European Journal of Human Genetics (1999) 7, 212–216
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ARTICLE

Feasibility and acceptance of screening for fragile X mutations in low-risk pregnancies

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Fragile X syndrome is the second leading cause of mental retardation after Down syndrome. Most women carriers of the fragile X mutation are unaware of their condition. We critically evaluated whether screening pregnant women at low risk for *FMR1* mutation would be feasible as a routine part of antenatal care in general practice. We also studied acceptance and attitudes to gene testing. From July 1995 until December 1996, a carrier test was offered at the Kuopio City Health Centre free of charge to all pregnant women in the first trimester following counselling given by midwives on fragile X syndrome. All women found to be carriers of *FMR1* gene mutations underwent detailed genetic counselling and were offered prenatal testing. Attitudes towards the gene test were elicited by questionnaire. Most pregnant women (85%) elected to undertake the gene test. Six women were found to be carriers (a rate of 1 in 246), and all subsequently accepted prenatal testing. Three foetuses had a normal *FMR1* gene, one had a large premutation, one a 'size mosaic' mutation pattern, and another a full mutation. This observational and interventional study demonstrates that antenatal screening provides an effective way of identifying carriers and incorporating prenatal testing into this process.

Keywords: attitude; genetic counselling; genetic screening; fragile X syndrome; prenatal diagnosis

Introduction

Fragile X syndrome (fra X) is characterised by moderate to severe mental retardation, large ears, prominent jaw, macro-orchidism, high-pitched jocular speech, and behavioural problems.[1] The underlying pathology of this syndrome is related to a lack of protein expression by the *FMR1* gene. The absence of the protein (FMRP) in the full mutation hinders development of the neuronal network, which is important for intelligence.[2]

In the normal population, the CGG repeat of the *FMR1* gene is polymorphic and varies between 6 and 55 units, with an average of 30 copies.[3] Fragile X premutations have been defined as being 55–200 CGG repeats (r) in size. Although premutations are usually not associated with any clinical phenotype, they can become full mutations when transmitted by a carrier woman.[3] Expansion of the copy number is accompanied by hypermethylation of the repeat sequence itself and its flanking region, which in turn shuts down the transcription of the gene.[4,5]

Patients with a full mutation and a premutation simultaneously are often referred to as 'size mosaics'.

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Received 16 June 1998; revised 19 October 1998; accepted 22 October 1998

This pattern is observed in 20–40% of male and 9% of female subjects.[6] Because the premutation alleles undergo normal transcription and translation,[7,8] mosaic males are capable of producing FMR1 protein in some cells. As a result, the behaviour of mosaics, as a group, is less impaired than that of non-mosaic full mutation males.[9] Another mosaic mutation group is comprised of 'methylation mosaics'. These individuals have intercellular variations for the methylation status of a full mutation. They produce a variable amount of FMR protein, and therefore a less severe or even normal phenotype can be expected.[10,11]

Affected individuals, especially younger children, need special education and training. Speech therapists or physiotherapists can help with language and motor development, and fluoxetine is an effective and relatively safe medication for treating depression and mood lability in heterozygotes and aggression in males.[12]

We have previously studied fra X families with *FMR1* mutations using cascade screening, in which a precondition for screening was a close relative with the syndrome.[13] Even if the syndrome is known in the family, information on the genetic risk may only be disseminated to a minority of those relatives at risk. Therefore, the present study focused on population-based screening of pregnant women within the general population. Although this policy might induce fear or resistance in expectant mothers, it also enables potential carriers to receive counselling and provides the option of prenatal diagnosis. This study seeks to determine whether such a policy would be feasible in general practice.

Subjects and Methods

The study was approved by the Research-Ethics Committee of the Kuopio University Hospital. All subjects gave informed oral consent before being enrolled in the study. Women with a family history of fra X seeking prenatal diagnosis were excluded from the study.

