Prevalence and Clinical Characteristics of Fragile X Syndrome at Child Development Clinic, Ramathibodi Hospital

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Abstract

Fragile X syndrome, the most common cause of inherited mental retardation, is an X-linked genetic disorder caused by an expanded CGG repeat in the fragile X mental retardation 1 gene. It is characterized by mental retardation, behavioral features, and physical features, such as a long face with large protruding ears and macro-orchidism. A screening for the syndrome was conducted in a representative sample of pediatric patients, who had developmental delay or mental retardation with unknown cause, at the Child Development Clinic, Ramathibodi Hospital. The DNA test was performed on all patients using PCR and southern blot techniques. Five positive cases were detected from 114 screened subjects, and more four cases confirmed among other family members. Two of five positive families initially denied a family history of mental retardation. Among 9 cases of fragile X syndrome, four had hyperactivity and two had autistic like behavior. More than half had rather a long face or prominent ears. Three boys had macro-orchidism.

Key word : Fragile X Syndrome, Mental Retardation

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Mental retardation is a common disorder with a prevalence of 2-3 per cent[1]. In order to provide proper services to the patients and their families, etiologic work-up should be appropriately performed according to pertinent signs and symptoms. Fragile X syndrome (FXS) is the second most common genetic cause of mental retardation[2]. Although Down syndrome is more prevalent than FXS, most cases of Down syndrome are not inherited[3]. Due to its heredity, diagnosis of FXS would therefore contribute to prevention of the disease.

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In Thailand, there have been two studies reported. The first case was reported by Wasant et al in 1992[4]. Jinorose et al studied in patients at Songklanakarind Hospital by using cytogenetic method and some patients were confirmed by molecular method. The prevalence among the developmentally delayed and mentally retarded children was 2.7 per cent[5]. However, in the previous screening study, there were no details of the clinical characteristics, particularly developmental-behavioral phenotypes.

The aim of our study was to establish a project screening for FXS in pediatric patients, who presented with developmental delay or mental retardation. Clinical data was collected and analyzed in order to provide some hints for early detection and to gather clinical information of Thai pediatric patients. All patients were studied by using molecular technique, which is now accepted as the standard method.

SUBJECTS AND METHOD

Studied population

The studied population included 129 children who attended the Child Development Clinic, Ramathibodi Hospital from July 1997 to December 1998. Clinical indications included globally developmental delay (at least two areas of development were delayed), mental retardation (IQ less than 70), history of being a full-term baby and no known etiology of developmental delay. Clinical characteristics, including age, sex, pedigree, developmental level, IQ, physical and behavioral features, were prospectively collected. Informed consents were required before obtaining blood samples from the children and their parents.

Genetic counseling prior to DNA testing provided information about the FXS, its inheritance and possibilities of molecular diagnosis were given to the families. If the child was positive for the syndrome, any other family members at risk were offered testing.

Laboratory method

DNA was extracted from whole blood using the phenol/chloroform method. The modified non-radioactive PCR method was used to amplify the CGG repeat region of Fragile X Mental Retardation 1, *FMR1* gene[6]. Repeat sizes were compared with known size samples. EcoRI/EagI double digestion and southern blot analysis was tested in all affected FXS individuals and suspected PCR results[7]. StB12.3 probe was labeled with Fluorescein and detected using the Gene Images CDP-Star protocol from Amersham. StB12.3 probe was supported by Dr. W. Ted Brown with Dr. L. Mandel's permission.

RESULTS

There were 129 children eligible for the study, but 15 denied to be tested. There were 114 subjects enrolled in the study, 94 boys and 20 girls. Seventy one (62.3%) children aged 6 years or less, forty three (37.7%) were more than 6 years. Table 1 shows the clinical characteristics of all subjects. The five index boys were confirmed to be FXS giving the prevalence in this group of patients as of 4.4 per cent. Since all positive cases were boys, the frequency of FXS among male patients was 5.3 per cent. Three FXS boys had an IQ in the range of severe mental retardation. Two could not be tested by a standard intelligence test due to their low functioning, which was less than half their chronological ages.

After knowing the results, siblings and other family members at risk were offered testing. Three younger male siblings and one female cousin were positive for FXS. Among three families with two positive children, their second child was not yet born by the time the parents were concerned about their first child's developmental delay. However, there was no laboratory setting available in Thailand at the time.

