such an effect were operating, it would falsely elevate the premutation prevalence.

Whichever estimate turns out to be best, there remains a large difference in the prevalence of the premutation, **as defined by total repeat length**, and the prevalence of the full mutation manifesting as the fragile X syndrome. As discussed earlier, this can only mean that a substantial proportion of women with a premutation (defined solely by total repeat length) have a much lower risk of having a child affected by fragile X syndrome than family studies would suggest. The current inability to identify this lower risk group would pose some counselling difficulties in any general population screening programme.

Key messages

- Past calculations of the prevalence of fragile X syndrome, based on cytogenetic detection of fragile sites, are overestimates.

- Reanalysis and new studies based on DNA testing give a prevalence of about 1 in 4000 males.
- About 1 in 8000 females can be expected to have learning difficulties caused by fragile X syndrome.
- In the presence of some established professional awareness of the fragile X syndrome, screening children with learning difficulties reveals a '**new**' case of fragile X syndrome in about 1%.
- Fragile X syndrome occurs in all major ethnic groups.
- The estimates of the prevalence of the premutation in females range from 1 in 271 to 1 in 550.
- The big difference in the prevalence of the premutation and the full mutation indicates that a proportion of premutation carrier females have a lower risk of an affected child than family studies would suggest.

Chapter 5
Technology of testing

Is there a role for cytogenetic analysis?

DNA analysis to detect the size of the CGG repeat and its methylation status if there is a large expansion is the definitive test for fragile X syndrome. Traditional cytogenetic analysis no longer has a role in either testing or screening for fragile X syndrome *per se*. As discussed in the previous chapter, cytogenetic analysis based on the detection of a fragile site at Xq27.3 led to a very significant overestimate of the incidence of fragile X syndrome. Part of this was caused by the detection of nearby fragile sites, FRAXE and FRAXF. Only FRAXE might be relevant in terms of diagnosing the cause of learning difficulties. It is much rarer[153] than FRAXA and a specific DNA test can be multiplexed with the FRAXA analysis if this was needed. However, standard cytogenetic analysis is an appropriate test in the diagnostic work-up of a person with learning difficulties and, in the past, this raised the question of whether it was useful to add a cytogenetic search for fragile sites at the same time. This is no longer sensible from either the point of view of diagnostic accuracy or laboratory cost-effectiveness. The test for fragile X syndrome should be DNA analysis of either a separately collected sample or an aliquot of the sample sent to the cytogenetics laboratory.

The above view is supported by the 1998 review of practice in the 22 UK regional genetics centres (see chapter 8). Of these, 19 had already switched to only DNA analysis for fragile X testing and the remaining three centres were planning to complete the switch soon.

Outline of molecular genetic analysis

At the present time no single test is ideal for all aspects of fragile X analysis and information from Southern blotting and a PCR-based test may need to be combined. CGG tracts of more than approximately 150 repeats are usually difficult to amplify and will not give a detectable product after PCR. It is just these larger expansions that can be resolved by Southern blotting but the technique cannot measure the repeat size in the normal (and low premutation) range.

Southern blotting

Restriction-digested DNA (usually using enzymes EcoRI or HindIII) is separated on agarose gels; the size of the CGG repeat that contains *FMR1* fragments is estimated by transfer to a nylon membrane and hybridisation with a radioactive DNA probe. A double digest, including a methylation-sensitive restriction enzyme, enables detection of the methylation of the CpG island in the CGG region. This permits detection of those males with incomplete methylation of a full mutation ('methylation mosaics') and can help resolve an unmethylated large premutation and a small methylated full mutation.

Southern blotting:

- requires a high quality DNA sample which, in practice, must usually be from a blood sample
- is time-consuming (1 week from receipt of sample) and labour-intensive (maximum of 100 analyses/week/full-time worker)
- remains the method of choice for detection of full mutations and mosaicism, and also of the rare deletions
- may miss small premutations as the band shifts are very small, and should therefore be supported by PCR analysis when the aim is to detect carrier females who are at risk of having affected offspring.

PCR

This is a patented technology on which a royalty must be paid to Roche Diagnostics Ltd. The centrally negotiated cost to the NHS, at the time of writing, was £2.80 for a single PCR and £5.60 for multiple testing of a family.

- The number of CGG repeats is accurately determined by amplification across the repeat region.
- The products may be detected by being either (a) radioactively labelled – which requires an autoradiography step that adds to the time required (2 days) and to the cost; or (B) fluorescently labelled – which allows a result to be obtained on the same day but requires an expensive automated sequencer (£100,000) for detecting the fluorochrome. In the context of a large throughput testing/screening

programme, fluorescent labelling for PCR would be much preferable.

- Sample requirements are less stringent – blood or mouthwash samples with minimal preparation.
- Full mutations do not amplify. Additional blot hybridisation steps may allow detection of the full mutation[190,245,246]) but this is almost equivalent in labour terms to Southern blotting, as above. Large premutations may also be missed especially in women in whom there is strong preferential amplification of the normal allele. Even large intermediate alleles may occasionally be missed with poorer quality DNA from buccal cells, obtained by mouthwash or mouth-brushing, when a fluorescent label is used (although a more sensitive radioactive labelling approach may pick up the band). Larger premutations, however, will be detected by Southern blotting, so the two techniques are complementary.
- About 17–30% of women do not have two distinguishable alleles on PCR; these women are not distinguished from carriers of full mutations except by Southern blotting.

A possible protocol for screening women with high sensitivity would involve using fluorescent PCR, thus allowing exclusion of about 70% within 24 hours; subsequent Southern blotting of the remaining 30% would provide results in about 1 week.

Sequencing

In the future, intermediate and premutation alleles could be sequenced, and information on AGG interspersion used to give more accurate estimates of the true risk of expansion, should evaluation show that this is worthwhile. However, with current technology this would be too time-consuming for immediate application when, for example, dealing with a pregnant woman with an expanded repeat. Immediate management would therefore still need to be based on empirical risks on the basis of size alone.

Deletions

These will not be detected in women by any of these methods since the normal X chromosome will 'hide' the fact that a deletion exists on the other. Their extreme rarity does not justify further efforts to detect them in most settings.

FMRP antibody

This has potential as a rapid, blood-smear method of screening for fragile X syndrome in males with learning disability but is not suitable for detecting females with a full mutation or a pre-mutation.[247] A potential improvement is the use of the FMRP antibody on plucked hair roots.[248] This has the advantages of being less invasive than blood-taking, rapid and inexpensive. Preliminary results suggest that it can be used to detect females with the full mutation as well as affected males. Ten hair roots per male patient (20 for females) were used in this study.[248] They can be taken by non-medical personnel and easily transported by post.

Prenatal diagnosis

Prenatal diagnosis is performed in the context of a high risk to the fetus, and most laboratories use a combination of PCR and Southern blotting.[249–253] When a premutation is detected by chorionic villus sampling (CVS), it raises the question of how reliable a reflection this is of the fetal genotype – could somatic instability have led to a full mutation in some of the fetal tissues? For this reason, some confirm the premutation in amniotic fluid or a fetal blood sample before completely reassuring the mother.[253] Discordance between the methylation status of a full mutation in CVS and fetal tissue has been observed. A full mutation was unmethylated in a CVS at 11 weeks' gestation but methylated in fetal tissues;[129,251] hence, reliance cannot be put on the CVS methylation status.

Practical experience of FRAXA analysis within a research project

Since large-throughput analysis for fragile X syndrome does not currently occur in a service setting, information was obtained (for which the authors are extremely grateful to Anna Murray, Sheila Youings and Pat Jacobs) on success rates from the Wessex research study of boys with learning difficulties and their mothers.[153] Initial screening with a fluorescently labelled primer in the PCR reaction, to allow detection and measurement of the size of the PCR product on gels run on an ABI 373A machine (Applied Biosystems Ltd), was followed by Southern blotting when an expanded allele was suspected. To date, Professor Jacobs and her team have screened 3238 boys and 2546 mothers. For subject acceptability and logistics reasons, the primary DNA sample is obtained using a mouth brush, with blood samples only being requested when a repeat mouth-brush sample has also failed to produce a result. With

mouth-brush DNA, the initial PCR fails in about 20% and therefore needs to be repeated. If it fails a second time, then the DNA is 'cleaned up' using a commercial kit and the PCR repeated. A much smaller proportion of samples fail repeatedly, triggering a repeat mouth-brush sample or, eventually, a blood sample. The initial PCR failures with mouth-brush DNA contrast with DNA from blood, in which the initial PCR fails in about 5%. It is worth noting that, with the relatively inefficient amplification of the larger alleles, these may occasionally not be detected with the fluorescent primers used, and a conventional ^{32}P-labelled PCR reaction is required.[131] The fluorescent label is usually a single 'end label', in contrast to the radioactive label which is incorporated throughout the PCR product, such that the larger the allele the greater the amount of label. Overall, the Wessex team considered that a radioactive PCR would detect a larger allele in the 40–50 repeat range, that had been missed by fluorescent PCR, about 1% of the time.

When there is no amplification on PCR in males or only one band in females (other than for the commonest alleles, in which homozygotes are expected), a blood sample is obtained for Southern blotting. Southern blots fail about 7% of the time because of a partial digest or other artefact and, in addition, whole blots fail about 10% of the time because of, for example, probe labelling problems and buffer contamination. The Wessex research study used an analytic strategy that would be very similar to a service screening approach, except for reliance on mouth-brush samples for DNA. Learning-disabled individuals may not be able to cope with a mouthwash sample for DNA (even if this did prove better than mouth-brush), so it is likely that blood samples would be regarded as the optimum from the analytic point of view, even in a screening programme. This would minimise the initial PCR failures.

The current methods can be adopted for fairly high throughput analysis with reasonable proficiency. Wildhagen and colleagues,[155] in their detailed cost-effectiveness analysis of general female population screening, adopted a failure rate of 10% for PCR amplification and for Southern blotting. They also took a very conservative figure of 40% for those samples that failed to show two clear bands on PCR (and therefore needed further analysis); these were caused by a woman having both alleles the same size, or differing in just one CGG repeat, or having one allele expanded beyond the size that is easily amplified by PCR.

Laboratory costs

These have not been analysed in detail and, anyway, the cost per test result could well fall in the context of high throughput and efficient use of resources. To illustrate relative costs, Wildhagen and colleagues[155] gave the following cost estimates in a screening situation – US$ 9.00 for DNA extraction, US$ 24.04 for the PCR test and US$ 75.36 for the Southern blotting. These costs accord roughly with what several laboratories considered reasonable. The Wessex 1998/99 'effectiveness–cost ratio' charge for DNA analysis for fragile X syndrome is £50 per genotype (Murray A, *et al.*, Wessex Regional Genetics Laboratories, Salisbury: personal communication, 1998).

Key messages

* There is no longer a central place for cytogenetic analysis in the diagnosis of fragile X syndrome.
* The definitive test for fragile X syndrome is DNA analysis, which can reliably detect full mutations, premutations and measure the total length of the CGG repeat sequence.
* Currently, no single test is ideal for all aspects of fragile X analysis. Information from Southern blotting and a PCR-based test may need to be combined.
* Repeat lengths of more than approximately 150 repeats will not give a detectable product after PCR, so PCR tests are only suitable for normal and intermediate alleles, and most premutations.
* Southern blotting can detect full mutations (> 200 repeats), methylation status and the longer premutations.
* About 17–30% of women do not have two distinguishable alleles on PCR and need Southern blot analysis to exclude a full mutation on one X chromosome.
* Southern blotting has a 10% initial failure rate and needs DNA from a blood sample or CVS biopsy. Southern blots cannot be done on Guthrie cards.
* Initial PCR failures (5–20%) increase with mouthwash/scrape samples and larger allele detection is less reliable with fluorescent rather than radio-labelled probes.
* *FMR1* antibody analysis of a blood smear provides a rapid screening test for full mut-ations in males. A similar antibody test on plucked hair roots has even more potential for screening for full mutations in males (and females) since it is less invasive, does not require medical personnel and the specimen can be easily transported.

Chapter 6

Principles of screening in
fragile X syndrome

Screening and prevention

The UK NSC defined screening as "the systematic applications of a test or enquiry, to identify individuals at sufficient risk of a specific disorder to warrant further investigation or direct preventive action, amongst persons who have not sought medical attention on account of symptoms of that disorder".[15] Screening is thus the systematic study of undiagnosed people to identify high-risk individuals, so that specific management can be applied to them which is not generally available.

At this point it is worth reiterating how the term 'screening' is interpreted in this report and hence the breadth of the remit that has been assumed. To take a very narrow view of screening would be to overlook the special context in which decisions about fragile X syndrome screening must be taken. This special context stems from the inheritance pattern of the fragile X syndrome. As previously indicated, the definition in the NSC's first report has been taken as the starting point. However, the nature of fragile X syndrome, which puts certain members of the extended family at high risk, means that a systematic approach to relatives who have not sought medical attention or are unaware of their genetic risk can also be regarded as a form of screening. This is called 'cascade counselling' or 'extended family follow-up'. Screening can also refer to the systematic testing of those with learning disabilities to discover those who have the fragile X mutation, particularly as patients with fragile X syndrome represent a small percentage of this population group and are not readily distinguishable from the others on clinical features alone.

Screening is closely allied to prevention and, with the identification of affected individuals, leads to an intervention with the aim of reducing pathology. Prevention may be primary or secondary: that is, the disorder may be completely avoided or may be ameliorated by intervention precipitated by diagnosis at screening.

Screening criteria have usually developed in relation to presymptomatic detection of treatable diseases, so do not sit comfortably with genetic screening programmes in which one of the interventions triggered is the offer of prenatal diagnosis and selective abortion of an affected fetus. This difficulty is recognised in the NSC report (paragraphs 3.5.2–3.5.5).[15] However, the NSC's discussion is couched in terms of antenatal screening alone (e.g. for Down's syndrome) and this is only part of the picture in a disorder where the extended family is at high risk. In a condition like fragile X syndrome, the family is the unit of attention as much as the individuals within it, and any benefits that flow from diagnosis in one member may be greatest for other family members.

In the context of the fragile X syndrome, screening could have an element of primary prevention, since one of the outcomes is the identification of carriers who are not at risk of the disorder themselves but may modify their reproductive behaviour to avoid the birth of affected individuals. They may seek to do this by limitation of family size or use of donor eggs as well as prenatal diagnosis and selective abortion. The term 'screening' may also be used more loosely to refer to systematic case identification of affected individuals with fragile X syndrome. This might generate some limited benefits for the affected people themselves in terms of more appropriate educational/social management (i.e. limited secondary prevention) but its main benefit is that it allows identification of at-risk relatives if testing is offered to relevant family members. Some testing for case identification is part of normal clinical practice. However, the term 'screening' is used in this report for case-finding when it is undertaken on a systematic basis at the instigation of the medical services (with informed consent), rather than because of a request from the patient or their carers.

As already discussed at the beginning of this report, some authors suggest that screening for genetic disorders has the reduction of affected births as its ultimate purpose.[4] Others promote screening for genetic disorders to allow individual reproductive choice, with the principle aim of

satisfying unmet (and initially, unrecognised) needs for genetics services. Since these services include prenatal diagnosis, it is acknowledged that there may be a reduction in birth prevalence as a result.[13] This was recognised by the Department of Health[254] in *Population Needs and Genetic Services: An Outline Guide 1993* (paragraphs 5.4–5.8), although it is emphasised that it is a consequence, not the purpose, of genetics services. These differences in approach are most relevant when developing criteria to measure the success or failure of a genetic screening programme in the context of monitoring and quality assurance.

As indicated by the NSC,[15] a screening programme offered to a population would, ideally:

- be based on good quality evidence that it did more good than harm at reasonable cost
- accord with criteria based on those of Wilson and Jungner[255]
- be delivered within the context of an effective quality assurance programme.

Although it can be counter-productive to try too hard to 'shoe-horn' an analysis of genetic screening into the Wilson and Jungner[255] framework (which was not developed with genetic screening in mind), there is some merit in considering each criterion in turn, plus some additional criteria specifically relevant to the impact of genetic risk on the wellbeing and reproductive confidence of family members.

Criteria for screening for genetic disorders

In this assessment, the Wilson and Jungner principles for medical screening[255] were adopted as a starting point. It was noted that the NSC recommended a similar approach[15] and their criteria are added where appropriate below (NSC 1998). Somewhat reordered, the original Wilson and Jungner criteria (WJ 1968) are as follows.

- The condition sought should be an important health problem.
- There should be a recognisable latent or early symptomatic stage.
- The natural history of the condition, including development from latent to declared disease, should be adequately understood.
- There should be an accepted treatment for patients with recognised disease.
- Facilities for diagnosis and treatment should be available.

- There should be a suitable test or examination.
- The test should be acceptable to the population.
- There should be an agreed policy on whom to treat as patients.
- The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
- Case-finding should be a continuing process and not a 'once and for all' project.

When screening has a key objective of providing genetics information for the family, the following additional criteria can be added.

- The degree of risk to the extended family should be significant.
- There should be a reliable and accurate test for determining the risk.
- The condition should so alter the quality of life of the affected individuals and their families that, in a significant proportion, it decreases their reproductive confidence.
- There should be an accurate and acceptable mode of early prenatal diagnosis so that, when a couple is identified as being at increased risk, they have the opportunity of having their reproductive confidence restored.

Application of screening criteria to fragile **X** syndrome

Important health problem

> "The condition sought should be an important health problem" [WJ 1968].

> "The condition should be an important health problem" [NSC 1998 (para. 6.2.1)].

The term 'important health problem' implies significant morbidity or mortality and a high prevalence. Learning disability, usually with behavioural problems, is the major clinical manifestations of fragile X syndrome. Around 1% of affected males are able to live independently in the community when adult but the rest require a variable amount of supervision. As discussed earlier, the medical problems associated with the condition are not usually major or life threatening. Mortality figures show an overall reduction in life expectancy of 10 years,[256] although this is unlikely to be specific to fragile X syndrome and would most likely be found in a group of similarly handicapped individuals.

