DNA testing for fragile X syndrome: implications for parents and family

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Abstract

The fragile X syndrome is an X linked, semidominant mental retardation disorder caused by the amplification of a CGG repeat in the 5' UTR of the FMR1 gene. Nineteen fragile X families in which the mutated FMR1 gene segregated were evaluated. The implications of the diagnosis for the parents and family were studied through pedigree information, interviews, and questionnaires.

Information about the heredity of fragile X syndrome was only disseminated by family members to a third (124/366) of the relatives with an a priori risk of being a carrier of the fragile X syndrome. Twenty-six percent (94/366) of the relatives were tested. Transmission of information among first degree relatives seemed satisfactory but dropped off sharply with increasing distance of the genetic relationship, leaving 66% uninformed. This is particularly disadvantageous in an X linked disease. Of those subjects tested, 42% (39/94) had a premutation and 18% (17/94) had a full mutation. On average, in each family one new fragile X patient and two new carriers were found.

When people have the task of transmitting genetic information to their relatives, they usually feel responsible and capable; however, reduced acquaintance and contact with more distant relatives severely reduces the effectiveness of such transfer of information in fragile X families.

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The fragile X syndrome is a common cause of familial mental retardation with an estimated prevalence of 1/4000-1/6000 for males in western countries.[1-3] The main clinical features in males are mental retardation, macro-orchidism, and a long, narrow face with large everted ears.[4-6]

The mutation involved in this X linked disorder is characterised by the amplification of a trinucleotide (CGG) repeat in the 5' UTR of the FMR1 gene.[7 8] Normal persons have between six and 54 CGG repeats, phenotypically normal carriers of the premutation have between 52 and 200, and affected subjects have more than 200 CGG repeats in their FMR1 gene, the so called full mutation.[9] The cloning of the FMR1 gene in 1991 made determination of carrier status by DNA mutation analysis in both males and females possible.[10]

The fragile X syndrome is an X linked recessive disorder with some special features; 52-82% of the carrier females with a full mutation show mental impairment[11-13] and males can also be carriers of the premutation. These so called "normal transmitting males"[14] can transmit the premutation through phenotypically normal daughters to their grandchildren who are at risk of being affected. A diagnosis of fragile X in a mentally retarded subject will allow better support of behavioural and psychological problems related to the fragile X syndrome.[15 16] However, the diagnosis also has far reaching implications for the parents of an affected subject, such as considerations regarding future offspring. Furthermore, it may be relevant to inform relatives about the hereditary aspects of the fragile X syndrome. So far, few reports have studied the impact of the fragile X diagnosis on parents and family and the effectiveness of disclosure of information to relatives. Other studies on informing the family through relatives, such as in cystic fibrosis[17 18] or balanced chromosomal translocations,[19-21] have shown the general ineffectiveness and problems of such an approach.

We studied the implications for parents and family after a diagnosis of the fragile X syndrome. Parental adjustment, the dissemination of information in the family, and the uptake of genetic counselling or DNA testing by family members at risk for being a carrier of the fragile X syndrome are reported.

Subjects and methods

Between 1991 and 1995, 19 fragile X families were newly identified by DNA mutation analysis of the FMR1 gene and were counselled at our department by one counsellor (BdV). In all families but one the index patient was male. The ages of the index patients at the time of diagnosis varied from 3 to 57 years. All the families remained in contact with our department for at least one year. Consultands were asked to inform relatives with an a priori risk of being a carrier of the fragile X syndrome about the possibility of counselling and DNA testing. To help them in this task, they could either use a brochure on the fragile X syndrome or give the telephone number of the clinical genetic service. The relatives who contacted the genetic service were subsequently informed about the a priori risk of being a carrier before testing.

Table 1 Overview of informed and tested persons in a group of 366 relatives derived from 19 families with a risk for being carriers of fragile X syndrome

	Relation to patient with fragile X syndrome				
	1st degree (n=41)	*2nd degree (n=68)*	*3rd degree (n=97)*	*4th degree (n=160)*	*Total (n=366)*
Relatives informed	41 (100%)	40 (59%)	38 (39%)	5 (3%)	124 (34%)
Relatives tested	37 (90%)	25 (37%)	29 (30%)	3 (2%)	94 (26%)

The study was approved by the Medical Ethical Committee of Erasmus University and University Hospital Dijkzigt.