Annually, there are about 1100 deliveries in the city of Kuopio, with almost all pregnant women seeking prenatal care and being registered in antenatal clinics between the 6th and 10th weeks of pregnancy, since such registration forms a requirement for obtaining the maternity allowance provided by the state. Specially trained health care providers, mostly midwives, give counselling to all pregnant women on their first visit. The health care providers, who were trained by geneticists, underlined the voluntary nature of participation in the screening. During this visit, all women received a brochure describing fra X syndrome, and were offered an *FMR1* gene test free of charge on a strictly voluntary basis. Blood samples for gene testing were later taken at primary care centres and sent to the prenatal diagnosis unit of our hospital. The PCR test, previously described by Brown,[14] was used to analyse CGG repeat lengths. If both alleles were of the same size (only one band) or amplification failed, we employed Southern blot analysis to rule out a full mutation. PCR and selective Southern blotting were also used for prenatal diagnosis.[15,16]

In this study we refer to carriers as those women with a repeat size larger than 60, even though there might be unstable repeats between 40 and 60. All mothers with 40 or more CGG repeats underwent genetic counselling in the hospital with a geneticist. As a clinical guideline, prenatal testing was mainly offered only after 60 repeats. In cases of great maternal anxiety, we investigated the foetal *FMR1* gene even when the repeat size was less than 60. We stressed that although a CGG size ranging between 40 and 60 repeats could be within the normal range of variation, it could also be indicative of a small premutation. Parents were then allowed to decide whether to undergo prenatal testing.

After delivery, a questionnaire was sent to every woman with more than 50 repeats ($n = 18$) and to the controls. The control subjects consisted of those women receiving a normal result and who appeared on the laboratory record as having provided the three subsequent samples after each abnormal finding. All questions were based on structured question formats consisting of self-rating (questionnaire available from authors).

Results

The outcome of antenatal fra X screening along with the number of women undergoing invasive prenatal testing is shown in Figure 1. A total of 1477 women (85%) elected to undertake the gene test. Southern blot was carried out in 222 cases (15.0%) due to failure in amplification or homozygosity in the size of the repeat. Of those screened, 1416 (95.4%) had a normal *FMR1* gene, 43 (2.9%) were found to have a CGG repeat size ranging between 40 and 50 repeats, 12 women had a repeat size ranging between 50 and 60 and six had 60 repeats or more. The carrier frequency is therefore 1 in 246 in this group of women. No full mutations were detected among those screened.

Prenatal Diagnosis

In the group of 43 women having between 40 and 50 repeats, six women underwent prenatal testing. No change in the length of the repeat was found in any of the foetuses (Table 1). Prenatal testing in the group of 12 women having between 50 and 60 repeats revealed no change in the length of the repeat size in 11 of the foetuses: in seven cases, the foetus inherited the normal allele from the mother, whilst in four cases the expanded maternal allele remained constant (50–60 r). In one female foetus, a maternal repeat of 56 expanded into a premutation (76 r). In another six women antenatal screening showed a repeat size of 60 or

greater. Prenatal testing of these subjects revealed that three foetuses had a normal *FMR1* gene, one had a large premutation (100 r), one female foetus had a 'size mosaic' mutation pattern (full- and premutation) (the mother had 70 r), and one female foetus had full mutation (the mother had 90 r). All the patients decided to continue with the pregnancy.

Overall, 24 invasive tests were performed with no foetal losses. Among those women having repeats of size 50 or greater, the CGG repeat lengthened in four cases (22%): maternal 56 r to foetal 76 r; maternal 70 r to foetal 100 r, maternal 70 r to foetal size mosaic mutation and maternal 90 r to foetal full mutation. On average, the entire screening procedure, from the carrier test until final prenatal diagnosis, took three weeks to complete.

Attitudes

After the screening process, 16 of the 18 women (84%) with a repeat size greater than 50 (study group) responded to our questionnaire, whereas only 33 of the 54 controls (58%) responded. All women in the study group and 22 (67%) in the control group said that the carrier test was easy to take and that the decision to undergo testing was made mainly by the woman herself. Nobody had felt coerced by their partner, friends or by staff at health care centres. Most women in the study group (74%) and 40% of the controls would have liked to have received more information concerning the disease and the significance of carrier status. Although 12 of the study group (75%) were very anxious after receiving a positive test result, those confirmed by prenatal testing as having normal foetuses considered

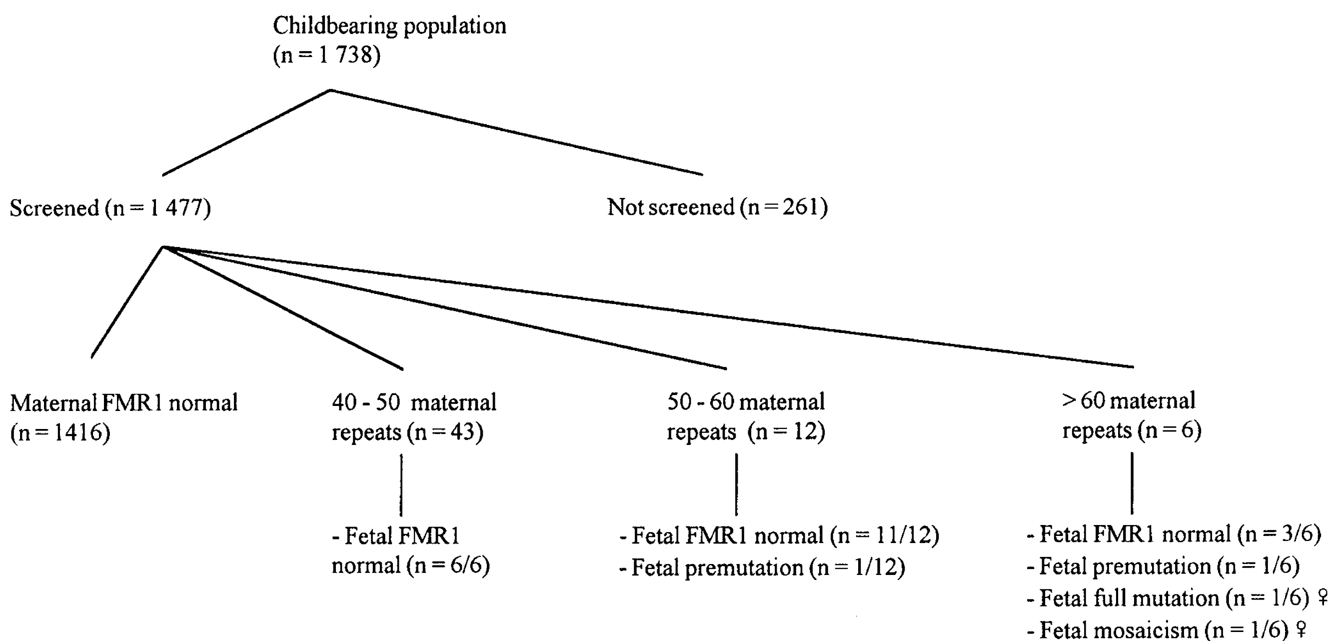

Figure 1 *Flowchart of antenatal fragile X screening.*

Table 1 Outcome of prenatal diagnosis for fragile X syndrome (*n*=24)

	Number of foetuses				
FMR1 *gene*	*Normal*	*Premutation*	*Full mutation*	*Mosaicism*	*Total*
Maternal repeats					
40–50	6	–	–	–	6
50–60	11	1	–	–	12
>60	3	1	1	1	6
Total (>40)	20	2	1	1	24

the test to have had an overall positive influence on their pregnancy. All but one of the fra X carriers intended to encourage their child-bearing friends to participate in the gene test.