Table 2 shows the clinical features and results of molecular study of all 9 positive children. Six were identified as full mutation and three as mosaicism (full mutation/premutation). Five mothers were identified as carriers, four were premutation and one was full mutation. Pedigrees of two families are illustrated in Fig. 1.

All affected children, except one mosaic case, started talking after 2 years of age. Their mentally retarded cognitive functionings were not diagnosed until they were of school-age. Most cases in this study did not have typical, classical facial features of fragile X syndrome. Four had rather long faces. Among eight children whose ears were either large or prominent, only five had definite large ears by measurement. Facial features of one boy and one girl are shown in Fig. 2. Aside from common phenotypes of fragile X syndrome, one had congenital heart defect, and another one

had precocious puberty. Two children had seizure disorders.

DISCUSSION

Fragile X syndrome is the most common inherited cause of mental retardation. Its prevalence from a survey using molecular study is 1:4,000-6,000 in males[2]. The physical features include long and narrow face, prominent mandible, large and prominent ears, and macro-orchidism. The clinical symptoms, which are usually of concern when the patients are young, are developmental delay, some atypical behaviors, such as hyperactivity, inattention, autistic-like behavior, and stereotypic movement. The diagnosis of mental retardation is not easily made during preschool years[8].

This genetic syndrome is one of the triplet repeat disorders with a polymorphic CGG repeat at the 5' untranslated region of the *FMR1* gene[9,10]. The normal chromosome contains 5-55 CGG repeats. Phenotypically normal carriers with repeat sizes of 56-200 are called premutation, whose offsprings are at risk of full mutation. Affected individuals or full mutation have more than 200 repeats and absence of the *FMR1* protein, which is caused by methylation of the adjacent CpG island[11,12]. All affected children, showed expansion of the CGG (>200). Three affected boys had mosaic premutation/full mutation. This phenomenon was common as found in the previous report[13]. Mothers in all five families were premutations, except the one in family I, who was a full mutation. Many other family members have not yet been tested, although genetic counseling has already been discussed.

There have been studies worldwide, including a study in Thailand, to screen for this genetic disorder among high-risk populations. The prevalence in mentally retarded or developmentally delayed patients is 0.8-6.4 per cent[2,14-16]. The figures varied according to the inclusion criteria of each study. Since most affected children with FXS are relatively severely retarded, and the subjects in our study were more severely mentally disabled (90.4%), the positive rate in this study was then on

Table 1. Clinical characteristics of 114 patients.

Characteristic	No.	%
Sex		
male	94	82.5
female	20	17.5
Age		
less than 3 years	21	18.4
3-6 years	50	43.8
7-14 years	43	37.8
Degree of DD or MR †		
mild	11	9.6
severe	103	90.4
Family history of MR		
X-linked	14	12.3
unidentified	35	30.7
none	65	57
Physical features		
long face	35	30.7
prominent and large ears	31	27.2
macro-orchidism	5	4.39
Behavioral features		
ADHD	58	50.9
autistic-like	49	43

† Severe degree of mental retardation means IQ is less than 50, or developmental level assessed by a screening test, Denver II, is less than half of the children's chronological age level

Abbreviations : DD = developmental delay; MR = mental retardation; ADHD = attention deficit hyperactivity disorder

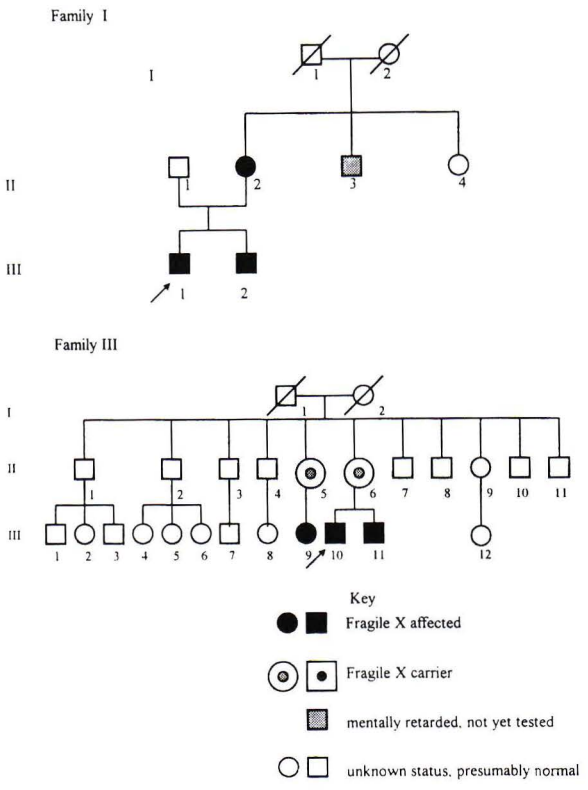
Table 2. Results of clinical features and molecular studies in all diagnosed cases.

