

Fragile X Syndrome in Korea: A Case Series and a Review of the Literature

The purposes of this study were to present DNA analysis findings of our case series of fragile X syndrome (FXS) based on methylation-specific polymerase chain reaction (MS-PCR), PCR, and Southern blotting alongside developmental characteristics including psychological profiles and to review the literature on FXS in Korea. The reports of 65 children (male:female, 52:13; age, 6.12 ± 4.00 yrs) referred for the diagnosis of FXS over a 26-months period were retrospectively reviewed for the identification of full mutation or premutation of fragile X mental retardation 1 (*FMR1*). Among the 65 children, there were 4 boys with full mutation, and one boy showed premutation of *FMR1*, yielding a 6.15% positive rate of FXS. All 4 children with full mutation showed significant developmental delay, cognitive dysfunction, and varying degrees of autistic behaviors. The boys with premutation showed also moderate mental retardation, severe drooling, and behavioral problems as severe as the boys with full mutation. Thirteen articles on FXS in Korea have been published since 1993, and they were reviewed. The positive rate of FXS was in the range of 0.77-8.51%, depending on the study groups and the method of diagnosis. Finally, the population-based prevalence study on FXS in Korea is required in the near future.

Key Words : *Fragile X Syndrome*; *FMR1*; *Fragile X Mental Retardation Protein*; *Mutation*; *Mental Retardation*

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INTRODUCTION

Fragile X syndrome (FXS) is the most common cause of inherited mental retardation and the second most common cause of genetically associated mental retardation following trisomy 21 (1). FXS is caused by a dynamic mutation which involves an unstable expansion of a trinucleotide CGG repeat at the 5'-untranslated region (UTR) of the fragile X mental retardation 1 (*FMR1*) gene, located at Xq27.3. The silencing of the *FMR1* gene leads to the deficiency of the fragile X mental retardation protein (FMRP), thus causing the classical FXS. The population-based prevalence study of full mutation ($CGG \geq 200$ repeats and cytosine methylation) of *FMR1* through DNA analysis was estimated 1/4,000 males and 1 in 8,000 females. Furthermore, there have been growing evidences of premutation (55-200 CGG repeats)-associated clinical phenotypes such as milder forms of FXS including autistic features, developmental delay and latter phenotypes of fragile X tremor ataxia syndrome, and premature ovarian failure. