DNA testing for fragile X syndrome in school for severely emotionally handicapped children in Korea

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Though Fragile X syndrome is one of the most common inherited causes of mental retardation, it is not much detected yet in Korean population. One of the reason may be that the syndrome is not well known to the special education teachers as well as to the clinicians in this country. Thus, molecular test was undertaken to screen out fragile X syndrome in 122 children of two Korean schools for severely emotionally handicapped children. The subjects were all boys, previously known as having pervasive developmental disorder with or without mental retardation. Southern blot analysis of peripheral blood showed the abnormally enlarged (CGG)n repeat sequence associated with fragile X syndrome in two children. This finding suggests that the DNA testing for fragile X syndrome is warranted for Korean high risk population and that more concern about this syndrome is needed for the professionals who work for mentally handicapped children. The issues involved in genetic counseling for fragile X syndrome are discussed.

Keywords: fragile X syndrome, DNA screening, pervasive developmental disorder, mental retardation

INTRODUCTION

The recognition of fragile X syndrome unfolded gradually over the last decade. Now, fragile X syndrome has been recognized as one of the most common inherited causes of mental retardation, affecting one in 1,250 males and one in 2,000 females (Webb, 1991). Although phenotypic expression is variable, characteristic features include mental retardation, macro-orchidism (rare in young children), large ears and long face (seen in about 60-80% of affected males) (Laxova., 1994). Moreover affected individuals also show increased rates of psychiatric difficulties including abnormal speech and language, difficulties in social relationship and attention deficit hyperactivity disorder. Many affected individuals also show autistic features such as gaze avoidance, hand flapping, tactile defensiveness and perseveration (Merenstein *et al.*, 1996; Hagerman, 1997).

Fragile X syndrome is characterised by the associated cytogenetic abnormality; the presence of a fragile site on the X chromosome. Until recently, definitive diagnosis was dependent on cytogenetic confirmation. However, it is important to note that detection of the fragile site is not a reliable marker on carrier status. A gene, FMR-1, has now identified at the fragile X locus. Within the first exon of this gene, an unstable repetitive sequence of trinucleotide (CGG) appears to be the mutation associated with the fragile X phenotype. Normal individuals possess between 6 and 54 copies of the CGG repeats on the X chromosome. However, fragile X carriers show a 'premutation', characterised by an elongated sequence of repeats that shows a tendency to increase in size, especially when transmitted by a female. It appears that when the repeat sequence reaches a critical length, probably about 200 copies, it becomes unstable and methylated. It is the presence of this large mutation that is associated with phenotypic expression of fragile X syndrome, and it now seems that the length of the repeat sequence correlates with the cytogenetic level of fragile X expression (Turk, 1992; Laxova, 1994).

In Korea, this syndrome is not well known to the special education teachers as well as to the pediatricians and child psychiatrists. Therefore, the prevalence rate of this syndrome is not known yet in Korean population. One of the reasons why fragile X syndrome is beyond the interest of many professionals may be the fact that the molecular work on fragile X syndrome is often not available yet in

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this country.

Thus, the aims of the present study were to (1) screen for fragile X syndrome in high risk population using DNA techniques, (2) provide further tests for the relatives of the identified children, and (3) evaluate the results of genetic counseling.

SUBJECTS AND METHODS

Subjects

In this study, the subjects were children attending two schools for severely emotionally handicapped children in Seoul. The children were mainly male who were known as having pervasive developmental disorders with or without mental retardation of unknown etiology. Because fragile X syndrome is more prevalent in male than in female, it was decided to study the male students first. A letter was sent to the parents of 302 male students, providing a brief information about fragile X syndrome and requesting the permission to obtain a venous blood sample. This was granted for 122 (40.3%) children (age range 5-18).