Discussion

Although the mothers of retarded children in Finland say that they love their child, most also feel that they would not wish to have another child with a handicap. Therefore, many mothers hoped that antenatal care could be developed to enable detection of serious conditions earlier in pregnancy. However, for most recessive conditions we normally have no clue as to which specific prenatal diagnostics should be carried out. Today, with the progress that is being made in mapping the human genome, public health applications can be developed which will make it possible in the near future to detect carriers of many recessive gene defects. All women with mutations of the *FMR1* gene could theoretically be detected before pregnancy using an accurate and comparatively inexpensive PCR test. Carrier screening should at least be considered by those women registering for chorion villus or amniotic fluid sampling.

Preconceptual screening would allow adequate time for genetic counselling and eliminate any possible adverse consequences that might result from receiving distressing news during pregnancy. It would also offer at-risk couples more reproductive options, as well as provide sufficient time to discuss these options. However, this would be difficult to practice, since most women carriers of fra X syndrome are not only unaware of their risk (about 1:20) in each pregnancy of transmitting a full mutation to their child, but also lack any clinical sign of premutations, or are unaware of anyone in their immediate family ever having been diagnosed with fra X syndrome. Consequently, screening could constitute the only means for detecting pregnant women at risk.

Although the carrier frequency is likely to differ across populations due to a dependence on the chance occurrence of a germline mutation, the carrier frequency reported here is comparable to that found in a population of French Canadians.[17,18] It is also noteworthy that 85% of the pregnant women were willing to participate in the screening process. This may reflect mothers' confidence in Finnish maternity care, as well as the general tendency to assume that whatever care is offered has been well planned and likely to be the best possible.[19] However, this high participation rate may also reflect women's concern about the outcome of their pregnancy.

Most women in the study group (76%) were very anxious after receiving the test result, compared with only 4% of the controls. However, this anxiety was ameliorated by a subsequent normal finding on the foetal test. Much of this initial anxiety could be allayed by providing effective counselling.[20,21] Nevertheless, it is likely that some carriers may remain worried throughout their pregnancy, and may have been overlooked in this study due to our small sample size and limited questions. Greater attention to human genetics in elementary and secondary schools might provide future adults with a better understanding of the facts about genetic screening tests and encourage more informed decisions. However, despite their initial fears, afterwards most women regarded the gene test to be worthwhile and would encourage their colleagues and friends to participate in it.

This study found that the total cost of DNA testing, including PCR and selective Southern blotting, was approximately £70 000 (£45/woman). The cost (per woman) of PCR and Southern blot testing was £36 and £72, respectively. When prenatal diagnosis is also included, the total cost of detecting one full-mutated foetus rises to £34 000. In New South Wales, it was estimated that a cascade screening programme to prevent one affected birth through prenatal diagnosis would cost £8100 ($A14 200).[22] In comparison, lifetime costs for the care of an affected individual exceed £570 000–£700 000.[22,23] If avoiding the treatment costs incurred by an affected individual is seen to be a benefit for society as a whole, then screening seems economically justifiable. Furthermore, screening is necessary only in the first pregnancy, not in subsequent ones, though we should take into consideration the possibility that many carriers will probably ask for prenatal testing in all subsequent pregnancies.

Any screening protocol needs to balance the ability to detect an affected foetus against the risk of amniocentesis or chorion villus sampling. In maternal serum screening for Down syndrome, it has become widely accepted to have 50 invasive tests (amnios or CVS) to detect one case of Down syndrome. Our model of the antenatal screening process suggests that the performance is certainly comparable with trisomy screening, since it requires only three invasive tests in order to detect one full mutation case, when amnios or CVS are only offered to women with 60 or more CGG repeats.

This study shows the feasibility and acceptability of screening for fragile X mutations in low-risk pregnancies.

Acknowledgements

We wish to thank Professor Leena Peltonen, University of Helsinki, for her generous advice and support and for her kind help with the questionnaire. We also thank Dr Bernt deVries, Erasmus University, Rotterdam, for his kind encouragement and advice during this study. The study was supported by grants from the Paulo Foundation and Kuopio University Hospital, Finland.

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