UPTAKE OF DNA TEST AND TEST RESULTS

Relatives were divided into four groups based on their relationship to the fragile X patient: first, second, third, or fourth degree relatives. In each group the number of relatives informed about the risk by one of the primary consultands was determined based on information given by the family during the counselling process. The uptake of DNA mutation analysis and its result were evaluated.

DNA ANALYSIS

Genomic DNA (8 μg) isolated from blood leucocytes[22] was digested with the restriction enzyme *Hin*dIII according to the manufacturer's instructions, separated by gel electrophoresis, and subjected to Southern blot analysis according to standard procedures.[23] The intragenic DNA probe pP2 was used for analysis of the FMR1 gene.[24] The probe was labelled by the random oligonucleotide priming method.[25] PCR analysis of the CGG repeat was performed according to Fu *et al*[26] with modifications.[27]

INTERVIEWS

The impact of the test results and genetic counselling was evaluated in interviews with the parents of newly diagnosed patients by the psychologist in the team (AT). The interview addressed issues collected from a review of published reports and our own clinical experience. The interviews were semistructured and a checklist helped to complete coverage of the following areas: personal development, coping with stressful events, experience and coping with the fragile X syndrome, and personal risk, intimate relationships, and anticipating the test outcome. The interviews took one to two hours and the first 45 minutes were audiotaped with parental consent. A panel of three of the authors (MAvR, BBAdV, AT) have listed and categorised the content of the audiotapes with regard to the presence and quality of the subject areas addressed. Only those subjects are presented which were explored and judged in all interviews.

Twenty-three parents, representing 14 families (nine couples and five single parents), were interviewed. The remaining five families were not accessible (moved to another area (n=4) or declined an interview (n=1)). The participating parents consisted of 12 mothers, all carrying the premutation, and 11 fathers.

PSYCHOLOGICAL QUESTIONNAIRES

In addition to the interviews, the parents completed a questionnaire that assessed experience with the fragile X syndrome, attitude towards the fragile X syndrome, Impact of Event Scale (IES), the Beck Hopelessness Scale (BHS), and the Hospital Anxiety and Depression Scale (HAD). One female carrier did not complete the questionnaire because it provoked intrusive, unwanted feelings and one father because of language problems.

The Impact of Event Scale (IES) is a reliable, self report scale used to measure the current degree of subjective impact experienced as a result of a specific life event (in this case, fragile X syndrome).[28] The IES estimates the influence of a stressor on two dimensions: (1) intrusion of unwanted ideas and thoughts into consciousness and (2) conscious denial-avoidance. The IES consists of seven items that form the intrusion subscale (score range 0-35) and eight items that form the denial-avoidance subscale (score range 0-40).

The Beck Hopelessness Scale consists of 20 true/false statements used to measure hopelessness or the pessimistic expectations a person has for his/her future. A score of 9 or higher (range 0-20) is indicative of depression and possible suicidal behaviour.[29 30]

The Hospital Anxiety and Depression Scale is a self report instrument for screening for clinically significant anxiety and depression, and provides a valid measure of the severity of these mood disorders. A score above 10 on either scale (score range 0-21) is indicative of severe anxiety or depression.[31]

Owing to the small sample size, the data from these questionnaires were not further analysed at a group level. However, scores on BHS and HAD were considered as indicative of psychological well being.

Results

UPTAKE OF DNA TEST AND TEST RESULTS

Up to the fourth degree, the 19 families studied consisted of 504 relatives: 251 females, 248 males, and five relatives whose sex had not been recorded. The 19 index patients, 78 relatives without any risk of inheriting the mutation, 36 mentally normal children (aged under 18), and the five relatives of unrecorded sex were excluded from further analysis.