Laboratory methods

Genomic DNA was prepared from peripheral blood leukocytes according to the methods of Gross-Bellard *et al*(1973). Southern blotting was performed according to standard techniques (Maniatis *et al.*, 1982). The following laboratory methods using the probe Ox1.9 to detect the fragile X mutation gene was according to the methods of Iida *et al.* (1994). In our experiment, DNA was digested by the restriction enzymes *Eco*RI. Ox1.9 hybridizes to a 5.1 kb *Eco*RI fragment in normal individuals. The restriction map surrounding the CpG island and the position of the probe is shown in Figure 1.

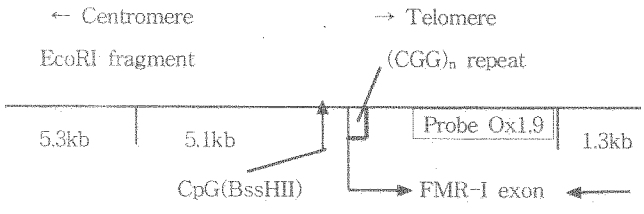

Fig. 1. Restriction map of the FMR-1 gene and the location of the probe Ox1.9. Ox1.9 is located downstream of the BssHII site. The probe detects the 5.1kb fragment in the EcoRI- digested DNA, Iida(1994).

RESULTS

Laboratory studies

From the 122 male children with pervasive developmental disorders, 2 males were detected with an expansion in the FMR-1 gene corresponding to the full mutation of the fragile X syndrome (Fig. 2 and 3).

Genetic counseling

In both cases, the mothers were informed first of the genetic disorder of their offsprings. At first, they were very despaired and then showed guilty feelings because their children's developmental disorders had been proven to be heritable.

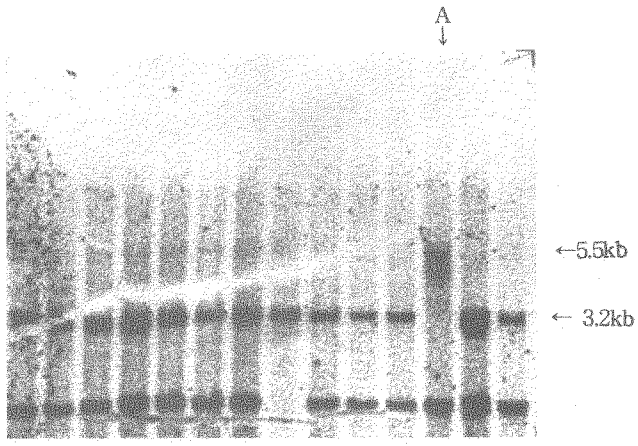

Fig. 2. Southern hybridization analysis of fragile X child(A) and control. The smear band around 5kb represents the (CGG)n expansion in the FMR-1 gene.

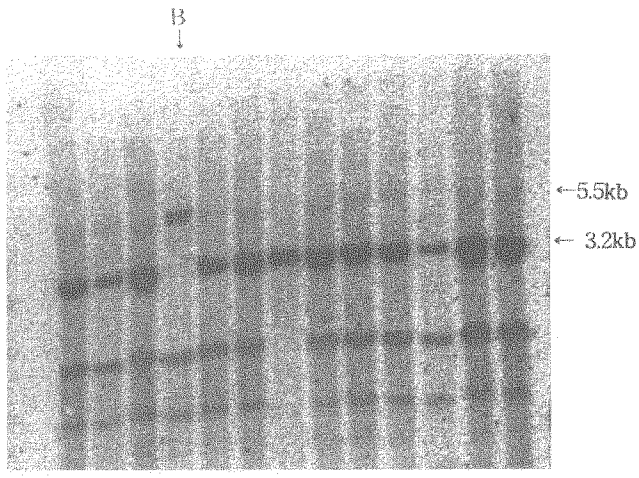

Fig. 3. Southern hybridization analysis of fragile X child(B) and control. The smear band around 5kb represents the (CGG)n expansion in the FMR-1 gene.

In addition, they were extremely worried about the situation that the results might be known to the rest of the family members. In one case the mother did not want it known even to the teachers of her child. Although repeated sessions of genetic counseling with more information and support had been conducted, the children's mothers refused to inform the results to the other family members. Thus, further DNA testing for the relatives of the identified children could not be obtained.

DISCUSSION

Fragile X syndrome is one of the leading inherited causes of mental retardation, with an estimated prevalence rate of 1 in 1,250 males and 2,000 females of general population. It was reported that about 2-7% of male and 0.3-10% of female intellectually impaired persons are positive for the fragile X syndrome (Jacobs *et al.*, 1986; Webb *et al.*, 1986a). Several reports have been published on the incidence of the fragile X marker in patients with autistic disorder. Positive results ranged from 0-53% of patients with intelligence ranging from normal to severe mental retardation (Blomquist *et al.*, 1985; Goldfine *et al.*, 1985; Fisch *et al.*, 1986; Wright *et al.*, 1986; Payton *et al.*, 1989). Thus any child or adult with autistic disorder or mental retardation of unknown cause should be considered for fragile X DNA testing (Hagerman, 1997). But, the general population prevalence studies have been restricted by the financial constraints of the relatively infrequent syndrome. Therefore, researchers have focused on institutional and school communities. Prevalence estimates based on these sources have ranged from 0.19 to 0.92 per 1,000 (Herbst and Miller, 1980; Blomquist *et al.*, 1983; Webb *et al.*, 1986a, 1991; Hagerman *et al.*, 1994; Mila *et al.*, 1997).

Our study is a preliminary study for estimating the prevalence of fragile X syndrome in Korean population. Since the school for severly emotionally handicapped children is mainly for children who have pervasive developmental disorders with or without mental retardation, the subjects could be considered as a high risk population for the fragile X syndrome. In this study, a population of 122 children attending for severly emotionally handicapped children was screened for fragile X syndrome using DNA techniques. Two new cases with full mutation were detected. The remaining 180 children were not screened, because the permission was not obtainable from their parents. The 1.6% (2 of 122) incidence of fragile X positive males in this sample of children with pervasive developmental disorders is consistent with some other previous reports (Borthwick-Duffy, 1994; Turner *et al.*, 1996).

One of the limitation of our study is that only the male subjects are included to the DNA testing. Although the children had been diagnosed as having pervasive developmental disorder once during their development, the reliability of the diagnosis should have been examined at the time of this study. Potential confounding variables in this study include the fact that the sample was biased toward severe behavior problems (attending school for severely emotionally handicapped children). Another explanation for the relatively lower incidence (1.6%) of positive fragile X subjects in this study compared to the average 7.7% found by others (Blomquist *et al.*, 1983; Goldfine *et al.*, 1985; Brown *et al*, 1986; Fisch *et al.*, 1986; Payton *et al.*, 1989) is that there is a real difference of the incidence of fragile X syndrome in Korean ethnicity.

There is an interesting response of mothers to the genetic illness to notice in this study. Through genetic counseling, information about the modes of inheritance of the fragile X syndrome was given to the mothers of the identified children. Much emphasis was placed on sharing the knowledge about the genetic illness to the rest of the family, especially to the female family members who could be pregnant eventually. However the response of the mothers was different from the expected direction. They felt very guilty and shameful to have a genetic illness in their family. In both cases, the mothers suffered from the false prejudice that genetic illnesses, especially the ones which are related to the low intelligence are inherited mainly through maternal transmission. Because the concern about the stigmatization within the family system was so great, they refused to inform the results to the other family members, even to the fathers of the children. Therefore further DNA study could not be conducted. It may be possible that both families were not psychologically healthy enough to handle this discouraging new facts and responsibilities of having a genetic illness that means additional painful experience in this society.

In summary, this study illustrates that screening of Korean special education school children for fragile X syndrome is warranted, and will identify previously undiagnosed cases. But a diagnosis of genetic disorder such as fragile X syndrome is not well accepted to mothers of the children. In Korean culture, there may be additional amount of social stigmata to have a genetic illness. This may suggest that more understanding about the culture bound illness behavior is needed to provide effective genetic counseling in Korean society.

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