Family	Parent	Children	Genotype	Development		Long face	Large, prominent ears	Macro-orchidism ‡	Behaviors	Other medical problems
				IQ	DA† (CA-months)					
I	mother		FM							
		son 1	PM/FM	46		±	+	+	ADHD	CHD
		son 2	FM		15 (38)	±	+	-	ADHD	
II	mother		PM							
		son 1	FM		18-36 (84)	-	+	-	autistic	seizure
		son 2	PM/FM		30-36 (52)	-	+	+		
III	mother 1		PM							
		son 1	FM	45		-	+	-	ADHD	
		son 2	FM		6-12 (15)	-	±	-		
	mother 2		PM							
		daughter	FM	47		±	-		ADHD	precocious puberty
IV	mother		PM							
		son	PM/FM	40		-	±	-		
V	mother		PM							
		son	FM	33		±	±	+	autistic/ADHD	seizure

† DA (developmental age) was estimated in months by using Denver II (a screening developmental test)

‡ Macro-orchidism is defined by using orchidometry

Abbreviations : FM = full mutation (affected); PM = premutation (carrier); DA = developmental age; CA = chronological age; ADHD = attention deficit hyperactivity disorder; CHD = congenital heart disease



the high side,4.4 per cent. The previous screening studies of FXS in an autistic population showed the prevalence of 0-2.5 per cent[17,18]. There were 49 children with some autistic behavior in this study, and the rate of positive result is 4.1 per cent. It is now concluded that the main deficit in FXS is intellectual impairment, not autistic disorder. So autistic children without mental retardation are not recommended to be routinely screened[19,20].

The typical physical features, including long and narrow face, large and prominent ears, and macro-orchidism, were not very commonly found in affected children in this report. In comparison with the study at Songklanakarind Hospital, the frequency of positive physical findings is comparable (Vasiknanonte, personal communication). The long and narrow face was the least common finding, and rather subjective. About half of the boys in both studies had macro-orchidism. Large and prominent ears were found in more than half of the cases. It is known that many phenotypes are not common in young children, and they are more commonly found at an older age[8]. Although this syndrome is X-linked dominant, three out of five families initially denied developmental delay or mental retardation in their pedigrees. Many probands, especially females,

Fig. 1. Pedigrees of the fragile X families I and III.

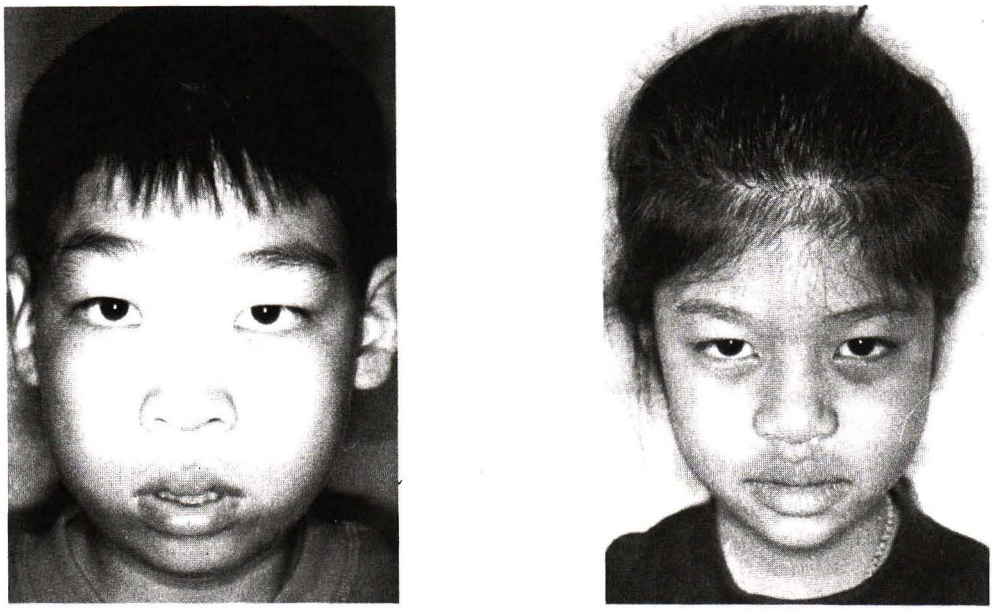


Fig. 2. Two affected children with some characteristic facial features of fragile X syndrome. Both have rather long faces, but only the boy has large, but not prominent ears.

might not be initially recognized in the pedigrees because of a mild degree of intellectual impairment[21,22].