The morbidity is such that fragile X syndrome generates significant health and welfare problems for close family members as well. As regards

prevalence, in genetic terms fragile X syndrome is of relatively high prevalence. If the estimated prevalence of 1 in 4000 males is correct, this compares to 1 in 3500 males with Duchenne muscular dystrophy, in which neonatal screening is being piloted, or 1 in 2000 babies with CF in Northern Europe, a condition for which several screening programmes have been piloted. However, the relevant figure with respect to introducing a screening programme may be the prevalence of 'as yet undiagnosed' cases or carriers. If three criteria listed in the 1998 NSC report are met,[15] namely:

> "6.2.3: All the cost-effective primary prevention interventions should have been implemented as far as practicable

> "6.2.10: Clinical management of the condition and patient outcomes should be optimised by all health care providers prior to participation in a screening programme

> "6.2.17: All other options for managing the condition should have been considered (e.g. improving treatment, providing other services)"

then improved regular paediatric referral and clinical genetics services with respect to diagnosis and family follow-up, should diagnose at least 50% of families. The more relevant prevalence figure for this assessment may therefore be less than 1 in 8000 males.

Detectable latent stage and known natural history

> "There should be a recognisable latent or early symptomatic stage" [WJ 1968].

> "The natural history of the condition, including development from latent to declared disease, should be adequately understood" [WJ 1968].

> "The epidemiology and natural history of the condition, including the development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage" [NSC 1998 (para. 6.2.2)].

These criteria can be considered for the affected individual and also in relation to the whole family. Although most males with fragile X syndrome demonstrate developmental delay from early infancy and the genetic defect is present from before birth, there is still a latent period between the first concerns about development and diagnostic confirmation of fragile X syndrome. During this time the family members may be either totally unaware of their increased genetic risk or have unresolved general concerns about 'it happening again' in the family. There is, therefore, a period of unrecognised genetic/health risk.

In a retrospective questionnaire-based study of members of the UK Fragile X Society,[257] the reported lag time (analysed by year of birth) between the first concern by the parents about their child's development and the diagnosis of fragile X syndrome fell from an average of 8.3 years (range 4.0–12.3 years) for those born in 1980 to 1.2 years (range 2 months to 3.0 years) for those born in 1990. Lag times in individuals born before 1980 were inevitably longer since the disorder was not widely recognised at this time. The longest lag time in the survey was 44 years. Clearly Fragile X Society data only include those in whom a diagnosis of fragile X syndrome was eventually made. However, it is still true that individuals remain undiagnosed and, in the UK, the extent to which this is true is crucial to this screening assessment exercise.

Under-diagnosis of fragile X syndrome in the population

The estimated number of males in the UK who should be known to have fragile X syndrome, assuming recognition by 5 years, a 10% reduction in life expectancy and a prevalence of 1 in 4000, is about 5525. This figure is obviously greatly dependent on the overall prevalence estimate that is used, plus the assumption that already-diagnosed families are not greatly modifying their reproductive behaviour. Unfortunately, systematic collection of data from all the UK regional genetics centres has not proved possible, because what data they have are not readily accessible (see chapter 8). Thus no direct estimate of under-diagnosis can be given.

Most children with developmental delay are investigated for possible causes, although clinical practice in terms of the investigations undertaken is very variable.[256] However, learning-disabled adults are rarely re-evaluated for diagnostic purposes. Children whose mothers are themselves intellectually impaired, as may be the case in fragile X syndrome, may be less likely to present for investigation.

Natural history

The natural history in fragile X syndrome is well defined in affected males and, although the prognosis in affected females is rather unpredictable, the range of the disorder is clearly documented. Nevertheless, the genotype–phenotype correlation in females with the full mutation is poor. There is no good, population-based information on the behavioural/cognitive phenotype of premutation carriers but there is emerging evidence that female premutation carriers will have POF.

From a family perspective, 'the natural history' of an intermediate allele and (small) premutation over the generations is poorly understood where the general population is concerned, although there is adequate information for nearly all counselling situations when dealing with relatives of an affected individual.

Accepted treatment or intervention

"There should be an accepted treatment for patients with recognised disease" [WJ 1968].

"Facilities for diagnosis and treatment should be available" [WJ 1968].

"There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment (intervention) leading to better outcomes than late treatment" [NSC 1998 (para. 6.2.8)].

Interventions that modify the outcome for affected males (and females) are reported, although there is still relatively scant evidence of measurable improvements in function. There is no curative treatment. Whether there are adequate resources to provide for the needs of individuals with fragile X syndrome and their families is also open to discussion. Even without diagnosis, most affected males will have a statement of special educational needs (or the equivalent) and require special provision at school. This means that the specific diagnosis of fragile X syndrome is unlikely to alter the level of educational and social provision needed by affected males. A specific diagnosis in a female with milder learning difficulties might well trigger additional help to meet her needs.

There are, of course, effective interventions that can be offered to family members as the result of the diagnosis of fragile X syndrome in an affected individual or indeed in an unaffected carrier (with a suggestive family history). The most striking examples of better early outcomes are:

(i)   the exclusion of carrier status in a female relative who was very concerned about her genetic risk and consequent restoration of her reproductive confidence

(ii) the forewarning of a relative of her carrier status before any pregnancy, so she can make an informed reproductive choice between several options, in a supportive environment.

A suitable test

"There should be a suitable test or examination" [WJ 1968].

"The test should be acceptable to the population" [WJ 1968].

"There should be an agreed policy on whom to treat as patients" [WJ 1968].

"There should be a simple, safe, precise and validated screening test. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed. The test should be acceptable to the population. There should be an agreed policy on further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals" [NSC 1998 (para. 6.2.4–7)].

Screening for fragile X syndrome in those with learning difficulties could be attempted by a range of approaches, as discussed below. Although a clinical checklist could be used as an initial screening test before proceeding to molecular analysis, the most direct method would be an assay for the expansion mutation. Currently this is best done by DNA analysis, using a PCR method followed by Southern blotting, in those with suggestive results. This is a reliable and valid technique with high sensitivity and specificity. There could be a role in the future for initial screening of males using the FMRP antibody test.

DNA analysis can be carried out on a small sample of blood (ideally taken after application of a local anaesthetic patch) or on buccal cells obtained using a small brush on the buccal mucosa or by a mouthwash. Such methods of obtaining samples are likely to be acceptable in most circumstances; however, obtaining any sample from an adult with a learning disability can be difficult and, if consent by the affected individual is withheld, constitutes assault.

As soon as one moves from the affected individual to testing unaffected relatives or to screening people without affected relatives, the issue of the 'suitable cut-off' in terms of the number of repeats becomes critical. In terms of genetic counselling and the offer of further interventions (including register-based follow-up) to a particular relative of an affected person, a reasonable cut-off could be agreed based on empirical data from affected families. The Wessex service uses 51 or more repeats as the criterion for register-based follow-up (Dennis N, Princess Anne Hospital, Southampton: personal communication, 1998).

The grounds for choosing a particular cut-off in general, low-risk populations are less clear.

Acceptable cost

"The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole" [WJ 1968].

"The opportunity cost of the screening programme (including testing, diagnosis and treatment) should be economically balanced in relation to expenditure on medical care as a whole" [NSC 1998 (para. 6.2.14)].

The costs, both financial and human, are considered in the next chapter. Wildhagen and colleagues[155] concluded that "from an economic point of view, there is no obstacle to fragile X screening. The decision to screen or not can (and should) therefore concentrate on discussion of medical, social, psychological and ethical considerations." This economic balance in favour of screening in the general population depends on the prevalence of any 'as-yet-undiagnosed carriers' in that population. As alluded to above, this figure falls as case-finding and cascade counselling of the family become better established in regular clinical practice.

A continuing process

"Case-finding should be a continuing process and not a 'once and for all' project" [WJ 1968].

"There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards" [NSC 1998 (para. 6.2.15)].

"Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme" [NSC 1998 (para. 6.2.16)].

The prevalence of 'as-yet-undiagnosed' individuals or carriers will always be a relevant factor in deciding whether or not to initiate, or indeed continue, general population programmes such as prenatal or neonatal screening. In many sporadic or autosomal recessive conditions the prevalence of such cases/carriers is little altered by the population screening process itself. It may be altered by other factors but there is a reasonable expectation that the prevalence will remain high enough to justify continuation of the screening process for decades once started. The same may not hold for fragile X syndrome, because each diagnosis of a full mutation or a premutation will trigger cascade family testing, if the NSC principle above is followed. The nature of the inheritance of fragile X syndrome means that this family follow-up can be expected to reveal several more people with 'high-risk' genotypes. 'High risk' is put in quotation marks because the instability of repeats in the 50–60 range, for example, when found in people without a family history of fragile X syndrome (the majority from a population screening programme) is still unclear. Nevertheless, screening-triggered family follow-up will gradually reduce the prevalence of 'high-risk' genotypes (however defined) still to be discovered by the population screening process. Thus, for fragile X syndrome, monitoring the screening programme may detect a drop in prevalence of the 'high-risk' genotypes to a point where continuation of the screening programme must be questioned.

Active case-finding among mentally retarded adults (which is a screening programme, in one sense) could significantly reduce the number of 'as-yet-undiagnosed' individuals or carriers in the population through cascade family testing over a 5-year period. Such a programme could be an example of an acceptable 'once-and-for-all' project, given that 'screening' these adults becomes less productive as paediatric case-finding improves. However, paediatric case-finding would continue.

The degree of risk to the extended family should be significant

In fragile X syndrome, no affected child has been born to a mother who is not a carrier of a premutation or full mutation detectable by DNA analysis. Thus, unlike Duchenne muscular dystrophy and most serious X-linked disorders, no 'new mutations' (that by their very nature cannot be anticipated) have been diagnosed. Furthermore, the fully expanded *FMR1* allele of the affected person can be traced back through the mother to either grandparent who is, at least, carrying a premutation, with a high chance of having transmitted it to their other offspring. Thus confirmation of the fragile X syndrome means that a number of relatives are likely to be at high risk of carrying the expansion mutation (see *Figure 1*). Male and female siblings of an affected individual have a 50% risk of inheriting the mutant X chromosome (usually a full mutation) and maternal aunts have a 70–75% chance of carrying the mutant X (usually a premutation). In terms of the risk of an affected child in any pregnancy that the maternal aunt might have, the figure is about 25%. These are very high genetic risks. A comparable prior risk of the maternal aunt's child being affected, after the diagnosis of CF in the index case, is about 1 in 160 or 0.6% in the UK. This is only 2.4% if all classes of first cousin are included.

There should be a reliable and accurate test for determining the genetic risk to relatives

The first step in **excluding** a high genetic risk in a relative is to establish their position in the pedigree in relation to the affected individual(s). As an X-linked condition, the mutant *FMR1* allele cannot be transmitted from father to son. For example, if it is known that the premutation came from the affected child's grandfather, then that grandfather's sons and their descendants are not at increased risk of fragile X syndrome compared with the general population.

The DNA technology used for diagnosis of affected individuals is applicable to determining carrier status. When two 'normal' alleles are detected in a woman, DNA analysis is extremely useful for **excluding the risk of** fragile X syndrome. If she has inherited the normal X chromosome (as oppose to the mutant one), then a woman has about a 94% chance of both her alleles being in the 'common' or 'minimal' range (< 41 repeats) and unambiguously stable. As regards predicting an increased risk, the results again are usually clear-cut when undertaking the analysis within an affected family. Even using the lower figures of expansion from premutation to full mutation, calculated by Murray and colleagues[4] and given in *Figure 2*, the risks to offspring are nearly always at a level at which people would accept a discussion of reproductive options as useful rather than meddlesome.

The condition decreases reproductive confidence

Data from the fragile X syndrome case-finding and cascade screening programme in New South Wales, Australia, demonstrate a 20% reduction in pregnancy rates in women in known fragile X syndrome families.[259] This probably reflects a loss of reproductive confidence in this group, an interpretation supported by the fact that 78% of women who do become pregnant seek prenatal diagnosis.[17,259] The reduced pregnancy rate associated with knowing their risk of fragile X syndrome may in part be due to reduced fertility in this group of women rather than loss of confidence, since as many as 16% of those carrying a premutation will have POF.

There should be an accurate and acceptable method of early prenatal diagnosis

Prenatal diagnosis of fragile X syndrome is widely available and in general is reliable for establishing the genotype. Proper follow-up should permit most women in known affected families to be offered prenatal diagnosis by CVS biopsy at about 11 weeks' gestation. There is a miscarriage risk of about 1% associated with the procedure.[260] Part of the biopsy will normally be cultured for later cytogenetic analysis to exclude major chromosome abnormalities and confirm the sex of the fetus. There may well be circumstances in which establishing the sex of the fetus represents a distinct first phase of the procedure, because the mother does not want further genotyping if the baby is female. Rapid sexing of the fetus can be done by direct chromosome examination (increasingly by using X- and Y-specific, fluorescent DNA probes for *in situ* hybridisation) or DNA analysis using Y (and X) probes. As indicated earlier, distinguishing a normal female fetus with two similar alleles from one with a full mutation requires a Southern blot, a test in which an expanded smear is easy to miss if the quality is poor. Prenatal results are required urgently and, for this reason, many laboratories supplement direct detection of the expansion mutation by linkage analysis to establish whether the female fetus has inherited the high- or low-risk X chromosome from its mother.

Many women at risk of transmitting fragile X syndrome are aware of affected females from their own experience. Demonstrating that a female fetus carries the full mutation predicts that there is a 50% risk of some mental impairment (see page 12) but there is no test to refine this risk or to predict the degree of impairment, if any. A decision about continuing such a pregnancy or opting for abortion in such circumstances must be very difficult. There are no systematic reports in the literature about how carrier women cope with these possible outcomes of prenatal diagnosis. Murray and colleagues[4] collated information from seven papers[164,246,252,261–264] that included outcomes after prenatal diagnosis of a full mutation in 47 female fetuses, and about 60% of these pregnancies were terminated. However, this figure does not take account of women who have decided to continue the pregnancy once they know that they are carrying a girl, and so DNA analysis of the *FMR1* gene is not completed.

Amniocentesis after 15 weeks' gestation can also be used but results take 3–4 weeks to become available, as sufficient cells need to be grown for the investigations. Amniocentesis also carries an attributable risk of 0.5–1.0% of inducing abortion.[260] Cordocentesis or fetal blood sampling is also used as an alternative to amniocentesis in cases of late presentation, because it allows a more

rapid result, but the expertise for doing this safely is not widely available. Concern about the stability of a premutation during fetal life has led to the precautionary practice of offering confirmation by amniocentesis or fetal blood analysis, when a premutation allele only was seen in the CVS biopsy.[253] This concern about missing an affected 'size' mosaic may lessen as more information on stability and possible tissue-specific mosaicism is obtained.

The above outline of some of the clinical management issues in prenatal diagnosis for fragile X syndrome highlights the importance of careful counselling and careful forward planning to ensure that complications are anticipated and can be overcome rapidly. There is a world of difference between a woman from a known fragile X syndrome family embarking on an 'at-risk' pregnancy, fully informed about the planned prenatal diagnosis, and a woman who discovers her risk, like a bolt from the blue, in mid-pregnancy. The former is likely to be regarded as much more acceptable than the latter, quite apart from the possibility of increased organisational and other errors caused by pressures of time.

Preimplantation genetic diagnosis is awaited by many families as an acceptable alternative to prenatal diagnosis and selective abortion. This is still in its infancy as a service and was the subject of a public consultation document prepared by a joint subgroup of the Human Fertilisation and Embryology Authority and the ACGT in 1999. Currently there are five centres in the UK that are licensed to perform the technique but less than 50 babies have been born in the UK after preimplantation genetic diagnosis for a single gene disorder. In many of these, it was used for simple sexing to select female (and therefore unaffected) embryos for transfer, the disorder in question being inherited in an X-linked recessive fashion. In the context of fragile X syndrome, this procedure would involve ova from a carrier woman being fertilised *in vitro* and, at an early embryonic stage, a single cell being removed for genotyping. In the simplest procedure of embryo sexing and transfer of only females to the mother's uterus, there remains the 25% risk of learning difficulties. In terms of mutation detection, there is uncertainty about the timing of the expansion from premutation to full mutation and single-cell analysis for a full mutation is not yet possible. An indirect linkage analysis for diagnosis is likely to be used for the foreseeable future. This 'gene tracking' approach would distinguish the normal X chromosome from the one carrying the expanded

allele and allow, on average, 50% of embryos to be transferred. Although this option is reported to be attractive to women at genetic risk, demand is likely to be limited by financial costs, valid concerns over the current lack of data on reliability and long-term safety, and the relatively low pregnancy rate of *in vitro* fertilisation set against the availability of early CVS biopsy.

Potential screening opportunities for fragile X syndrome

Preconceptional screening

Advantages and disadvantages

Identifying women who are fragile X syndrome carriers and offering them counselling before they become pregnant has the advantage of offering them the full range of reproductive choices. Two such screening strategies were used as models in a recent assessment of costs, effects and savings in fragile X syndrome screening – namely school screening and other preconceptional settings, for example, those who present for contraceptive advice.[155] With regard to the latter, the absence of systematic preconceptional consultations and the experience of pilot studies of CF carrier screening[264–267] suggest that these approaches would not reach the majority of the population and detection rates would be low. Although approaching girls in their last year at school provides an organisational framework that might facilitate screening, it would raise many difficult ethical issues relating to privacy, confidentiality, peer pressure and stigmatisation at a time of rapidly changing personal and emotional circumstances. For either of the above approaches, a huge amount of education about fragile X syndrome and its inheritance would be needed.

Practical experience

There are no detailed reports in the literature of attempts to offer screening for fragile X syndrome in this kind of population. However, a recent report on 108 prenatal diagnoses for fragile X syndrome[182] made it clear that these 107 women (one with two pregnancies) carrying intermediate or premutation alleles were from a group of 10,587 Israeli women who had accepted an offer of fragile X screening when not pregnant. Reported details of this programme are very limited. Between 1995 and 1998, healthy Israeli women with no (overt) family history of mental retardation were offered fragile X syndrome 'carrier testing' on a self-pay basis. The programme was specifically designed to pick up intermediate alleles, premutations and full mutations by

performing Southern blotting first. When larger bands were detected, the exact number of repeats was established by PCR testing. The 138 individuals who carried one allele with ≥ 51 repeats were offered a free prenatal test, in the event of their becoming pregnant during the study period, and 107 women underwent prenatal testing. The only other relevant comment in the report was that from June 1999 the policy in Israel changed, so that for women without a family history of MR, prenatal diagnosis was only offered to those carrying an allele of ≥ 60 CGG repeats. There was no discussion of how the counselling for those with 51–60 repeats was handled.

CF screening has been studied in school populations,[268] where it was suggested that most young people would welcome the opportunity for testing; however, the different (recessive) inheritance of this condition means that caution should be used in extrapolating from these results to X-linked conditions, in which the women shoulder the burden of transmitting the disorder.