The remaining group of 366 relatives consisted of 41 first degree, 68 second degree, 97 third degree, and 160 fourth degree relatives (table 1). All first degree relatives (41/41) were informed about their risk of being a carrier of the fragile X syndrome. In second, third, and fourth degree relatives these percentages were respectively 59% (40/68), 39% (38/97), and 3% (5/160). Overall, 34% (124/366) of the relatives were informed about their carrier risk. Almost half of the uninformed relatives (103/242) lived abroad.

Overall, 26% (94/366) of the relatives were tested for carriership. The participation was highest among first degree relatives, 90% (37/41). In second degree relatives, 37% (25/68) were tested and in third degree

Table 2 DNA test results in relatives of fragile X patients

	Relation to patient with fragile X syndrome								
	1st degree		*2nd degree*		*3rd degree*		*4th degree*		
Test result	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*Total*
Normal	6	8	8	0	11	4	1	0	38 (40%)
Premutation	14	0	13	3	4	3	2	0	39 (42%)
Full mutation	7	2	1	0	3	4	0	0	17 (18%)
Total	27	10	22	3	18	11	3	0	94

Table 3 Post-diagnosis attitude of parents towards fragile X syndrome (n=21)

	Agree	
	No	*%*
I was relieved by the DNA test result in my child	15	71
The test result has improved my relationship with my child	11	52
I am worried about the implications of the test results for the family	10	48
The family has a right to know about the inheritance of the fragile X syndrome	20	95
It is difficult for me to inform relatives about the prevalence of the fragile X syndrome in the family	5	24
I (we) feel responsible for informing other relatives about the inheritance of the fragile X syndrome	21	100
I (we) encourage relatives at risk to have themselves tested for carriership of the fragile X syndrome	20	95

relatives 30% (29/97) applied for DNA testing. In the group of fourth degree relatives three out of 160 relatives at risk were tested for carrier status.

In the group of tested relatives, 40% (38/94) had normal FMR1 genes, 42% (39/94) had the premutation, and 18% (17/94) the full mutation (11 males and six females) (table 2). In the latter group, 14 persons were mentally retarded (11 males and three females).

INTERVIEWS

Experience before test result

Retrospectively, most parents (11/14) reported underestimation of the problems of their child by health care professionals or school teachers or both and this reawakened resentment towards health care in general. The prominent feeling was, especially for mothers, that they had had to convince others that something was wrong with their child. Four participants felt abandoned by family and friends.

Before a definite diagnosis, different explanations for the problems in their child were believed: lack of oxygen (3) or brain injury after traumatic delivery (6), age over 40 years of the father (1), a disease in the mother (1), etc. The desire to learn more was counteracted by the fear of new stressful and unproductive medical procedures. Also, most medical specialists did not usually have suggestions on how to cope with the child's anxiety, panic, and behavioural problems.

The influence of the affected child on family life was pervasively strong. The most difficult decisions were about schooling or admission to an institution, and throughout there was a lack of emotional and social support in these experiences (11/14 parents). Learning to love their retarded child and its associated strong feelings made them more acutely aware of how their child would be dependent on them for life. The fear about reduced awareness of future society of the needs for the handicapped was frequently expressed (12/14 parents). One couple (both with extremely pessimistic future expectancies as measured by the BHS) hoped to survive their child. Another couple anticipated the future by developing a training programme to make their child more independent of them.

Experience after the diagnosis of the fragile X syndrome

Long years of uncertainty, differing medical opinions, and guilt feelings were ended by the diagnosis. Moreover, the limitations of the child, his restless behaviour, and anxious moods were now confirmed as the reality.

Some couples (10/14) only communicated the test results and the clinical genetics department's offer of genetic counselling to their close relatives, while others (4/14) communicated the test result to relatives and were also able to inform and support them. A few couples (2/14) were afraid to burden relatives with the information and to provoke adverse reactions. Coping with the test result and informing relatives about the diagnosis and its hereditary aspects at the same time was regarded as a problem. Some reported resentment and disapproval by relatives (5/14), while most experienced positive reactions. Parents emphasised the need for additional support in regard to disclosure of the hereditary aspects to the family.

PSYCHOLOGICAL QUESTIONNAIRES

The most important result was the expression of relief (a cause was found) in 15/21 parents (table 3). About half of the parents (11/21) stated that the diagnosis had improved their relationship with their affected child.

The impact of this diagnosis on relatives was generally acknowledged. The majority of the parents (18/21) informed close relatives within a few days of the diagnosis. The family's right to know about the genetics was generally acknowledged (20/21), including the responsibility to inform their relatives at risk (21/21) and to encourage them to be tested (20/21) (table 3). Half of the parents worried about the consequences of the result for their relatives and a quarter found it difficult to inform their relatives.

When asked, a minority (8/21) found pregnancy termination acceptable in the case of fragile X syndrome.

On the BHS, IES, and HAD questionnaires two couples and one single female carrier expressed great fear and pessimistic expectations. Both couples had BHS scores higher than 9 which indicates an increased risk of depression or possible suicidal behaviour. Three carriers had extremely high intrusion scores on the IES which reflects suffering from untoward feelings and thoughts about fragile X syndrome. Their HAD scores were higher than 10 indicating a severe depressive and anxious mood. Although the group is small, three out of 12 parents interviewed (25%) reported extreme psychological problems. No differences were observed between couples who experienced severe reactions and those who did not with respect to family structure and support.

Discussion

The diagnosis of the fragile X syndrome usually meant the end to a long period of uncertainty and anxiety among parents and disbelief by professionals. Parents were relieved and could adjust their expectations towards the affected child. However, little support was experienced, either from family or friends, or from professionals, especially for the problems associated with raising a mentally retarded child. Family life was strongly affected in different ways and nearly all parents expressed their worries about the future. Moreover, some participants reported severe psychological problems. Therefore, it is essential to pay attention to psychological support and follow up evaluation of the impact of a fragile X diagnosis.

Cascade screening within families in combination with counselling is a way to detect carriers and patients. In the present study, in more than half of the tested relatives mutations in the FMR1 gene (pre- or full mutation) were detected; however, on average, only one new fragile X patient and two additional carriers were diagnosed per index patient. The latter low yield is probably caused by the small number of people informed and tested per family. Over all, only one third of the relatives at risk were informed. However, first degree relatives were completely informed within a short period of time, but second degree and more remote relatives were not approached in the initial period after the diagnosis. More relatives might have been informed later on, but our centre did not receive later "spontaneous" requests for carrier testing in more distant relatives because of information on one of the index cases. The pattern of loose contact between relatives, migration, and influences of communication styles and family conflicts contribute to the poor transmission through the family grapevine. Such family dynamics can play an important role when dealing with a heritable disease.[32-35] Feelings like denial, blame, and guilt can interfere with good diffusion of information. Also the possible impairment of female relatives who are carriers of the full mutation and the complex genetics of the fragile X syndrome might influence the perception of the information. Nearly all parents are initially well aware of the impact of the fragile X syndrome on the family and their responsibility to transmit the information. However, the result is influenced by inability to inform or reach relatives, their perception and understanding, etc. Counselling after a genetic diagnosis should also address these family dynamic aspects of the parent's responsibility to inform relatives. Standard information procedures should be developed to assist parents in informing their relatives. Genetic associate nurses might play an important role in extending the family contacts. Besides family dynamics, the high rate of migration certainly influenced the ability of the consultands to inform their relatives.

McConkie-Russel *et al*[36] gave some useful guidelines to facilitate the disclosure of information, such as informing different branches of the family by different relatives. If the genetic counsellor takes the initiative to inform relatives at risk, more people would be able to consider genetic 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.

A different way of identifying people at risk is active screening for the fragile X syndrome among the mentally retarded, for example in special schools and institutions.[37 38] Subsequently, the fragile X diagnosis in a patient would allow genetic counselling and DNA testing in relatives.

It is most important that first degree relatives (parents and sibs) are properly informed and that they are supported in disseminating the information to other relatives. The genetic counsellor should play an important role in optimising and supervising the process of transmission of genetic information.

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