The common presentation of most cases was cognitive deficit with an intelligence quotient (IQ) of less than 50[23]. Five out of nine affected children, whose cognitive level could be assessed by administering a standard test, had an IQ of less than 50. Other common behavioral problems such as attention deficit hyperactivity disorder (ADHD), autistic-like behaviors were also found in this group of patients with the frequency of 55.6 per cent and 22.2 per cent respectively. ADHD is positive in 54-80 per cent of reported studies[24,25]. A few large studies reported a prevalence of 16-17 per cent for autism in FXS[26,27]. Due to the uncommon physical features in younger ages, children with significant globally delayed development should be thoroughly evaluated, especially those with hyperactivity, autistic behavior, or a positive family history of mental retardation.

There were other associated findings found, both common and uncommon findings, in this report. Two boys had seizures, and were well controlled with anticonvulsant drugs. One of them, who was mentally retarded and autistic, made remarkable developmental progress after the seizures were controlled. Seizure is the most common neurological abnormality in FXS with the frequency of 17 per cent[8]. Conotruncal heart defect and precocious puberty were rare medical conditions found in two affected children. Although cardiovascular abnormalities are not uncommon in FXS; most are mild abnormalities such as mitral valve prolapse, aortic root dilatation[28,29]. The first boy (III-1) in family I had Tetralogy of Fallot with severe pulmonary stenosis. The only girl with full mutation had been diagnosed and treated for her precocious puberty a few years before being diagnosed as FXS. It is hypothesized that hypothalamic dysfunction is the cause of this endocrinological abnormality, which was previously reported in a few girls[30,31].

Mental retardation is a life-long morbidity. People with severe mental retardation are the burden of both families and society in general. Fragile X syndrome is a new genetic etiology that can be prevented by a prenatal diagnosis[32]. Knowledge of FXS and information about the availability of diagnostic techniques in Thailand are still limited among physicians.

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ความชุกและลักษณะทางคลินิกของกลุ่มอาการโครโมโซมเอ็กซ์เปราะที่หน่วยพัฒนาการเด็กโรงพยาบาลรามาธิบดี

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กลุ่มอาการโครโมโซมเอ็กซ์เปราะเป็นสาเหตุซึ่งพบบ่อยที่สุดของภาวะปัญญาอ่อนที่ถ่ายทอดทางพันธุกรรมเป็นชนิด X-linked dominant เกิดจากสาเหตุมีจำนวนซ้ำ CGG (cytosine-cytosine-guanine) มากกว่าปกติที่ตำแหน่งยีน fragile X mental retardation 1 ลักษณะทางคลินิกที่สำคัญคือ ปัญญาอ่อน มีปัญหาพฤติกรรม ความผิดปกติทางร่างกายบางอย่าง เช่น หน้ายาว หูใหญ่และกาง ขนาดอัณฑะใหญ่กว่าปกติ คณะผู้วิจัยทำการศึกษาหาความผิดปกติชนิดนี้ในผู้ป่วยเด็กที่มาตรวจที่คลินิกพัฒนาการด้วยปัญหาพัฒนาการช้าหรือปัญญาอ่อน โดยใช้การตรวจทางห้องปฏิบัติการที่เป็นมาตรฐานคือ PCR และ southern blot ผลการศึกษาพบผู้ป่วย 5 ราย จากทั้งหมด 114 ราย และตรวจพบเพิ่มอีก 4 ราย จากญาติพี่น้องผู้ป่วย ก่อนทราบผลการตรวจ 2 จาก จำนวน 5 ครอบครัวปฏิเสธปัญญาอ่อนในครอบครัว ในจำนวนที่ผิดปกติทั้งหมด 9 คน 4 คนมีพฤติกรรมชนอยู่ไม่สุข 2 คนมีลักษณะของออทิซึม มากกว่าครึ่งของเด็กทั้งหมดมีใบหน้าค่อนข้างยาวหรือหูกาง และ 3 คนมีขนาดอัณฑะใหญ่กว่าปกติ

คำสำคัญ : กลุ่มอาการโครโมโซมเอ็กซ์เปราะ, ภาวะปัญญาอ่อน

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