Prenatal screening
Advantages and disadvantages
Prenatal screening for fragile X syndrome as a potential strategy has been the subject of several analyses.[4,155,269] Testing all pregnant women for fragile X premutations and full mutations would identify all carriers, since no affected child has been born to a woman who is not a carrier of a premutation or full mutation. The prenatal population is already exposed to a number of screening procedures to check maternal and fetal wellbeing and, in general, these investigations seem to be well accepted. However, the cost of ultrasound screening in terms of maternal depression over (trivial) deviations from the expected 'norm' is still an issue under investigation.

The principle disadvantages of screening during pregnancy are the uncertain risk associated with alleles of 50–65 repeats, the volume of information that needs to be assimilated, particularly by those women identified as being at increased risk (some of whom will be intellectually impaired), the short-comings of the prenatal test that can be offered to them and the increased anxiety associated with all of the above.

Selective prenatal screening could be restricted to those with a family history of learning disability. This might reduce the difficulty, faced by all general population screening strategies, of predicting the instability of alleles of 50–65 repeats in length

but only in the small proportion of women in whom the familial learning disability is shown to be fragile X syndrome. Urgent family studies during pregnancy are problematic and expensive, although when such pregnant women are referred to genetics clinics this would be current practice.

Practical experience
Although the Israeli fragile X syndrome screening programme led on to prenatal diagnosis, the screening was offered when the women were not pregnant. There is only one set of data based on offering a test for fragile X syndrome to pregnant women in a general low-risk antenatal clinic.[154] *Figure 4* is reproduced with permission from the paper by Ryynänen and colleagues[154] and summarises the outcome of offering fragile X syndrome testing, free of charge, to all pregnant women in the first trimester who attended the Kuopio City Health Centre, Finland, between July 1995 and December 1996. As noted earlier, 1 in 246 had a premutation with an expanded allele (> 60 repeats), even though women with family histories of fragile X syndrome were excluded from the study. This relatively high figure is comparable with the figure of 1 in 259 in a French Canadian study.[209] It should be noted that in Kuopio nearly all women registered at the antenatal clinic between their 6th and 10th week of pregnancy in order to claim state maternity benefit. Some 85% took up the offer of screening and it is evident that those with > 40 repeats were given detailed genetic counselling. Although prenatal testing was offered to those with > 40 repeats, the counsellor stressed that 40–60 repeats could be within the normal variation. The uncertainty of the risk and associated difficult counselling of those within these 'grey area' intermediate alleles was reflected in the authors' comment: "As a clinical guideline, prenatal testing was mainly offered only after 60 repeats. In cases of great maternal anxiety, we investigated the fetal *FMR1* gene even though the repeat size was less than 60." In the event all 12 women with repeat size 51–60, and 6/43 (14%) of those with alleles of 41–50 opted for prenatal diagnosis. One woman in the 51–60 range had a female fetus with a 76 repeat premutation, a degree of instability that would justify the offer of family follow-up. As indicated in *Figure 4*, all six women with premutations > 60 repeats had prenatal diagnosis, and two female fetuses had expansions to full mutation size (one a size mosaic). All those having prenatal diagnosis continued their pregnancies to term.

Ryynänen and colleagues[154] sent a structured questionnaire to the 18 women who had repeats

of > 50 (16 replied) and to 54 controls with < 40 repeats (33 replied) to assess reactions to the screening procedure. The authors report that "Most carriers (76%) were very anxious after receiving the test result, compared to only 4% of controls … However, despite the great concern, most women regarded the gene test to be worthwhile and would encourage their colleagues and friends to participate in it."

Another report related to the experience of offering tests in pregnancy in rather selected circumstances. Spence and colleagues[211] offered fragile X syndrome testing to women presenting to a genetics institute for prenatal diagnosis in pregnancy. This was a facility in which the pregnant women paid for procedures carried out. Uptake was low, at 21% from over 3000 offered the test. This may be because of the financial cost. One-third of those who opted for the test had a relevant family history. The rate of detection of carriers was about 0.5%. The three women diagnosed as premutation carriers did not have a relevant family history.

Neonatal screening
Advantages and disadvantages
As a potential approach, neonatal screening has the advantage of an existing screening framework with a population familiar with Guthrie blood spot screening for phenylketonuria and hypothyroidism. Although little can be done to ameliorate the affected child's learning disability, the lag time to diagnosis would be reduced to a minimum and counselling offered ahead of any further pregnancy the mother might have. On questioning the parents of affected individuals, through the UK Fragile X Society, 85% were in favour of introducing routine neonatal screening for boys but, interestingly and quite unprompted, 19% commented that a neonatal test is 'too late'.[257] Neonatal screening without the offer of prenatal screening may be regarded as unacceptable.

There are a number of potential problems. Currently, it is not possible to do all the DNA analysis required to diagnose fragile X syndrome on the filter blood spot. If PCR analysis does not result in unambiguous detection of normal alleles, then a blood or buccal sample will be needed for Southern blot analysis. This would be particularly troublesome for girls, as about 20–30% will appear 'homozygous' and need a further sample. An additional problem with testing girls in the neonatal period is the inability to predict whether or not they will have learning difficulties from

the genotype in those who test 'positive' with a full mutation. For this reason, testing might be restricted to boys but this would only give an opportunity of identifying half of the carrier mothers who would otherwise be detected. Nevertheless, it has been suggested that neonatal screening of males may be useful, once systematic case-finding and family cascade counselling have been fully exploited.[17]

It has been suggested that the rapid FMRP antibody test on blood smears could be useful in neonatal screening of boys[247,270] as it measures deficiency of the *FMR1* protein – the fundamental cause of the fragile X syndrome clinical features.

Practical experience
Neonatal screening for fragile X syndrome has not been undertaken. There is some experience of screening for genetic purposes in another X-linked condition in the neonatal period. In Wales, screening for Duchenne muscular dystrophy has been offered to newborn boys with an uptake of 95%[271] (Clarke A, University College of Medicine of Wales: personal communication, 1998). The identification of affected boys offers little in the way of therapy but does allow families to make informed choices in future pregnancies that might occur before the symptoms of muscular dystrophy trigger a clinical diagnosis. In contrast to fragile X syndrome, half of all female carriers for Duchenne muscular dystrophy represent 'new mutations' and, therefore, cannot be forewarned by family history, and there is no simple test to identify female carriers as opposed to affected males.

Cascade screening in affected families
The rationale
Cascade screening refers to the process whereby, once an individual has been shown to be affected by, or a carrier of, a genetic disorder such as fragile X syndrome, testing is offered to those relatives at high risk of being a carrier. As close relatives have their carrier status established, so some other relatives' risk drops to that of the general population and they can be reassured, while others shift into the high-risk category and can be offered testing. In fragile X syndrome, for example, the risk shifts to a particular set of relatives once it is known whether the grandmother or grandfather passed the (pre)mutation to the mother of an affected boy. Cascade screening has the advantage that those who are tested are at high prior risk, and often already have some information and experience of the condition within their family.

The process described above is screening in the sense that the relatives have not actively sought testing but are approached in a systematic way as a result of a medical initiative taken during the first consultation with the family. In practice, the initial approach to each relative is via a family member but the clinical genetics team is proactive thereafter. As a means of meeting **population** needs for genetics services, the success of cascade screening in identifying carriers depends on:

- the efficiency of case diagnosis
- the proportion of carriers who have an affected relative
- the effectiveness of the cascade counselling in reaching all relatives at risk.

Each of these three elements is considered in turn.

Case-finding in regular paediatric practice

Testing for fragile X syndrome is undertaken by many paediatricians as part of their assessment of children with developmental delay. Recently, however, Corrigan and colleagues[272] called into question the value of this investigation in children with mild to moderate delay. Gringras,[258] in investigating the practice of community paediatricians, found that about half would request fragile X syndrome testing in a child with mild to moderate delay. It is clear that there is no systematic approach to testing in paediatric practice.

Prevalence studies of fragile X syndrome have shown that the detection rate is relatively low in populations of children at special schools or in institutions (*Table 4*). The recent review by de Vries and colleagues[152] of DNA-based analyses in schools/institutions in England, Australia, USA and The Netherlands puts the figure of newly diagnosed cases as ± 1%. The large number of children who needed to be tested for each positive result may explain the reluctance of some paediatricians to use fragile X syndrome testing routinely in the investigation of developmental delay.

It is clear from the above that existing UK paediatric practice does not represent the efficiency of case diagnosis required to meet most population needs for genetics services via cascade counselling.

Case-finding in regular adult practice

Although a number of fragile X syndrome families are found because an adult is diagnosed with the condition, the investigation has usually been instituted because of reproductive concerns of a family member. In the UK there is no evidence of any systematic approach to diagnosis in mentally handicapped adults. Many involved with the care of this population express reluctance to initiate investigations with no perceived benefit to the individual in question, nor do they wish to encourage 'medicalisation' of learning difficulties. The low level of actual medical problems in fragile X syndrome means there are few opportunities to combine mutation screening with other medical tests.

The number of carriers with an affected relative

The unusual inheritance pattern of fragile X syndrome and the fact that affected males can rarely live independently and have a near-normal life expectancy mean that the majority of carriers of a full mutation will have (or will have had) a relative with overt learning difficulties. In a clinical cytogenetic (and subsequent DNA) screening of 1100 people attending three different local services for people with learning disability in an Essex Health District,[229] all 24 individuals (of whom 23 were males) who tested positive for fragile X syndrome were found to have at least one other family member reported as having a learning difficulty, even though a positive family history was not a selection criterion for testing. The proportion with a positive family history will of course be affected by changes in average family size.

The proportion of females with a premutation who have a positive family history will depend on the definition of premutation. If the definition is a **transmission unstable**, expanded allele, then the proportion will be substantial and much greater than with relatively stable alleles in the 50–65 range.

Despite the premutation definition problems, fragile X syndrome is well suited for a cascade screening approach to meeting the genetics service needs of the at-risk population. This is principally because those premutation carriers at greatest risk of an affected child are those most likely to have an affected relative. It is this relationship that makes cascade testing a logical contribution to reaching the at-risk population, quite apart from the fact that extended family testing is expected as good clinical practice. That said, a recent simulation by Wildhagen and colleagues[273] did not paint a very encouraging picture of what can be achieved by cascade testing from an index case. They concluded that: "In the start-up phase of the testing programme, 18%

of couples who will have a fragile X syndrome child are detected. After this phase the (stabilised) cascade testing programme detects 7% of undetected couples who would have a fragile X child if only first degree relatives were tested, 12% if first to third degree relatives were tested, and 15% if first to fifth degree relatives were tested." They predicted that testing only in the current generation with fifth-degree relative testing, including the start-up phase, would detect 48.1% of all carriers. In their theoretical population, 60% of the premutations were in the 55–59 repeat range, the group that, with their very low risk of expansion to full mutations, contributes most to the poor 'identification rate' of their simulated cascade testing, despite the best-case scenario of all index cases being diagnosed soon after birth and all relatives agreeing to be tested. This brings us to the third point relating to the success of cascade screening.

The effectiveness of cascade counselling in reaching relatives at risk

There are few reported data on the proportion of at-risk relatives counselled and tested as part of the extended family counselling of individuals diagnosed with fragile X syndrome at UK regional genetics centres. In a report from Rotterdam,[274]

the outcome of attempted family cascade counselling was described, starting just from newly identified fragile X syndrome patients in 19 families between 1991 and 1995. The consultants were asked to inform the 366 relatives at risk, and 100% of 44 first-degree relatives of the affected patient were informed, together with 59% of 68 second-degree, 39% of 97 third-degree, and 3% of 160 fourth-degree relatives. On average, in each family, one new fragile X syndrome patient and two new carriers were found. The authors expressed the view that "if the genetic counsellor could take the initiative to inform the relatives, more people would be able to consider counselling and DNA testing. However, this approach bypasses the principle of medical confidentiality, which could be solved by obtaining permission to contact the relatives by the genetic counsellor."[274] The guidelines for genetic counsellors derived from a workshop at the 4th International Fragile X Conference[275] favour a family member making the initial approach to other family members. As described below and in *Figure 5*, the New South Wales programme appeared to have somewhat better success in reaching extended family members but the way in which the data are presented does not allow a direct comparison.[17]
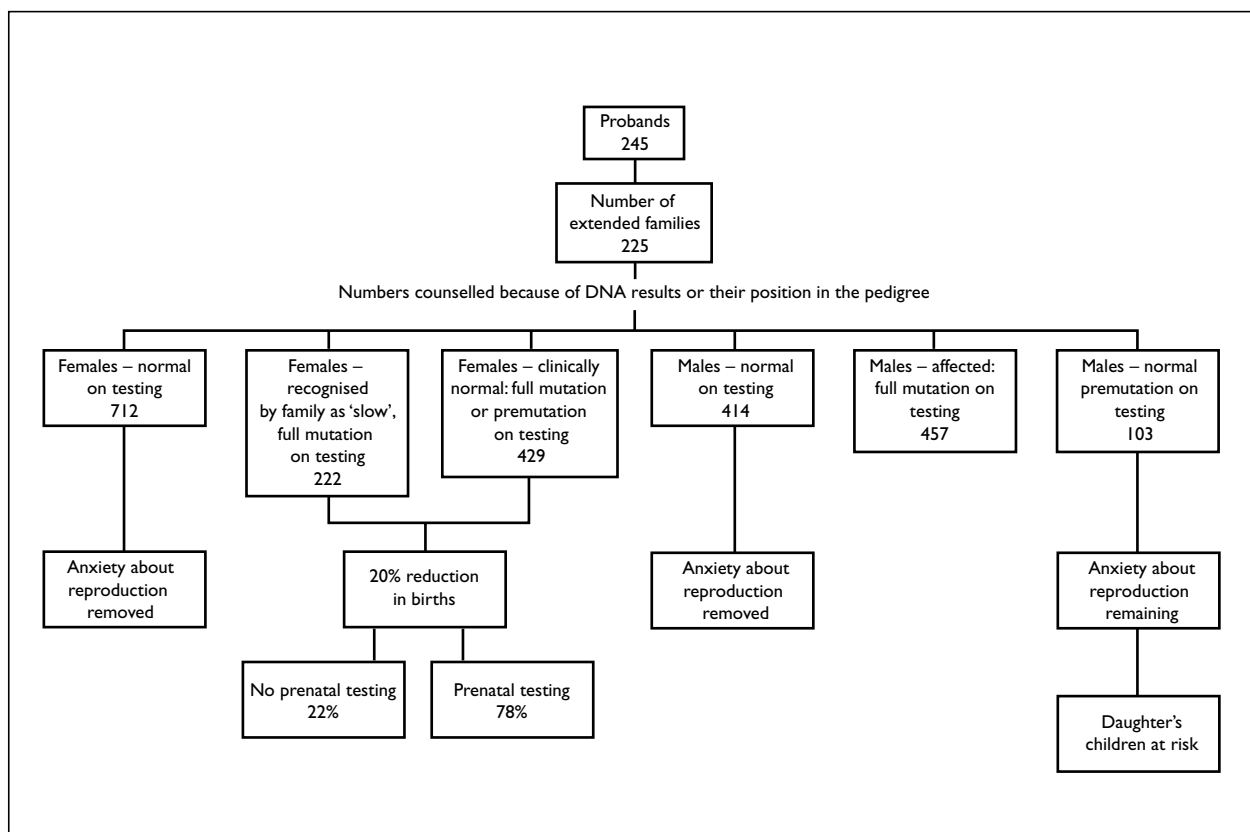
FIGURE 5 *The outcome of cascade testing and its effects in New South Wales, Australia*[17]

The proportion of 'at-risk' women ascertained by current UK practice

The three individual elements that are relevant to estimating what proportion of women at high risk of a child with fragile X syndrome are being offered genetics services, are discussed above. In the better staffed and organised UK regional genetics centres, there is the capability for effective cascade counselling but little progress has been made in the last 3 years (see chapter 8). The greatest shortfall is likely to stem from the low efficiency of case diagnosis. In The Netherlands, where clinical genetics services are comparable with those in the UK, it is estimated[227] that about 36% of males with the fragile X syndrome are known to the genetics services. However, this figure is heavily dependent on the prevalence and, using their 95% confidence limits for prevalence, the figure for known cases varies from 23% to 60%.

In the Wessex region, where the estimated population prevalence of fragile X full mutations is 1 in 5530 (95% CI, 1/4007 to 1/8922), there has been an interest in fragile X syndrome for many years. This team's comprehensive case-finding research study[225] identified 20 boys with full mutations. Interestingly, 17 (85%) were already known to the clinical genetics service. It may be that when the condition is given a high profile with active extended family follow-up by the clinical genetics team, case-finding through all routes improves.

Systematic case-finding combined with cascade screening
Advantages and disadvantages

The advantages of a programme of systematic case-finding and active cascade counselling are that a greater proportion of the at-risk population are offered genetics services and, as a consequence, more women are reassured when found not to be carriers and more carrier women are offered timely reproductive options, including prenatal diagnosis. The disadvantages stem principally from the (perceived) intrusiveness of a proactive case-finding programme in non-medical settings, with the risk of discomfort and embarrassment, and possibly stigmatisation as far as the affected person is concerned, and the emotional upheaval of diagnostic reassessment (often inconclusive) for the family.

Overall practical experience

The most substantial experience comes from New South Wales, Australia, where a state-wide, active, systematic case-finding programme in both adults and children has been established for more than a decade.[16,17,223] In their recent review,[17] Turner and colleagues concluded that the programme had identified about 75% of families who were affected and that cascade testing of first-, second- and third-degree relatives by genetic counsellors was well accepted. Of the 225 families, 16% had minimal testing; in 24% just first-degree relatives were tested; in 40% first-, second- and third-degree relatives were tested; and in 20% cascade testing was even more extensive. The cascade testing and its effects in the 225 families is summarised in *Figure 5*. On average, two affected males (and one intellectually 'slow' female) were diagnosed per family, which supports the view expressed earlier (page 38) that the great majority of clinically normal carriers of a full mutation and significantly unstable premutation will have an affected relative. On average, two such unaffected female carriers were diagnosed by cascade counselling per family. Importantly, on average, three females per family were reassured by carrier exclusion.

Other groups have not had quite such a positive response. In New York, Nolin and colleagues offered diagnostic testing to adults with mental handicap, and counselling and cascade testing to relatives shown to be at risk of fragile X syndrome.[276] No families were traced for 4/17 identified males and, of the rest, half declined further testing. Similarly, in a study in Spain, about a third of those tested apparently had no at-risk relatives.[277] Case-finding with clinical pre-selection (see below) in this Spanish study proved difficult and all available males were eventually tested. This compares with 43% in the New York study, which was based on pre-selection by physical examination.

A Finnish study did not actively seek cases but offered testing in families with a diagnosed proband from chromosome studies.[262] Relatives with a risk greater than 1 in 8 were invited for testing and, from a total of 1017, about half were tested.

The studies published in 1997 by the Rotterdam Collaborative Fragile X Screening Study group[227,274] have been quoted in relation to prevalence (see page 21), clinical pre-selection in systematic case-finding (see page 41) and the effectiveness of cascade counselling (see page 39). Since 1992, this group have conducted a screening programme for fragile X syndrome in five institutions giving residential care and 16 special schools. After some basic clinical pre-selection (see below),

65% (2189 individuals) were eligible for testing and 70% of the parents/guardians consented to testing. In addition to 32 previously diagnosed fragile X syndrome patients, 11 new patients (nine males) were diagnosed.

An example of a smaller systematic case-finding programme, focused on adult males, is reported by Arvio and colleagues.[226] In Southern Hame, Finland, 541 learning-disabled adult males, older than 16 years, were known to the District Organisation for the Care of the Mentally Retarded. Of these, 197 had a confirmed diagnosis, including 20 with the fragile X chromosome. In the remaining 344, six new fragile X syndrome cases were found.

Experience of clinical pre-selection in systematic case-finding

In general, one or two levels of clinical pre-selection have been employed before testing in systematic case-finding in schools, institutions and adult training centres for people with learning disabilities. The first level of selection is to exclude those with a specific known cause for their learning disability (e.g. Down's syndrome) and, of course, those who already have had DNA testing for fragile X syndrome with a negative result (an increasing number). This first level may also exclude more non-specific groups, such as, in the Rotterdam study, cerebral palsy with quadriplegia.[227] The second level is to positively select a subset for testing on features that are typical of individuals with the fragile X syndrome. A number of prospective studies in the era of cytogenetic diagnosis evaluated the use of a checklist to establish a clinical score for pre-selection.[271,278–280] Using a checklist and scoring system adapted from Laing and colleagues,[278] de Vries and colleagues[227] (who only used level one exclusion to obtain their eligible group of 2189 individuals for consent for DNA testing) assessed the potential usefulness of clinical classification for pre-selection. The individuals actually tested (1531) were divided into: 'low' (474) – when dysmorphic features suggested a diagnosis other than fragile X syndrome; 'moderate' (923) – in the absence of specific dysmorphic features; and 'high' (92 or 10.6% of males; 42 or 6.3% of females) – in the presence of characteristic features of fragile X syndrome. All nine of the newly diagnosed males and one of the two females were in the 'high' group. Clearly there could be a place for level two clinical pre-selection in systematic case-finding. At level two, Arvio and colleagues[226] used a clinical checklist completed by a nurse specialist to select 41% (140/344) of individuals for physical examination by a physician experienced in fragile X syndrome, who in turn

selected 44 subjects for testing. The Leuven group, in Belgium, have developed and validated a 28-item checklist (seven physical and 21 behavioural features) that seems a reliable screening instrument.[281] However, as high throughput DNA (or FMRP) analysis becomes more cost-efficient, so the case for seeking to test all 'as-yet-undiagnosed' **males** becomes stronger. Offset against this is the desire to keep invasive tests to a minimum. It may be that the FMRP antibody test on plucked hair roots[248] provides a more acceptable approach in these circumstances.

Case-finding in females with learning difficulties poses problems. Clinical pre-selection is more difficult and yet the DNA analysis is currently more complicated and costly. The New South Wales case-finding programme, responding to, among other things, the trend for those with learning disabilities to be integrated into mainstream schools, has only targeted boys.[17]

Key messages

Application of screening criteria to fragile X syndrome

- Screening criteria have usually developed in relation to presymptomatic detection of treatable disease and pose difficult issues for genetic disorders in terms of 'prevention'.
- In fragile X syndrome, each criterion needs to be assessed in relation to both the affected person and their extended at-risk family.
- Fragile X syndrome is an important health problem, because affected males cannot live independently and each diagnosis creates substantial genetics service needs for the extended family.
- There is an average lag time of a year or two in making the diagnosis but the natural history is known.
- A diagnosis allows appropriate management of the affected person but no specific treatments are currently available. The greatest opportunity for intervention is in cascade counselling of the extended family.
- As monogenic disorders go, fragile X syndrome is relatively common but the prevalence of as-yet-undiagnosed cases will fall as regular paediatric case-finding and clinical genetics services for known families improve.
- Although DNA-based tests are valid, reliable and acceptable in the family with a known fragile X syndrome member, the significance of the 40–60 repeat alleles in the general population is poorly understood.

- A detailed Dutch exploration of the costs, effects and savings of screening females suggests that there are no economic obstacles to screening.
- Fragile X syndrome is the example, par excellence, of high genetic risks to the extended family and, in this context, tests are valid and reliable.
- Knowledge of fragile X syndrome carrier status reduces reproductive confidence and prenatal diagnosis is often sought.
- Prenatal diagnosis of a **female** with the full mutation predicts only a 50% chance of learning disability.

Potential screening opportunities for fragile X syndrome

- *Preconceptional screening* There are no detailed reports of the practical experience of either screening females in schools, where ethical and social issues make it problematic, or in family planning and other preconceptional settings, where organisational frameworks are poor. Israel had a self-pay screening programme for non-pregnant women, in which those with ≥ 51 repeats were offered free prenatal tests in the study period 1995–98. Over 10,000 women were screened but no practical details have been published.
- *Prenatal screening* The only real experience comes from the screening of 1477 women in Kuopio, Finland. Although 85% of women accepted the offer, considerable difficulties and anxieties were created by the discovery of alleles in the 41–60 repeat range, where one-third proceeded to prenatal diagnosis. The Israeli experience of 108 pregnancies provides the best population data on allele instability to date.
- *Neonatal screening* There is no practical experience of this. It would be problematic for girls but the majority of fragile X syndrome families questioned favoured its introduction for boys.
- *Cascade screening in affected families* This is already part of good clinical genetics services. In terms of meeting **population** needs for genetics services, success would depend on (i) the efficiency of case diagnosis, (ii) the proportion of carriers who have an affected relative, and (iii) the effectiveness of cascade counselling in reaching all relatives at risk.
- Case-finding is variable in paediatric practice and poor in adult practice.
- The proportion of female (pre)mutation carriers who will have an affected relative increases the more unstable the premutation. While this means that cascade testing will tend to identify those premutation carriers at greatest risk of an affected child, overall the strategy would only pick up about half of all the women with ≥ 55 repeats, if index cases in the family were used as starting points and testing extended to fifth-degree relatives.
- Experience, especially from New South Wales, suggests that cascade counselling can make an important contribution. Importantly, three females per family were reassured by carrier exclusion and two unaffected female carriers for family were detected and offered help.
- Clinical pre-selection in systematic case-finding is valuable in reducing unnecessary tests.

Chapter 7
The costs of fragile X syndrome

Costs of diagnosis of fragile X syndrome

To individual and family

Although the diagnosis of fragile X syndrome can bring benefits both to affected individuals and their families as discussed later, there are some potential costs that are mostly human rather than financial. The first of these is stigmatisation of both the affected individual and their family. The second is the guilt that many mothers feel on learning that they have transmitted a genetic condition, albeit unwittingly, to their child.

However caused, a handicapped child generates emotional distress within a family and many parents experience a bereavement reaction. These and other stresses can contribute to marital breakdown. Parents with a disabled child or children may find there is less time for their other children. Opportunities for paid employment of parents may be impaired. Families also experience increased financial demands over and above those of raising an unaffected child, for example, extended childcare arrangements or a need to avoid public transport. The above costs, of course, are not specific to fragile X syndrome.

To community and NHS

The costs of providing for an individual with fragile X syndrome are borne mainly by government from education, health and social services budgets. These costs again are not specific to fragile X syndrome but are similar for all individuals with intellectual disabilities of a comparable degree.

The educational costs will vary and depend on whether the educational needs are met in a mainstream school or in special school. The authors were not in a position to assess the costs properly and recognise that what follows is just a brief comment on what is a very complex subject. However, when it comes to decisions about screening, the critical factors are the cost of identifying an 'as-yet-undiagnosed' carrier of a premutation or a full mutation and the costs of realising the benefits that can flow from such a diagnosis. These, in turn, depend on how good our estimates are of the prevalence of fragile X syndrome full mutations

and of allele instability; hence the emphasis in this report on integrating all these aspects.

Information from MENCAP (the UK charity for those with learning difficulties and their families) indicated that the annual cost per adult with moderate fragile X syndrome would be at least £20,000 at 1995 prices. If the life expectancy of a fragile X syndrome male is 60 years, then the cost of his lifetime of care is about £1 million. Other estimates for lifetime costs range from US$1 million to $4 million[223,276,282] but by far the best analysis is that of Wildhagen and colleagues.[155] Their analysis took account of where people with fragile X syndrome lived; they reported that, in The Netherlands, 38% of males and 8% of females are in institutions, 18% of both males and females stay with surrogate family units, 35% of males and 38% of females live with their parents, while 9% of males and 36% of females are (financially) self-supporting. Taking the known costs for these different services, they adjusted the age- and sex-specific costs for the survival figures of the general population. They used a 3% annual discount rate to transform the streams of future costs (and savings but not effects) to the present value at the point of screening and estimated the so-called lifetime costs of care for fragile X syndrome patients as US$957,734 for males and US$533,673 for females.

Costs of screening for fragile X syndrome

To individual and family

The costs of screening to individuals and families are again mainly human and centre on the process of testing. In whatever population is screened, anxiety is engendered by the testing procedure. For most this will be short-lived but for a minority it will continue. Although this minority will include those who are shown to be carriers or have equivocal results, it would be wrong to assume that the anxiety associated with testing always disappears on receiving a normal result. In the Kuopio, Finland, prenatal screening study,[154] 4% of those with normal results were reported to be "very anxious after receiving the test result", although the details are not given and their anxiety may be linked to other factors.

For women identified as carriers who decide to have tests in pregnancy (or pre-implantation genetic diagnosis or donated eggs), there are the additional costs of the procedures used, of which the most obvious is the risk of procedure-induced miscarriage with prenatal diagnosis. With experienced operators, this risk is similar to that for CVS biopsy and amniocentesis, and is about 0.5–1.0%.[260] There has been some evidence to suggest there is also a small increased risk of limb defects after CVS biopsy when performed before 10 weeks' gestation[283,284] but, overall, the procedure does not seem to be associated with an increased risk.[285]

For women who have a prenatal diagnostic procedure and are predicted to have an affected fetus, there are further human costs in making a decision on whether to continue with the pregnancy or to opt for an abortion.

In systematic case-finding, the clinical pre-selection procedure and testing can be upsetting for the affected individual, and the consent decision by the parents can sometimes be a stressful and disturbing event that brings feelings of guilt and sadness to the surface. When cascade screening is used, there are potential costs of fractured family relationships as a consequence of the issue of testing being raised.

To community and NHS

The costs of screening will depend both on the method used and on the ability to define what additional costs screening will bring over and above current clinical practice. Since preconceptional, prenatal and school screening for fragile X syndrome are not performed at all in the general population, estimating the additional costs is possible. These three screening strategies have been the subject of detailed analysis by Wildhagen and colleagues.[155] For each strategy, based on the Dutch situation, they calculated the expected costs, effects and savings for a 1-year screening period. The limited experience of fragile X syndrome screening to date meant that they had to make a large number of assumptions in their analysis. However, they conducted a sensitivity analysis of these assumptions to test the robustness of the results. Their analyses predicted that prenatal screening will detect most carriers and will lead to the highest number of avoided fragile X syndrome patients. Using a nominal population of 100,000 couples, the costs per detected carrier are quite similar for all screening programmes – about US$45,000. All screening strategies had a favourable cost–savings balance (prenatal US$14 million; preconceptional

US$9 million; schools US$2 million) and remained favourable within quite a wide variation of the initial assumptions made. Wildhagen and colleagues[155] concluded that economics *per se* is no obstacle to fragile X syndrome screening and the decision to screen or not can (and should) be based on medical, social, psychological and ethical considerations.

In practice, all screening programmes have to be supported by a well-developed and adequately funded service that can meet the needs of the affected families identified. Current good practice would include offering genetics services to relatives at high risk through family cascade counselling. The way clinical services have evolved in the UK in the last 25 years means that the only service that can, in practice, provide family cascade counselling for fragile X syndrome is the national network of regional clinical genetics services. As discussed in chapter 1, the case for creating fragile X syndrome centres that might handle all these aspects (along the lines of haemophilia centres, for example) is hardly justified in the absence of complicated ongoing therapies for fragile X syndrome. It would be wrong to say that the clinical genetics services alone can achieve the necessary increase in detection of carriers of fragile X syndrome (pre)mutations, although they certainly have a central role in coordination and professional education. Case-finding occurs in several clinical disciplines. Selective genetic testing, as an aid to diagnosis, is increasingly part of paediatric practice when a child has learning difficulties and perhaps up to 50% of new paediatric cases of fragile X syndrome are being diagnosed. The NSC criteria state that:

> "Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme" (paragraph 6.2.1.6).[15]

If the equivalent staff and facilities were available for meeting the genetics service needs of fragile X syndrome families, even without systematic case-finding, a significant proportion of carrier women in the population will have already been diagnosed. Thus the relevant prevalence figure in the screening cost–savings analysis is 'as-yet-undiagnosed' female carriers.

Wildhagen and colleagues[155] assumed a 1 in 4000 prevalence of full mutations in females. The threshold value for a favourable cost–saving balance is about 40% of this prevalence figure (39% for prenatal screening and 43% for

preconceptional screening). A key question, therefore, is whether 'as-yet-undiagnosed' full mutation carriers have a prevalence that is more or less than 1 in 10,000. Based on Wildhagen and colleagues' assumed female prevalence of premutations (> 55 repeats) of 1 in 435, the equivalent threshold value for premutation carriers would be 1 in 1088. In the UK overall, assuming a prevalence of affected males of 1 in 4000, it is unlikely that these threshold frequencies of undiagnosed carriers have yet been reached and there is no longer a favourable cost–saving balance to screening. If complete case-finding and cascade counselling within the extended family were capable of identifying 80% of all women at 'increased risk' of an affected child in the population, we would fall below the saving–cost threshold of screening only when 75% of families in the population were known to genetics services and there had been optimal cascade counselling. However, this conclusion is critically dependent on the true prevalence. If the prevalence of affected males is 1 in 6000 as estimated for The Netherlands[227] (and the Wessex estimate of 1 in 5530 is very similar[225]), then only some 50% of families in the population need to have received cascade counselling before the rarity of undiagnosed 'at-risk' women starts to make screening uneconomic. The above assumption that, in theory, 80% of 'at-risk' women could be reached via case-finding and cascade screening, is probably overoptimistic, particularly in view of the simulation studies by Wildhagen and colleagues.[273] However, if this figure is set at 50%, and 75% of all families are known to the genetics services and received cascade counselling, as occurs in the New South Wales case-finding programme,[17] then again the prevalence of 'as-yet-undiagnosed' female carriers (1/9600 for full mutations, 1/1186 for premutations) drops to a figure at which screening starts to become uneconomic. It is worth reiterating that the Wessex genetics service had very good evidence for saying it already knew of 85% of the fragile X syndrome males, independent of its population-based research programme.

The above brief exploration of the economics model developed by Wildhagen and colleagues[155] shows the importance of assessing general population screening strategies in the light of existing clinical genetics practice and what has been achieved, elsewhere, by a programme of systematic case-finding and cascade counselling.

The additional costs of systematic case identification in the paediatric population are more difficult to predict, because of the uncertainty about how widespread testing is in current paediatric practice. This could well vary geographically. The additional cost of systematic paediatric case-finding could be relatively small but can only be estimated once most, or at least several, UK regional genetics centres have accessible systematic data on referrals for fragile X syndrome testing in those with developmental delay and can integrate these data with population statistics for those with learning disabilities. There would also be additional costs in cascade screening and, in order to make this more efficient than at present, additional counsellors would be needed who could work with families to provide information and support to those who are tested. As indicated later in chapter 8, the New South Wales' experience indicated that a systematic case-finding/cascade counselling programme needs one dedicated counsellor per regional genetics centre, support costs and moderate additional laboratory costs.

Whichever screening strategy is used, there are costs from the additional prenatal tests carried out. These comprise costs of counselling both before and after testing, costs from obstetrics services providing the tests, technical costs of laboratory studies and costs of abortion.

Key messages

- The costs of fragile X syndrome diagnosis to the individual and family are mostly human rather than financial.
- The costs of fragile X syndrome diagnosis to the community and government are mainly borne by the education, health and social services budgets.
- The costs of screening for fragile X syndrome for unaffected individuals are mainly those of anxiety generated by the testing procedure. This may not resolve after a normal result.
- The estimates of the healthcare costs of prenatal and other population screening strategies suggest there is a favourable cost–saving balance. However, these estimates are based on a prevalence of 1 in 4000 males having the full mutation and 1 in 270 to 1 in 435 females having the premutation. If the prevalence of males with the full mutation were ≥ 1 in 6000 and 50% of at-risk women will eventually be reached through case-finding and cascade counselling, the lower prevalence of 'as-yet-undiagnosed' carrier females would tip the cost–saving balance.

Chapter 8
The benefits of making a diagnosis

Although there is no curative treatment for fragile X syndrome, there are benefits resulting from the diagnosis both to the affected individual, to their family and to the wider community. Most of the benefits derive from knowing that the cause of the learning disability is the fragile X syndrome.

To the affected individual

There are no treatments for fragile X syndrome that can correct the underlying pathology or abolish the symptoms but a number of approaches can ameliorate the problems associated with the diagnosis.[286] Drug therapy with folic acid has been the subject of a sustained debate in the literature. Lejeune first reported an effect of folic acid on the clinical manifestations of fragile X syndrome in uncontrolled trials of oral and intravenous folic acid.[287] Subjective assessment showed improvements in behaviour in seven of eight individuals studied. Controlled trials of folic acid have been carried out but it is difficult to design trials that are free from bias. It is important not only to have control groups (matched for age, intellectual handicap and behavioural problems, and with and without fragile X syndrome) but also to have a run-in period prior to any treatment, since the process of testing and assessment can, in itself, exert a placebo effect. Many of the tests used to assess intellectual function can show an apparent improvement due to learning after repetitive administration.

In the largest trial to date,[288] 25 fragile X syndrome males aged from 1 year to 31 years were recruited in a double-blind trial of oral folic acid given over a 6-month period. Assessments included haematological indices, language skills and measures of IQ and behaviour. Of those who completed the trial, nearly half showed no change. The others were reported as showing some improvements, although these were mostly not detectable on formal testing, only by parental report. Similar experience has been reported in other trials, with none demonstrating improvements in intellectual function but most reporting subjective changes, with some parents withdrawing from the double-blind trial at the crossover point because their child lost improvement when coming off what they believed (correctly) to be folic acid. Murray and colleagues[4] collated the results of seven, double-blind, placebo-controlled, crossover studies of affected males.[288–294] These covered a total of 65 patients aged 1–31 years in whom there was some objective evidence in 12% and a subjective impression in 46% of an improvement in (behavioural) symptoms. The general consensus is that, although parents and carers do perceive an improvement at least in the behaviour of pre-pubertal fragile X syndrome males who have been treated with folic acid, this has not been confirmed in double-blind trials. Nevertheless, it remains a treatment prescribed for some affected individuals and has no significant side-effects.

Other drug therapies have been tried and there is some evidence that stimulants, such as Ritalin® (Novartis Pharmaceuticals) (methylphenidate), improve hyperactivity and attention deficit, and therefore may allow better learning to occur. There are unwanted side-effects to these therapies, which makes the benefits of long-term therapy uncertain, but these drugs, already popular in North America, are increasingly prescribed in the UK. Propranolol therapy has been reported to improve aggressive and self-injurious behaviour in fragile X syndrome males (and non fragile X syndrome individuals), possibly due to a reduction in arousal induced by social demands,[295] but there are no other reports of its use.

Other strategies used in the management of individuals affected by fragile X syndrome are mainly behavioural and educational.[296,297] There is increasing evidence that the approach to this aspect of management is not the same for all individuals with learning disabilities but that certain specific strategies are beneficial in fragile X syndrome.[298] There may be specific educational strategies that help with learning, such as the recognition of the visio-spatial problems and the difficulties with sequencing. More remedial help will become available as new methods of teaching in this condition emerge. There is some evidence that fragile X syndrome children do not benefit

from the traditional approach of breaking down a task to its component parts but achieve more when the overall aim is presented.[299] There are few scientifically rigorous data to support this assertion and it merits further study.

There is also scope for management of the medical problems that present in fragile X syndrome.[286] Professionals involved with, in particular, affected children need to be aware of complications; for example, appropriate monitoring for glue ear and visual problems should be considered.[300]

Last but not least, recognition and molecular confirmation of the diagnosis can stop further investigations for developmental delay that can sometimes be invasive and unpleasant.

To parents

After parents have recognised that their child has a learning disability, for most there is a desire to understand the cause. They often have feelings of guilt over what they did or did not do in pregnancy or consider that the slowness is caused by the way they are rearing their child. In some cases this is relieved by the diagnosis of fragile X syndrome but, for some mothers in particular, a different guilt feeling may be aroused. Knowledge of the cause helps them pass through the grieving period for the healthy child they hoped for and halts the search for a cause that always carries with it the hope that there might be a cure.

Knowledge of the cause can help in coping with the behavioural problems associated with fragile X syndrome, such as hand-flapping, overactivity and gaze avoidance. In some cases, referral for professional assistance in these areas can achieve considerable improvement. Realistic goals can be set and plans for future provision made, both in education for the child and for living arrangements and gainful employment for affected adults.

There are direct practical benefits if, as a parent, you have a diagnosis – when someone asks, you have an answer. When a medical person asks, the diagnosis is provided so that he/she can listen to the problems in the context of the diagnosis and, hence, may be more understanding and so of greater assistance. When applying for state benefits and planning educational provision, a diagnosis is much more acceptable and more

likely to result in the application for special help being accepted.

For many parents, and in the wider family context, contact with a support group (the Fragile X Society in the UK) allows families to share experiences and coping strategies, and many families find this promotes their ability to deal with day-to-day difficulties and anxieties.

Knowledge of the cause and the mode of inheritance allow rational, informed decisions about future reproduction.

In a retrospective home-visit interview, 3 years after a diagnosis made as a result of a voluntary screening programme for those with learning difficulties in New South Wales, 100 parents were asked the question, "If you were offered participation in this screening programme again would you have agreed to it?" For 95% the answer was "Yes". Results from an open-ended question in a questionnaire sent to members of the UK Fragile X Society[257] suggested that more advantages than disadvantages were perceived as stemming from a diagnosis (*Table 6*). Respondents were not provided with a list of suggestions but suggested, unprompted, that diagnosis allowed appropriate intervention for affected individuals, promoted tolerance and support across the family and from other affected families, allowed genetic counselling and improved prospects of financial assistance. As mentioned earlier (page 43), a number of disadvantages to diagnosis were cited, particularly stigma, reduced expectations of affected individuals and guilt and anxiety of other family members. The response rate to the questionnaire was only 61% (254/413) and there is a possible bias, in that only those relatively satisfied with the diagnosis may have reported their perceptions.

To siblings

The benefits to siblings are similar to those for parents. First, understanding of the cause of their sibling's disability can improve tolerance, both their own and that of their friends, who can be given an explanation for the problem. Second, the diagnosis can alert families to other affected siblings, particularly mildly affected girls who may benefit from a diagnosis through the opportunity to have appropriate educational help. Finally, a diagnosis provides siblings with access to genetic counselling and, hence, appropriate choice in reproduction.

TABLE 6 *Benefits and disadvantages of knowing the diagnosis: results from a questionnaire*[257]

To child		To parents		To siblings		To wider family	
Benefits							
Appropriate intervention	146	Understanding needs	101	Genetic counselling	90	Genetic counselling	90
Tolerance	25	Genetic counselling	56	Understand needs/	63	More supportive	40
Financial help	7	Information	46	more tolerant		Diagnosed subsequently	3
		Have a reason	37	Helps explain to friends	7		
		Relief not at fault	26	Diagnosed subsequently	5		
		Contact/support	26				
		from other families					
		Financial help	14				
Total respondents	**162**	**Total respondents**	**192**	**Total respondents**	**126**	**Total respondents**	**123**
Disadvantages							
Stigma	14	Guilt	25	Stigma	4	Family tension	21*
Label	8	Anxiety about	2	Anxiety about	3	Guilt	8
Reduced expectations	3	other children		carrier status		Anxiety	1
		Stigma	1	Potential problems	1		
				with insurance			
Total respondents	**27**	**Total respondents**	**30**	**Total respondents**	**9**	**Total respondents**	**30**

Includes four families for whom there was complete loss of contact with relatives following diagnosis

To the extended family

Female relatives usually want information about their carrier status and, through testing, gain some resolution of the uncertainty that comes from realising that they may be facing a genetic risk. They usually want the chance of learning their carrier status and information about fragile X syndrome before having a family. Female relatives are not usually offered carrier testing until they are mature enough to give informed consent. Occasionally, parents may wish to have the information earlier, mainly because they have concerns that their daughter may be a carrier because of learning problems. Males may wish to know whether they carry a premutation because, if they do, any of their daughters could be at risk of having affected children. Males in the family, who are known to be handicapped but with no clear diagnosis (or perhaps a misdiagnosis), may now learn the correct diagnosis.

For a female relative who is a carrier, this knowledge will influence their decisions about reproduction; whether to forego having children, or to use prenatal diagnosis and selective abortion or some other option. Knowledge of an expected early menopause may be of benefit in their family planning.

To the wider community

One of the consequences of meeting the clinical genetics needs of the community can be a reduction in the birth prevalence of serious untreatable disorders, along with an increase in healthy children born to couples at risk.[301] This seems to be the case with fragile X syndrome,[17] so the main benefit to the wider community of making the diagnosis is the reduction of costs to health service providers, consequent on a reduction of prevalence. There is also a potential reduction in the costs of investigation of children with developmental delay. *Figure 6* shows the reduction in prevalence in identified families in New South Wales, Australia, following the introduction of a systematic case-finding and cascade counselling service in the state in 1985.

Key messages

- To the individual and family, most benefits come from knowing that the cause of a learning difficulty is fragile X syndrome and, therefore, having a better understanding of the needs of the affected person and the importance of genetic counselling.
- The benefits to the extended family are those of resolving concerns about their carrier status or forewarning female relatives of their genetic

Incidence per 10,000 births
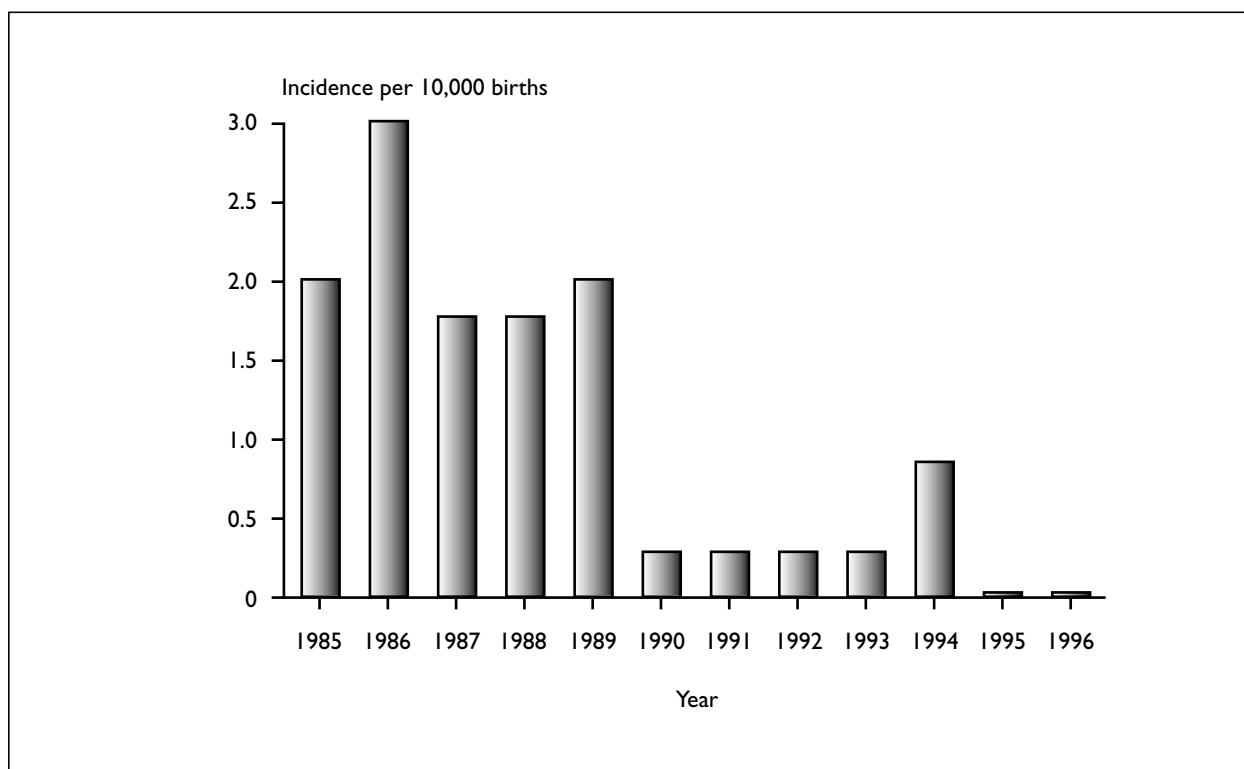
FIGURE 6 *Determination of prevalence by comparing number of males with fragile X syndrome by year of birth with male birth rate/10,000 in New South Wales, Australia[17]*

risk and offering prenatal diagnosis. Both activities help restore reproductive confidence in the face of genetic risks.

• The benefit to the community at large of diagnosis and cascade counselling is

a reduction in prevalence of fragile X syndrome. The New South Wales programme has seen an estimated reduction in prevalence of affected males from 1 in 4000 to 1 in 10,000.

Chapter 9

Meeting the needs of those at risk of a child with fragile X syndrome

Common scenarios of unmet need

It cannot be emphasised enough that, whatever the route to the diagnosis of a full *FMR1* mutation or premutation, that person and/or their immediate family will need information and (ongoing) support from the appropriate clinical services if their needs are to be met in full. The complexity of the inheritance of fragile X syndrome and the counselling in the face of uncertainties about risks to offspring and relatives means that regional clinical genetics services will need to be involved. It would be unusual if the regional genetics centre could provide all the help that is needed but it plays a pivotal role in coordinating services. In assessing the possible role of screening in improving services to 'at-risk' families, the following points should be borne in mind.

1. At the present time, the actual provision of services are such that families range from those whose needs are well met to those who are totally unaware of their special risks and needs.
2. The proportions of fragile X syndrome cases known to regional genetics centres probably range from 25% to 85%. As an indication of the background level, a study[223] in New South Wales, Australia, published in 1986, estimated that only 25% of affected males were diagnosed. This figure rose to 74% after a decade of active case-finding.[17] In The Netherlands, an estimated 36% of cases, and certainly < 50%, are diagnosed.[227]
3. Increasing awareness of fragile X syndrome, ongoing case-finding and cascade counselling within known families will continue to reduce the prevalence of undiagnosed cases for some time to come. This in turn reduces the 'yield' from screening or systematic case-finding programmes.

It is nevertheless a useful exercise to set out the various scenarios of unmet need and note which policy (in theory, at least) would address the problem. A general schema illustrating the five policies is presented in *Figure 7*.

Problem situation 1

Families who know the diagnosis of fragile X syndrome in the affected child, but who may not have had extended family studies, may not have a regular contact person and a planned follow-up, and may not have had a cytogenetic diagnosis confirmed or the extended family restudied by DNA analysis.
Solution: Policy I (see below).

Problem situation 2

Families who have a member with a learning disability of unknown cause, for whom the diagnosis is actually fragile X syndrome (with all its genetic implications to the extended family). It is estimated that, currently, 50–75% of fragile X syndrome families with an affected member are in this category.
Solution: Policies II and III, then I (see below).

Problem situation 3

Females who are unaware that they are carriers and who do not have (or know of) a history of learning disability in the family.
Solution: Policies IV and V, then I (see below).

Problem situation 4

Fragile X syndrome represents only about 25–30% of the proportion of severe learning disabilities caused by mutant genes on the X chromosome.[119] The families of people with these other entities also require a diagnosis, carrier detection (if possible) and ongoing support. In practice, meeting some of these needs cannot easily be divorced from active case-finding for fragile X syndrome, since any learning-disabled person with a family history suggestive of X-linked inheritance will be tested for an *FMR1* mutation and, even if the test is negative, the family will be offered follow-up counselling on account of female relatives facing high risks.
Partial solution: Extension of Policy I (see below).

Services currently provided by UK regional genetics services

At the onset of this assessment, in the summer of 1995, a questionnaire was sent to all 22 UK
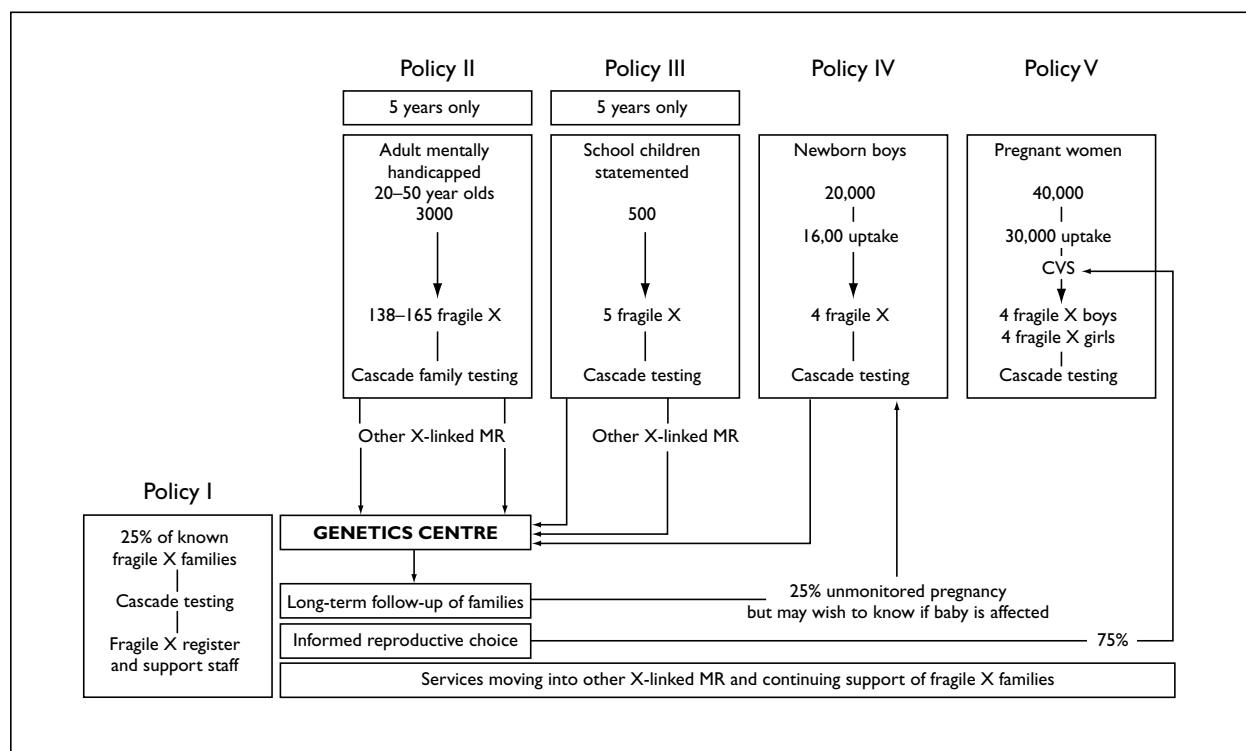
FIGURE 7 *Different screening approaches for fragile X syndrome (3 million population)*

regional clinical genetics centres to try and estimate how many fragile X syndrome individuals had been ascertained in each region and to gather basic information on fragile X syndrome services. Two of the authors (GT and BC) also visited many centres but it proved impossible to collect useful information because:

- information was not kept in a standard form
- few centres had information on a database
- where information was on a database, it was not always clear whether cytogenetic tests had been confirmed with DNA testing **or**
- if cytogenetic cases were duplicated in the DNA register **or**
- if the database was complete, with cases entered retrospectively.

Some centres provided information about the numbers of families known to them, with estimates of how many affected individuals there were in each family; others gave total numbers, without a breakdown into males and females, and it was not always clear whether cases counted as positive carried a full or premutation. While it might have been possible to get more consistent information, it was clear that this could only be done by individuals spending time going through case records, and it was not considered reasonable to ask them to do this. At one centre, at least, the only way to

get an accurate assessment required laborious comparison of results from the DNA laboratory with cases known to the cytogenetics laboratory. It was not always possible to identify samples that had been tested in both laboratories and input was required from a geneticist who knew the families. The two centres that were able to provide the most detailed information most easily were undertaking grant-funded fragile X syndrome work.

Ascertainment could be improved if all families with a positive laboratory result were referred for genetic counselling. It was clear that this did not happen routinely in some regions. At one centre, at which laboratory and clinical staff provided separate information, there was a marked disparity between the numbers of affected families. The clinical staff reported '10–15 affected families', while the laboratory register recorded 55 different pedigrees. In another region, 9/22 cases diagnosed from 1989 to 1995 were not referred for genetic counselling. This disheartening picture is supported by a questionnaire study of the members of the UK Fragile X Society.[257] Of the 153 families who did not receive the diagnosis at a genetics centre, 35 (22%) stated that they had not been referred for genetic counselling.

Any advance in regular, basic clinical genetics services for fragile X syndrome families, let alone

the adoption of screening programmes, will need systematic collection of information on known families on one integrated database at regional genetics centre level, at least. This would provide data that could be used to compile national statistics. To assess the general level (and any improvement/decline) of register-like activity with respect to fragile X syndrome families, one author (MEP) interviewed senior staff from each of the 22 regional genetics centres in September/October 1998. The results are summarised in *Table 7*. The results are not very encouraging even though, in addition to the two centres with fully established registers, a further eight centres reported having a fully integrated database between clinical, cytogenetics and molecular genetics sections of the service. All centres had moved over to molecular analysis as the method of diagnosis (although three were struggling to completely replace cytogenetic studies). Only five (23%) reported that systematic cascade counselling for families had improved since 1995 and three (14%) considered it had actually got worse.

Policy 0: no extra staff and facilities provided for fragile X syndrome related services at regional genetics centres

The present provision of services for known fragile X syndrome families varies in quality between the different regional genetics centres (*Table 7*). All have access to DNA diagnostic facilities for diagnosis and prenatal diagnosis of fragile X syndrome. All centres offer counselling and at least some testing to the families referred. However, as indicated earlier (see page 51), many centres fall short of providing even adequate services to the extended families. The clinical pressures on limited resources over the 3-year period 1995–98 have resulted in only five of the 22 centres reporting an improvement in systematic cascade counselling, and three centres considered that cascade counselling services had deteriorated.

Consequences of Policy 0
- There will be some decline in the prevalence of new affected individuals in the families of the 30–50% of cases already identified and in those cases newly diagnosed by paediatricians and school medical officers.
- There should be a slow increase of awareness in the medical community through the under-graduate teaching of genetics, continuing medical education programmes and through the medical literature.
- There may be litigation because of inadequate extended family counselling or inappropriate reliance on cytogenetic results.
- There will be a worsening quality of fragile X syndrome services as clinical pressures (e.g. cancer genetic referrals) exceed staffing and infrastructure resources.
- The quality of UK fragile X syndrome services relative to other countries will fall.

Policy I: active cascade counselling and testing of the families known to genetics centres

In the New South Wales, Australia, experience[16] this requires one full-time genetics counsellor per 3 million population. It involves networking with other similar genetics counsellors for extended family studies. Much of the work is done by home visits, since many of these families have few resources for travelling and it is difficult to travel with a handicapped child or children on public transport. The one other important advantage of the home visit is that it can be arranged at a time when the father can be present and sometimes when other relatives are visiting. After explanation and consent, collection of DNA may be undertaken in the home.

Independent transport is essential for the counsellor, as is secretarial assistance for corres-pondence, the maintenance of files, pedigrees and cross-referencing of all names, including maiden names. Positive results are always immediately followed-up by interview, written information, a contact phone number and written information to the general practitioner (GP) (if the patient wishes). The counsellor is the contact person to support and facilitate decisions concerning pregnancy.

There has been recent debate in the UK about how best to organise such extended family follow-up for all relatively common disorders, in which the diagnosis in the index case immediately puts certain classes of relatives into a high-risk category (mainly autosomal dominant and X-linked dis-orders). The issue is whether to have relatively separate disease-specific registers, with associated disease-specific staff, or a generic patient and family management system. It can be argued that all extended families at risk should have the same active follow-up, not just those with disorders that are common enough to have a designated register. Whatever the approach adopted, some

TABLE 7 *Genetic register-type activities for fragile X syndrome at UK regional genetics centres, September/October 1998*

Regional Health Authority	A	B(i)	B(ii)	C(i)	C(ii)	D	E	F(i)	F(ii)	F(iii)	G
England											
1. South East Thames	No	Yes	No	No	No	Yes	Yes	Yes	No	No	No
2. South West Thames	No	*	No	No	No	Yes	No	No	Yes	No	No
3. North West Thames	No	Yes	No	No	No	Yes	Yes	*	No	No	No
4. North East Thames	No	No	*	No	*	Yes	Yes	Yes	No	No	No
5. East Anglia	No	Yes	No	No	No	Yes	No	Yes	No	No	No
6. Oxford	No	No	Yes	No	No	Yes	No	No	Yes	No	No
7. Wessex	*	*	No	No	No	Ye	Yes	No	*	No	Yes
8. South Western	No	No	No	No	Yes	Yes	No	Yes	No	No	No
9. West Midlands	No	No	*	No	*	Yes	Yes	No	No	*	No
10. Yorkshire	No	No	*	*	No	Yes	Yes	No	*	No	No
11. Trent (Sheffield)	No	Yes	No	No	No	Yes	Yes	No	Yes	No	No
12. Trent (Nottingham)	No	*	No	No	No	Yes	Yes	No	Yes	No	No
13. Trent (Leicester)	No	No	No	No	Yes	Yes	Yes	No	Yes	No	No
14. Northern	No	Yes	No	No	No	Yes	*	No	No	*	No
15. Mersey	No	No	*	No	*	Yes	No	No	Yes	No	No
16. North Western	Yes	No	No	No	No	Yes	Yes	No	Yes	No	No
Wales											
17. Wales	No	Yes	No	No	No	Yes*	Yes	No	Yes	No	No
Scotland											
18. Glasgow	No	No	No	No	No	Yes	No	No	No	Yes	No
19. Edinburgh	No	No	Yes	No	No	Yes	No	No	Yes	No	Yes
20. Aberdeen	No	Yes	No	No	No	Yes	*	No	*	No	No
21. Dundee	No	No	No	No	Yes	Yes	No	No	Yes	No	No
Northern Ireland											
22. Northern Ireland	No	Yes	No	Yes	No	Yes	No	No	Yes	No	No

* See notes below

A Active fragile X syndrome family register with:
  • designated staff member(s)
  • computerised integrated database of pedigrees known to all sections of regional genetics service
  • ability to list 'at-risk' females for the offer of cascade counselling
  • and produces an annual report

B Computerised list that could give confirmed fragile X syndrome cases (linked by family) known to the regional clinical genetics service:
  • with fully integrated database between clinical, cytogenetic and molecular genetics sections of the service
  • with separate listing of fragile X syndrome cases in two or more sections of the service

C A general patient referral/diagnostic computerised record system that could generate list of fragile X syndrome referrals/confirmed cases but would need reference to notes to confirm current diagnostic status, 'at-risk' females, etc.:
  • with fully integrated database between clinical, cytogenetic and molecular genetics sections of service
  • with separate listing of fragile X syndrome cases in two or more sections of service

D Uses only molecular genetics methods of diagnosis of fragile X syndrome, even though cytogenetic analysis may be part of work-up of case of learning disability

E Have had audit meeting on fragile X syndrome in last 2 years

F In terms of systematic cascade counselling for fragile X syndrome, are services better developed (funded) now than in 1995?
  (i) Better
  (ii) Same
  (iii) Worse

G Performing fragile X syndrome screening/systematic case-finding, other than through cascade counselling, following standard referral to genetics service.

TABLE 7 contd *Genetic register-type activities for fragile X syndrome at UK regional genetics centres, September/October 1998*

Notes	

The following data were collected from appropriate senior members of regional clinical genetics centres at face-to-face interviews (except Glasgow) by Professor Pembrey. Usually there was a clear yes or no but sometimes a respondent provided a useful qualifying statement, indicated by *. These short quotes are given below.

A* *'Almost a fully active family register but no annual report as yet. We have just decided that the cut-off for being an at-risk female on the register is ≥ 50 repeats.'*

B(i)/C(i)* *Some respondents could not decide whether statement B or C described their activity, e.g. 'We can produce a list of known fragile X syndrome cases and link family members, but may have to go to notes to confirm at-risk females, although most could be ascertained by the follow-up list produced by the computer.'*

B(ii)/C(ii)* *There is still not integration of cytogenetics, clinical and molecular genetics databases at many centres but respondents were keen to qualify their statements, viz.: 'about to be'; 'can be'; 'no but new system is planned by end of 1998'; 'different Trusts therefore different lists'.*

D* *'At recent review we noted that cytogenetics had not missed any in the past and, since molecular gives you unwanted information, we have discussed reverting to cytogenetics.'*

'While we have changed, the small outlying cytogenetics laboratories are still looking for fragile sites.'

'We are still trying to finally change over to only molecular.'

E* *'Not a formal audit but we have had meetings.'*

'Ad hoc meetings as new families arise.'

F(i)* *'Better developed, not better funded.'*

F(ii)* *'Same but greater uncertainty due to the new Government's ongoing reviews.'*

F(iii)* *'Slipped back because the experienced staff member who ran the fragile X syndrome side of things left.'*

'Worse because no additional staff or funds but extra pressure from cancer services.'

specialisation by genetic counsellors, when the disorder is common enough for substantial experience of the disease-specific issues to be acquired, is probably beneficial for both health professionals and families.

Consequences of Policy I

- The development of a network of family counsellors, experienced in working with fragile X syndrome families, with one at each genetics centre. These counsellors will communicate regularly to facilitate testing of extended families; develop guidelines for managing different counselling situations, and devise pamphlets and other teaching aids that can be used at all centres.
- Effective cascade testing and support of families, with ongoing counselling as illustrated in *Figure 5*.
- A decrease of about 80% in the prevalence of new, affected boys within known fragile X syndrome families, through reduced birth rate and the use of prenatal diagnosis.
- Increasing reproductive confidence of women from fragile X syndrome families, both in those shown not to be carriers and in carriers who find prenatal diagnosis acceptable.
- Increasing family confidence in the family network, as the problem is identified and solved for the next generation.
- An increase of knowledge in the community about fragile X syndrome because of talks and discussions with others from different areas of

service; e.g. teachers, prenatal clinics and adult community services for the learning disabled.

Costs (per centre serving a population of 3 million)
- Salary of one genetics counsellor (nurse or social worker).
- Travel or car allowance.
- Secretarial assistance, 20 hours/week.
- Cost of testing in 50 families: one in three – as little follow-up possible; one in three – 10 samples; one in three – 50 samples. PCR tests + 30% Southern blots.
- Further testing, for example, CVS × 5.

Policy II: systematic case-finding in adults with learning disabilities

If we consider the number of adults with learning disabilities in a population served by a regional genetics centre (about 3 million people), their estimated numbers and places of abode are shown in *Table 8*.

Only about 1% of adult males with fragile X syndrome live independently, so that almost all should be known to adult services and, if there is a well-run register, they should be registered. There is likely to be some baseline information on diagnosis (e.g. Down's syndrome) or, if not, a direct question to the guardian or carer would reveal that approximately 30% would have a

TABLE 8 *Estimates of the numbers and location of adults with learning disabilities (based on a total population of 3 million)*

MENCAP 1995 statistics relating to those with mental handicap – defined as being unable to live independently and requiring various degrees of care	
Total adult male population over 18	991,060
Total mentally handicapped population over 18	9,410
Assuming a male:female ratio of 4:3, number of males	5,079
Proportion in residential care	27%
Proportion in institutions	7%
Proportion living at home	66%
Prevalence of adult males with mental handicap	54/10,000
Prevalence of males with fragile X syndrome	2.5/10,000
Estimated proportion of the 5,079 with fragile X syndrome	4.6% (234)

substantiated diagnosis. If all parents, individuals and guardians were asked for informed consent for a limited physical examination and follow-up interview or telephone discussion, about 75% would give it. This figure, based on the New South Wales study,[16] is similar to studies (combined adult and child) in The Netherlands[227] (70%), Essex[229] (80%) and Yorkshire.[302] If a scoring system for clinical features of fragile X syndrome is used,[278] the New South Wales' experience is that about 77% of individuals can be excluded altogether (with a 1% false-negative rate) and only 42% of the remainder will score positive on the checklist, so the number requiring tests will be about 500 (5079 × 0.23 × 0.42).

It is worth remembering, however, that carers may have legitimate concerns about 'medical-ising' the learning disability and the use of in-vasive tests that are of little direct benefit to that person.

Systematic case-finding for adult females with fragile X syndrome may well not be justified for the following reasons.

- Affected females have half the prevalence of affected males and a proportion of those who are affected function without help in society and may not be known to the local services for the learning disabled.
- The phenotype is less obvious, which makes reliance on clinical pre-selection before testing unsatisfactory; for example, in the Rotterdam study[227] (of all ages) only one of the two affected females (out of 661) was in the group of 42 females designated as 'high' on features suggestive of fragile X syndrome.
- Examination of the data from New South Wales shows that only 4% of affected females would not have been identified through

family studies originating from a male proband[17] (GT: personal communication, 1996).

Depending on the approach adopted and the experience of the staff involved, seeking patients with fragile X syndrome will incidentally allow many other clinical diagnoses to be made (e.g. Williams' syndrome, Angelman's syndrome). Those with other syndromes that may be X-linked, prin-cipally on the basis of family history, could be identified and discussed with parents and relatives.

It might take 3–5 years to cover this backlog and would require extra staff for that period. It may best be undertaken as part of a combined team within the adult services, with one of their nurses being seconded and trained in taking family histories. This 5-year project would require the input of a medical officer, either in a post in which genetics and learning disabilities were combined or as (part of) a post for a clinical geneticist. Each region could negotiate such a position depending on the local situation. The fragile X syndrome genetic counsellor at the regional genetics centre (Policy I) would have to have his or her service enlarged to incorporate the number of families who would be identified and referred. Proactive case-finding for fragile X syndrome will, over the years, generate and train staff with the expertise necessary to provide services to high-risk families with other rarer diagnoses, particularly X-linked disorders. With the advances in medical genetics will come precise molecular diagnosis for both the affected and carrier individuals, and the ability to offer families useful information and services. Individually, these disorders are rare (although not so in aggregate), so diseases-specific screening will not be viable. Systematic clinical diagnosis (often by a dysmorphologist) and the family history will be relied upon to target specific molecular genetic tests.

Costs (per year for 5 years)
- One geneticist/medical officer
- One nurse counsellor attached to genetics/ adult handicapped services
- One secretary
- Testing 500 individuals using PCR + Southern blot
- Family studies: PCR tests + Southern blotting in 100 families
- CVS in ten individuals.

Policy III: systematic case-finding in children with learning disabilities

There is increasing awareness of the fragile X syndrome by the different groups of professionals who provide services for children with developmental delay. The effect of this is that the lag period between the mother requesting help and a definitive diagnosis is decreasing, although to what extent is uncertain. The data presented earlier reflect the experience of members of the Fragile X Society but may not reflect the level of service provided to all strata of society. An alternative to relying on a gradual improvement in the diagnosis of children with learning disabilities is to launch a proactive, systematic case-finding programme, linked to the educational assessment process. An estimate of the scale of the task is outlined in *Table 9*. This is based on the 'statementing' criteria as they were in the first half of the 1990s. There is now an initial 5-stage grading process in the assessment of children suspected of having learning problems,[303] which seems to be resulting in fewer children being 'statemented' as such. Nevertheless, the figures below provide an indication of the very small proportion of such children who will have fragile X syndrome (approximately 1%), even using an overall prevalence figure for fragile X syndrome of 1 in 4000 males. Screening of over 3738 boys with learning difficulties in Wessex, identified 20 with the full mutation (0.54%).[153,225]

Given the relatively low expected 'yield' from screening of 'statemented' children, a retrospective programme to screen all children **already** identified as having learning difficulties – to deal with the backlog, as it were – may not be the best way forward. The clinical commitment over the period of retrospective review would be very substantial. Parents and guardians of learning-disabled children (as opposed to adults) are more likely to want detailed explanation, further diagnostic work-up and genetic counselling, once diagnostic review of their child is raised, regardless of the negative result of the pretest clinical check or DNA-based *FMR1* analysis.

Rather than attempting mass screening in this population, it may be better to promote the proper investigation of developmentally-delayed individuals as a service component of the school medical service. All children identified as having significant long-term delay, and who have no clinical diagnosis, should have a family history taken, a karyotype and testing for fragile X syndrome. A combination of fragile X syndrome testing as part of the initial diagnostic work-up of developmental delay and a review of the diagnosis (and genetic implications for the family) at school-leaving age, would seem to be a realistic approach. However, the school medical service would have to work closely with the regional genetics centre.

There is a question that needs to be answered. What proportion of boys aged 18 years, who are now graduating from the school system, have not been diagnosed, and why has this not happened?

Consequences of Policy III
(i.e. if informed fragile X syndrome screening were a component of the 'statementing' of schoolchildren.)

- Fragile X syndrome detection becomes part of the diagnostic service of school medical services.
- There are increased numbers of referrals to regional genetics units.

TABLE 9 *Estimated number of 'statemented' children with fragile X syndrome*

Schoolchildren (in a total population of 3 million)	
Total male school population aged 5–18 years	149,457
Total expected to have fragile X syndrome	37
Proportion of schoolchildren 'statemented'	3.5%
Proportion 'statemented' because of slow learning	2.5% (3736)
Expected prevalence of fragile X syndrome in 'statemented' children	1 in 100

- Diagnosis of fragile X syndrome at younger ages makes the offer of prenatal diagnosis to the mother more relevant for some families.
- Permission for testing becomes part of the initial medical diagnostic work-up and is not dependent on obtaining permission (often by mail) in an educational setting, where testing may be perceived as 'out of context'.
- An unknown proportion of males with fragile X syndrome who are not 'statemented' will not be identified.

Costs

These would be for a full-time equivalent geneticist, who might be paediatrician with extra training and an interest in genetics, with a liaison supervisory and diagnostic role. The items to be funded would include the following:

- salary
- 500 PCR tests per year.

Policy IV: screening newborn boys

The technical feasibility of using a spare spot on the Guthrie card for *FMR1* analysis is not established but it may well become possible in the future. As discussed earlier (page 27), the initial PCR test on mouth-brush DNA fails in 20% and, if a repeat attempt fails, the DNA is 'cleaned up' before trying again. How feasible this step is using a dried blood spot is unclear. Certainly Southern blotting would be problematic. It is possible, however, that a separate blood smear could be taken for FMRP antibody testing,[247] which can be an effective first screen for fragile X syndrome in males.

As mentioned earlier (page 37), there is support (85%) for neonatal screening of boys from members of the UK Fragile X Society, although 19% regarded it as 'too late', thereby implying that it might not be acceptable in the absence of prenatal testing. Nevertheless, this generally favourable response and the fact that neonatal screening is being assessed in another X-linked disorder, Duchenne muscular dystrophy, makes it worth considering.

Consequences of Policy IV
- The parents would be saved, on average, 1.2 years of no diagnosis and many investigations.
- An opportunity would be provided for early intervention and management of behaviour, in the knowledge that their son will have learning difficulties.

- Potentially all boys with fragile X syndrome will be identified and through them the majority of high-risk fragile X syndrome families in the population.
- An opportunity would be provided for prospective testing in any future pregnancy the mother has and in members of the extended family.
- An as yet unknown reaction of families to the diagnosis, with possible effects on bonding.
- An as yet unknown false-positive rate of screening tests and unnecessary anxiety when follow-up shows no *FMR1* expansion mutation.
- An as yet unknown false-negative rate of screening tests.
- A big commitment to public education focused around the neonatal period.
- The potential for inadequate information being provided in the time available, such that a decision by parents to have their newborn son tested is not fully informed.

Costs (total population of 3 million)
The items to be funded would include the following.

- Information leaflets.
- The teaching of midwives, nurses, health visitors and others.
- Kits and mailing of blood smears to the laboratory.
- Assuming an 80% uptake in a population of 3 million with 40,000 births/year, approximately 16,000 FMRP antibody tests.
- The costs related to the, as yet, unknown number of positive screening results that require confirmation by a blood test and Southern blotting.
- The 3–4 true-positives a year would need referral to their GPs and clinical geneticists, and close follow-up.

Policy V: prenatal screening

Prenatal screening was piloted on a population of 1738 low-risk pregnant women in Kuopio, Finland,[154] and resulted in the conclusion that their approach "provides an effective way to find carriers and to incorporate prenatal testing into this process". The authors reported that "overall, the attitudes towards screening were encouraging". A pilot study in the UK has been suggested,[4] principally on the grounds that "A simple model of the screening process suggests that performance might be high and certainly comparable with

antenatal screening for Down's syndrome and CF". Performance in this context is in terms of detection and the expected rate of termination of affected fetuses. The detailed costs, effects and savings analysis by Wildhagen and colleagues[155] suggested that (within a wide range of assumed parameters) there is no economic obstacle to fragile X syndrome screening, and the decision to screen or not should be based on other considerations. Clearly prenatal screening is an option that has to be considered seriously.

In the flow chart (*Figure 8*), a woman's number of CGG repeats is used as a predictor of the degree of her risk. Those below 54 repeats are not offered further tests. Those with a CGG repeat number of over 90 have a 1 in 4 risk of having an affected son.

Those with a CGG repeat number under 90 are regarded collectively as facing a risk of 1 in 16 of having an affected son (assuming the prevalence figures of 1 in 4000 males with the full mutation and 1 in 250 women being premutation carriers).

Consequences of Policy V
- Theoretically, all women at risk of having an affected child could be detected.
- The offer will be universal to all women and only necessary in a first pregnancy, not subsequent ones.
- There will be a significant reduction in the birth prevalence of affected males through detection and abortion of affected fetuses.
- There will be a high demand for rapidly available information and counselling during
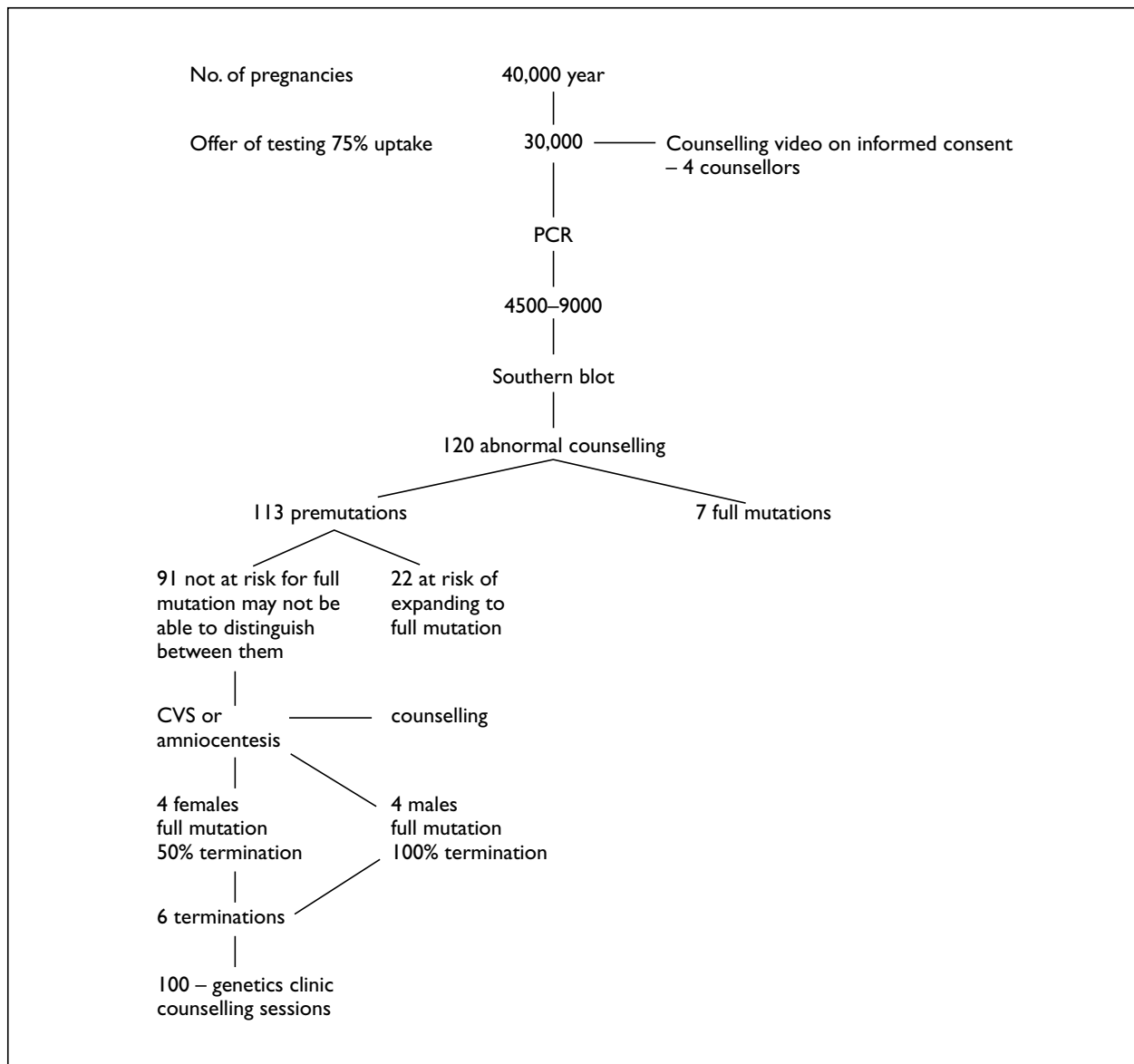
FIGURE 8 *Prenatal testing in a population base of 3 million*

the decision to take the test or not, and a demand from some for urgent and ongoing counselling and support after the test result.

- There will be an ongoing professional challenge to ensure that a woman's decision to have the test is fully informed, given the lack of public knowledge about fragile X syndrome compared with, for example, Down's syndrome.
- There will be high anxiety generated in most premutation carriers (approximately 4.0% of women), in many with intermediate-sized alleles (2.0–3.0% of women), and in some of those who show only one band on PCR and need (repeated) Southern blots (15–30% of women), thus having a delayed result.
- There will be a significant proportion of those with test-related anxiety, for whom the anxiety (and possibly associated depression) will not resolve on the birth (or prenatal diagnosis) of an unaffected baby.
- The non-predictability of future learning difficulties in a female fetus with a full mutation will generate the need for intensive counselling and support for recently diagnosed carrier women when their babies are shown to be female. Some of these women will not wish for further genetics information about their female babies; the remainder will need to be supported while making an informed choice.
- Cascade counselling of the extended family of those identified with a premutation or full mutation would have to be offered by the regional genetics centre. This is likely to be demanded urgently by family members, who are currently pregnant or are concerned about the development of a child.

Costs

There are few data on the costs of prenatal screening. The costs were reported by the Finnish pilot study[154] as £34,000 per full-mutation fetus diagnosed. This figure seems very low but a detailed cost analysis was not given, beyond a statement that the figure included the costs of the prenatal fetal diagnoses.[154] Wildhagen and colleagues,[155] in their comprehensive analysis of the Dutch situation, estimated the cost per carrier detected as £28,500 and the cost per avoided affected birth as £331,714 (or £170,250 in the unlikely maximum yield scenario). Murray and colleagues[4] estimated the average cost of preventing each affected birth as £111,600, assuming that half the females and all the males with a full mutation will be aborted. Proper assessment of all the costs, including those related to the great amount of information giving and counselling needed, is a large

undertaking. For the purposes of our general discussion of screening for fragile X syndrome, the Dutch figures are probably nearest the truth. The Finnish figure does not seem to represent the full costs.

Special population subgroups

To date, the only population subgroup in which an excess of *FMR1* expansion mutations has been properly established are women with POF. As discussed earlier, the excess only holds true for premutation carriers, there being no association between POF and full mutations. About 1–2% of sporadic POF cases and up to 16% of kindreds with familial POF carry *FMR1* premutations.[93] This raises the issue of offering fragile X syndrome screening and appropriate pre-test genetics counselling to women presenting with idiopathic POF. If this approach was adopted, there would need to be close liaison between the regional genetics centre and the clinicians seeing women with POF. Such a link would also support and inform the discussions with women who have been found to carry a premutation through fragile X syndrome family cascade counselling, for example, and who need to know the implications for themselves. Allingham-Hawkins and colleagues[95] reported that 16% of 395 women with the premutation had experienced menopause prior to age 40 years. Again this raises the issue of discussing the possibility of POF as part of genetics counselling for women with a premutation.

Increasing awareness

Preconceptional counselling

Preconceptional counselling clinics have been considered for many years but have not been all that successful. The main barrier to offering screening to adults prior to (potential) reproduction is the lack of a universal preconceptional consultation system. However, the study of adult female population screening (on a self-pay basis) reported from Israel[182] and discussed earlier may provide valuable information in due course. No details have been published to date. Screening in the last years of schooling has been considered as an option. Wildhagen and colleagues[155] considered both a school-based screening scenario and adult preconceptional approaches in their cost, effects and savings analysis of fragile X syndrome screening and concluded that there were no purely economic obstacles to such screening strategies.

Currently, from a policy point of view, pre-conceptional testing in the low-risk population is perhaps best left to just responding to individual requests, wherever these arise in primary care. However, as the public becomes increasingly aware of genetics issues and, perhaps, is subjected to promotional pressure to buy commercial tests via the Internet, a more systematic offer of pre-conceptional screening coupled with appropriate information and genetics counselling in primary care may have to be considered. The pressure for this service will increase if prenatal screening was introduced, since the latter will be seen as leaving it 'too late' by many. For example, the House of Commons Science and Technology Committee in their report, *Human genetics: its science and consequences*, stated that: "If [antenatal] carrier screening becomes routine, serious consideration should be given to offering it outside the context of antenatal care".[316] Unlike Down's syndrome (in which it is the pregnancy that is at risk) or autosomal recessive conditions (in which it is a **particular** couple who are at risk) a woman does not have to wait for a pregnancy or even a stable relationship before discovering her fragile X syndrome risk. Should a demand for fragile X syndrome testing arise in the future, it is unlikely to be properly met by 'over-the-counter' tests. The UK ACGT made it fairly clear in their *Code of practice and guidance on human genetic testing supplied direct to the public*[304] that it considered the provision of such tests for X-linked disorders as posing more difficulties than the carrier status of autosomal recessive disorders.

Professional education
GPs
If, on average, a GP cares for 2500 individuals, then every second practice should have one affected male with fragile X syndrome. Most will be adults and will not present in the surgery except for normal medical care. There may be eight other patients with obvious learning disabilities and 60 who are slow learners. Only rarely will a mother present complaining that her child seems to be slow in development and she is likely to be referred on to a paediatrician.

For the average GP, fragile X syndrome is a rare entity and one not likely to catch attention unless, by chance, there is a diagnosed individual in the practice. Currently, more relevant and practical genetics will largely centre around late maternal age and concerns over a family history of breast cancer or early heart disease, plus haemoglobinopathy carrier testing in certain ethnic groups. Raising general awareness of the need to refer patients to the regional genetics centres when there is a strong family history of any serious and relatively rare condition will be the main educational contribution to fragile X syndrome services.

School medical officers
An educational programme is appropriate to this group, whose responsibilities include the medical examination of 'statemented' children. It raises the issue of which professional service should coordinate such a programme. An increase in diagnosis rate at school entry is already happening. It is expected that in the next cohort of children diagnosis will be made earlier, in some cases before the mother has completed her family.

Paediatricians
Fragile X syndrome is now part of the working knowledge of most paediatricians. They may not be fully conversant with the genetics and do not usually see it as part of their medical responsibility to arrange for the extended family testing. The questionnaire survey of the Fragile X Society[257] showed that of those families diagnosed by paediatricians, 22% were not referred on to a clinical geneticist, so the importance of referral remains an important message.

Adult psychiatrists or doctors working in learning disability
This group's knowledge and interest in the underlying cause of learning disability is very variable. Some have the attitude that an aetiological diagnosis makes little impact on management; the families are not asking urgent questions about cause, so why pursue the question further? Others are interested and would like to know more, particularly now that specific behavioural phenotypes are being linked to specific genetic mutations. Further liaison and communication between this group and geneticists could increase the diagnostic rate in the adult population of the mentally handicapped.

Mental handicap registers
The care of the mentally handicapped in the adult population comes under the social services departments of local authorities. Since the 1970s, the policy for the care of the handicapped has changed gradually from institutional to community care, with adults being maintained either in group homes or with their parents. In some areas, there are registers that contain considerable information about the functional level of the individual and their needs including questions on diagnosis. One register (that of NW Thames Regional

Health Authority) records a prevalence figure of 4.4/1000. Most areas now have active registers starting from the age of 14 years, and a system of compulsory assessment has been introduced at age 18 years. If this system were well maintained in all areas, it would be possible to access those families who might wish to be investigated for diagnosis not only of fragile X syndrome but also the other causes of intellectual handicap.

Key messages

- Whatever the route to the diagnosis of a full mutation or premutation, a family will need information and ongoing support from the appropriate clinical services if their needs are to be met in full. The regional genetics services will be expected to be involved.
- The genetics services for fragile X syndrome families vary greatly from region to region. Only 2 of 22 regional genetics centres have a fully established register activity for fragile X syndrome. Only 23% reported an improvement in cascade counselling services for families between 1995 and 1998, and 14% thought they had deteriorated.
- Maintaining the *status quo* will result in only a slow rise in the proportion of fragile X syndrome families identified, in variable quality of fragile X syndrome services across the UK, with a possible increasing risk of litigation because of inadequate extended family counselling or reliance on inadequate, old cytogenetic results.
- A 5-year programme of systematic case-finding among adults with learning disabilities has the potential to substantially increase the proportion of fragile X syndrome cases known to the genetics services. It would also allow better nationwide assessment of the prevalence.
- A retrospective programme of systematic case-finding in children with learning disabilities would produce a low yield.
- Screening newborn males on the basis of the Guthrie card is unlikely to be feasible at present.
- Prenatal screening is likely to pose major difficulties by generating results that are uninterpretable for those with intermediate size repeats (approximately 4%), and in the uncertainty associated with the risk in women with 55–65 CGG repeats. The complex inheritance of fragile X syndrome and uncertain risks make it difficult to ensure that consent is fully informed.
- There is a possible case for offering screening for premutations to women with POF and for advising premutation carriers of their 16% chance of the menopause occurring before the age of 40 years.
- Enhancing professional understanding of fragile X syndrome and the needs of families, both in terms of initial diagnosis and subsequent referral for genetic counselling, is probably best targeted at special school medical officers, paediatricians and those working with learning-disabled students and adults.

Chapter 10

Conclusions – implications for healthcare and recommendations for research

The broader view of screening

The NSC recognises the difficulties of applying the traditional criteria for screening[15,255] to genetic disease. Fragile X syndrome, which is associated with high genetic risks to certain extended family members of an index case, presents a particular challenge in this respect. For this reason it has proved necessary to take a broad starting point for this assessment of screening strategies for fragile X syndrome and set the discussion against the existing practice and services for fragile X syndrome families in the UK. The European Society of Human Genetics (ESHG) has recently addressed the issues relating to population genetic screening programmes in recommendations that followed from extensive consultations and a major workshop funded by the Commission of European Communities (CEE BIO4–CT98–0550); these have been published.[305] In these recommendations it is noted that:

> "...systematic case-finding followed by systematic cascade testing is intermediate between population screening and testing of high risk individuals and should also be considered according to the same criteria as population genetic screening".[305]

The conclusions from our assessment are in line with this view, and this has implications for the NSC. It will have to decide whether systematic case-finding followed by systematic cascade testing is within its remit or, if not, on whom the responsibility should fall for advising on effectiveness, quality and value in this activity.

It is relevant that, unlike in Down's syndrome and the haemoglobinopathies, for which the case for population screening is fairly well established, a significant proportion of those born with fragile X syndrome represent **recurrences** in 'known' families. This raises the possibility of a rather different but nevertheless systematic approach to meeting the needs of those at risk of an affected child.

The clinical features of fragile X syndrome are subtle and there is commonly a delay in making a specific diagnosis. It is estimated from the experience in New South Wales, Australia, and The Netherlands that, overall, less than half of the families with one or more members with fragile X syndrome would be known to regional genetics centres (who, bar commercial testing, would be the service involved in making the necessary molecular/cytogenetic diagnosis). It needs to be borne in mind when considering how to improve the rate of diagnosis (and, in turn, target help for the extended family) that the Wessex regional genetics centre has independent evidence of the collective clinical services in **that** region having ascertained over 80% of fragile X syndrome families.[225]

Systematic case-finding

Both the NSC and the ESHG recommend that before starting any population genetics screening programme, all alternative options have to be explored. Should promotion of improved systematic case-finding be contemplated, the following points need to be borne in mind. The New South Wales evidence suggests that the yield from systematic case-finding is greater in adults than in children with learning difficulties. There is, however, variation in the (minimum) figures in UK studies using DNA analysis, from 1/179 (0.56%) in total adult male residents of two institutions for those with learning disabilities in Yorkshire[302] to 11/377 (2.9%) in total adult male residents in Essex (Sabaratnam M, Ealing Hospital, Middlesex: personal communication 1997).[229] The latter study, which undertook screening **with clinical preselection** throughout the North East Essex Health District, also found a minimum prevalence of 4/116 (3.5%) in men attending two local adult training centres, 3/38 (7.9%) in boys attending schools for those with mild learning disabilities, and 4/91 (4.4%) in boys and 1/47 (2.1%) in girls attending special schools for those with severe learning disabilities. However, in Wessex, testing 3738 of 5451 boys selected from the school population because of 'special educational needs' revealed only 20 with the full mutation or 0.53% of those tested.[225]

In contrast to testing a child who has been referred for paediatric assessment at the time when learning difficulties become apparent, systematic case-finding through schools, adult training centres or institutions raises all the issues of how to make the approach socially acceptable and the test as non-invasive as possible. However, as demonstrated by studies reported in this review, it can be done. One important healthcare question resulting from systematic case-finding for fragile X syndrome is what should be done for those people who are left with no specific explanation for their learning difficulty? Does this approach commit the case-finding team to making selected referrals of the families of fragile X **negative** patients to the regional genetics service? Referrals will, almost certainly, increase if only through active requests from family members. This aspect, combined with the complicated genetics counselling needed in fragile X syndrome and the link of a programme of systematic case-finding to systematic cascade testing of the family, means that in practice a central role would have to be played by regional genetics services if such a programme were promoted.

Extended family cascade testing

As far as the cascade testing of the extended family component is concerned, this review highlights the impact of the New South Wales programme[17] but also draws attention to the simulation study by Wildhagen and colleagues.[273] They reported this approach as having a relatively poor performance for forewarning all premutation carriers in the population of their risk of an affected child. This was based on a definition of a premutation as 55–200 repeats and principally (and reasonably) presents the results in terms of ascertainment of those couples who would have had a child with fragile X syndrome. The primary goal is, of course, to ascertain all premutation carriers in order to provide counselling, including the offer of prenatal diagnosis, but the overall figures are still not very encouraging as a means of reaching all premutation carriers in the population. However, other evidence reviewed in this assessment demonstrated that the higher the risk of a woman having an affected child, the greater the chance of her having a family history detectable through cascade testing. It is this and the high absolute prior risk for maternal aunts, for example, that makes the offer of at least limited family testing a matter of good clinical practice, once a person has been diagnosed with fragile X syndrome. Despite the disappointing population

coverage in the simulation study by Wildhagen and colleagues,[273] they estimated that cascade testing needed 35- to 40-fold fewer tests to detect one carrier couple (who would have had a child with fragile X syndrome) than general population screening.

If the NSC were to consider promoting trial programmes of systematic case-finding and cascade testing, then the New South Wales experience is able to provide much of the practical information needed to plan and cost it.[17]

Comprehensive evaluation of a programme of systematic case-finding and systematic cascade counselling and testing presents quite a challenge, given that there is unlikely to be an independent measure of the prevalence of fragile X full mutations in that region and that prevalence estimates, where they exist, have wide confidence limits. Nevertheless, the rate at which new cases are added to a regional fragile X syndrome register and the proportion of such cases that belong to already known families will provide some measure of impact. If all regional genetics services were able to have at least a simple fragile X syndrome register, then the figures might well identify differences that are unlikely to be due to differences in prevalence alone. Programmes introduced into regions where there appears to be significant under-ascertainment are likely to provide the clearest evidence of impact. As far as cascade testing is concerned, the number (per index case) of females at risk offered testing, and the number of those tested who have their prior risk excluded or a full or premutation confirmed, provide one measure of performance. A further measure over time is the change in reproductive behaviour (as an indication of reproductive confidence) of those who have had their risk excluded and of those at risk who have been offered counselling, prenatal diagnosis and general support through activities coordinated by the regional genetics centre.

Population coverage from systematic case-finding and cascade testing

Any attempt to estimate the proportion of those at increased risk in the population as a whole who have been covered by the case-finding/cascade testing approach will depend first on deciding who qualifies as being 'at increased risk'. For example, should the cut-off be > 55 or > 60 CGG repeats? Some 30–60% of

premutations fall in the 55–60 range. More simulation models are needed to explore the yield at different cut-off points for the at-risk group. The estimates from simulations will, in turn, depend on more empirical data for the chance of expansion to a full mutation on transmission by females with alleles in the 55–75 range. The collation of more family-based prenatal testing data in this range is needed.

Future research on the risk factors for trans-mission expansion in the 55–60 repeats group may allow selective extension of cascade testing to the descendants of those with additional risk factors in addition to their repeat size. Beyond the above question of the repeat size cut-off point (which can be tackled in parallel), there is really no basic research required before initi-ating UK trials of systematic case-finding and extended family cascade counselling and testing. There is considerable experience available from the New South Wales programme.

Premature ovarian failure

The link between a menopause age of 40 years or POF and the premutation alleles could be explored in relation to systematic fragile X testing. About 16% of premutation carriers have POF[95] and women ascertained for POF have a 2% chance of being a premutation carrier, which can rise to 16% for familial cases.[93] However, asking women the age at which the menopause occurred in their mothers, or asking women over 40 years of age about early menopause in order to raise reproductive issues for their daughters, adds intergenerational complications to what is already a complicated matter by population screening standards.

Population screening

The major conclusion of this assessment is that any trial of population screening would face serious challenges, if it were to meet the NSC and ESHG criteria. The first is that any screening programme would need to be able to deliver one of the key benefits, namely liaison with the person who has screened positive, to offer extended family cascade counselling and coordination of support for family members at risk on an ongoing basis. The clinical work is currently the responsibility of regional genetics services and the evidence indicates that they

would need more resources to undertake a trial of population screening. If it were to be built into some other existing or new clinical service, it would face even more challenges, as new referral patterns would have to be established, and training and accreditation arrangements set in place, to name just two aspects.

The second challenge relates to how to handle the uncertainty of the meaning of alleles in the 50–60 CGG repeats range **ascertained from the general population** in terms of the genetic risk they confer on the female carrier. Modelling from family-based data shows a mismatch with modelling from total population estimates of the prevalence of the premutation and the full mutation. Our knowledge of what contributes to transmission instability beyond an association with CGG repeat length is still very limited, so modelling is simplistic. Empirical data from non-UK population screening programmes are limited to 53 pregnancies in which the mother has transmitted an allele with 50–60 repeats and none of these expanded to produce a child with fragile X syndrome. The transmission risk experience in the 55–60 repeat range is < 20 pregnancies, too few to compare meaningfully with the affected family-based estimate of approximately 9%.

A third aspect relates to the prevalence of 'as-yet-undiagnosed' female (pre)mutation carriers. This cannot be estimated easily but it is the rele-vant figure to use when assessing the cost-effective aspects of introducing population screening. What is clear is that as case-finding and cascade testing improves so the prevalence of 'as-yet-undiagnosed' (pre)mutation carriers falls. The pressures of 'good clinical practice' and the threat of litigation should lead to variable improvements in case-finding and cascade testing, although they may not. Improvements up to a uniform high standard in at least the cascade testing are a prerequisite for any population screening by the criteria of the NSC or ESHG. As soon as these improvements are put in place, the prevalence of 'as-yet-undiagnosed' (pre)mutation carriers will start to fall.

By the time that systematic case-finding in the UK is reaching a level at which there are diminishing returns, improved modelling, based on better empirical data or molecular genetics insights, may allow a fair estimate of the prevalence of 'as-yet-undiagnosed' (pre)mutation female carriers on which to base decisions about population screening programmes in the future.

Research in progress and needed in the near future

One of the current debates centres around the claim, based on the Wessex study,[153,225] that intermediate alleles of 41–60 CGG repeats have a detrimental effect on learning. If this proves to be correct it will add another layer of explanation to any population-based screening programme. It will also greatly complicate the assessment of boys with learning difficulties and the associated explanation to the parents. The physician will no longer be able to say, with confidence, that the discovery of an intermediate allele on fragile X testing is a non-contributory, coincidental finding. However, it will still be true that, in most cases, there will be another more substantial cause of a boy's learning difficulty. There is a risk that, when further investigations draw a blank, a boy's learning difficulties will tend to be put down to the inheritance of the intermediate allele by the parents, even if advised differently.

The ongoing study by Professor Jacobs' laboratory and the ALSPAC team may well provide a definite answer. ALSPAC is a cohort study of approximately 14,000 children representing about 85% of the general population in three health districts around Bristol. Enrolment was in pregnancy for those mothers with an expected date of delivery between 1.4.91 and 31.12.92 (full details of the ALSPAC study are available on their website at www.ich.bristol.ac.uk). The comprehensive prospective data collection from self-completion questionnaires, school reports and tests and medical records is supplemented by direct cognitive and behavioural assessments at a half-day ALSPAC clinic at the age of 8 years (and at approximately 2-yearly intervals thereafter). On current estimates, DNA samples should be banked on about 6000 boys by summer 2001 and the *FMR1* alleles genotyped soon after. The cognitive and psychosocial performances of the approximately 120 boys with 41–60 repeats will be compared with those of boys with < 41 repeats. The comprehensive nature of ALSPAC means that there will be the opportunity to take confounding influences into account in any analysis.

ALSPAC is unlikely to have the power to clarify whether the premutation allele (> 60–200 repeats) has an effect on cognitive and psychosocial performance, unless the effect is marked (which family-based data suggest is not the case, although a small proportion of male premutation carriers probably do have cognitive impairment).[306,307] It is worth noting that in a recent study on males

with 100–200 repeats, an unexpected five-fold **increase** in *FMR1* mRNA levels was found, despite a lower percentage than normal of FMRP-positive lymphocytes.[308] The authors concluded that mechanisms other than reduced transcription (e.g. blocks in nuclear export or translation) are responsible for the FMRP deficit and, ultimately, for the clinical involvement in some premutation carriers. If these results in males are also true of females with a premutation allele, then this might underlie the association with POF. Although mRNA levels were mildly elevated in seven males with 61–100 repeats who were tested, there were no increases in four males with intermediate alleles (41–60 repeats).

There is some work on trying to develop a reliable PCR-based test for sizing the CGG repeat in dried blood spots from Guthrie cards[309] and on further applications of the FMRP antibody, such as detection on plucked hair roots.[248] It is also possible that fluorescent *in situ* hybridisation (or FISH)-based analyses of interphase lymphocytes[310] might exploit replication differences in chromosomes with significant expansions, to distinguish low premutation/full mutation female carriers from normal homozygotes and reduce the need for Southern blotting in the future.

As far as the screening issues go, informed decisions by health policy makers and by those individuals who have to make personal reproductive decisions will depend on the accumulation of more population-based empirical data on the probability of expansion of a short premutation (> 50 or 55–60 repeats) on female transmission. The smallest expansion that would lead to the full mutation on transmission is currently being sought through a worldwide collation of family-based data on > 2000 female premutation carriers. As yet, the survey has not collected precise data on the actual number of transmissions included but it is estimated to be over 1000 (Nolin SL, Institute for Basic Research, Staten Island, New York, USA: personal communication, 2000). Two cases have been found in which the mother had 58–59 CGG repeats but none have been reported below this figure.[311] The full analysis is still awaited but these data suggest that the chance of expansion to full mutation for women with < 60 repeats is low, perhaps less than 1%. When such data is being collected as a result of an offer of genetic screening/testing to the general population and it results in a subsequent offer of prenatal diagnosis, as in the (apparently ongoing) programme in Israel,[182] it will be important to try and extract some information, if only descriptive, of the

problems encountered. For example, with the change of policy in June 1999 to offer prenatal diagnosis only to women (without a family history of MR) who carry an allele with more than 60 CGG repeats, what do they say to those who have 55–60 repeats? Do any of these women express a wish to have prenatal diagnosis regardless of the policy? A fragile X syndrome screening study of newborn boys is underway in Cuba (Oostra B, Heredero L, National Centre for Medical Genetics, Havana Medical University: personal communication, 2000), so more empirical information may become available. This research programme uses the FMRP antibody test on neonatal blood samples and, to date, about 5000 boys have been tested with no positive test results.

There is a need to gather more empirical information from a British population on the probability of expansion of a short premutation (55–75 repeats) to a full mutation on female transmission. This should ideally be within a study population in which further research can be undertaken in the longer term, as possible risk factors for expansion are revealed by ongoing research. The latter would include molecular genetics studies on animal models[144] or further family-based analysis, following up preliminary data suggestions that greater maternal age and the paternal origin of the premutations in carrier women increase the risk of expansion to a full mutation.[213] Some very large population collections of mother/child DNA pairs would be necessary. They could be generated from maternal and cord-blood samples obtained during routine obstetric practice if appropriately coordinated, both being irreversibly anonymised before analysis.

Acknowledgements

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Appendix 1

The team who carried out this assessment

From the start of this assessment the aim was to benefit directly from the practical experience of those who have been working to meet the genetic service needs of fragile X syndrome families for many years. Not only would they already know the literature and many of the authors, they could bring clinical judgement, born of long experience, to bear on some of the issues.

Original applicants

Marcus Pembrey was, until 1999, Professor of Paediatric Genetics and Head of the Mothercare Unit of Clinical Genetics and Fetal Medicine at the Institute of Child Health, University College London. He has had a research and service interest in fragile X syndrome since a collaborative mapping project in 1982. With colleagues, he helped elucidate the unusual inheritance and, in collaboration with Professor Kay Davies, has contributed to the molecular genetics. As Director (until 1996) of the main component of the North Thames (East) Regional Clinical Genetics and DNA Analysis Service, he has focused on the development of DNA testing in clinical genetics, receiving (with Cardiff and Manchester) a 5-year Special Medical Development grant from the Department of Health in 1985. He helped plan and supervise Dr Sabaratnam's evaluation of clinical selection-based screening for fragile X syndrome in North East Essex. He was a member of the Advisory Committee on Genetic Testing (1997–99).

Angela Barnicoat is a Consultant Clinical Geneticist at Great Ormond Street Hospital for Children NHS Trust. She undertook a systematic study of referral and diagnosis of fragile X syndrome patients seen at the South Thames (East) Regional Genetics Service. This work, plus collaborative studies on the delineation of FRAXE, formed the basis of her MD thesis. At Great Ormond Street Hospital and the Institute of Child Health, she remains actively involved in FRAXA and FRAXE studies and is one of four specialist advisers to the Fragile X Society.

Martin Bobrow is Professor of Medical Genetics, University of Cambridge. When at the Paediatric Research Unit (PRU) at the United Medical and Dental Schools and the South Thames (East) Regional Genetics Centre, he developed a long-standing interest in the development and evaluation of genetics services and, with large cytogenetics and DNA analysis services together at the PRU, he was in a good position to compare cytogenetic and DNA methods of diagnosis. He worked with Dr Theresa Marteau to establish the Wellcome Psychology and Genetics Research Group, who are evaluating aspects of genetic counselling relevant to fragile X syndrome. He chaired a MENCAP workshop on fragile X syndrome and co-authored their submission on fragile X syndrome screening to the Nuffield Council on Bioethics (working party on genetic screening). He was a member of the Human Genetics Advisory Commission (1997–99).

Collaborators and co-authors of the assessment

Gillian Turner is Senior Staff Specialist at the New South Wales (Newcastle and Northern) Genetics Service, Australia. She has made many fundamental observations on the contribution of X-linked mutations to MR and did pioneering research that put fragile X syndrome on the map. She is the world authority on systematic screening of intellectually impaired individuals for fragile X syndrome, the New South Wales programme having run for over 10 years. She was able to bring unique experience and data to the assessment.

Barbara Carmichael is a clinical genetics Nurse Specialist in Southend and Essex and at the Institute of Child Health, London. She has been an active member of the Fragile X Society since 1990 and lectures widely on fragile X syndrome, being able to talk from both a personal family and professional point of view.

Appendix 2

Literature and peer review

The literature review was based principally on the MEDLINE searches under various names for fragile X syndrome, including FRAXA and Martin–Bell syndrome. There were no real surprises in the 2429 references obtained, since the applicants and Dr Gillian Turner had, between them, attended every one of the Biennial International workshops on fragile X and X-linked mental retardation.

Conscious of the fact that our search might not be ideal for the psychological aspects of screening for fragile X syndrome, in February 1996 Susan Michie, at the Psychology and Genetics Research Group, UMDS, kindly performed an additional search for us as indicated below.

An assessment of screening for fragile X syndrome: the psychological dimension

Aim
To identify the likely impact of three kinds of screening:

(i)   cascade screening of families of identified fragile X syndrome individuals
(ii) screening of adult mentally handicapped people
(iii) screening of children identified as having special needs at school.

Method
A literature search was carried out on three databases: MEDLINE (1980–August 1995), PsycINFO (1984–August 1995) and BIDS, using the keyword 'fragile X' and combinations of the keywords: screening, population screening, pregnancy, testing, abortion, termination, neonatal, newborn, risk, burden, genetic counselling, families, psychological, decisions, stigma, attitudes. Three references were retrieved; these did not yield further references.

Results
No psychological outcome measures were reported in the three references identified, all of which were already known to the authors.

A reference list prepared on the psychological aspects of genetic testing by Louise Nicol-Smith, National Institute of Public Health, Oslo, Norway, for the 6th meeting on Psychosocial Aspects of Genetics, Paris, September 1998, did not include anything specifically addressing fragile X syndrome screening/testing.

Peer review

An early (and substantially different) draft of this report was discussed at a specially convened workshop at the 7th International Workshop on the Fragile X and X-linked Mental Retardation, August 1995, Tromsø, Norway. Many written comments on participants' copies as well as verbal comments were received.

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Feedback

The HTA programme and the authors would like to know your views about this report.

The Correspondence Page on the HTA website (http://www.ncchta.org) is a convenient way to publish your comments. If you prefer, you can send your comments to the address below, telling us whether you would like us to transfer them to the website.

We look forward to hearing from you.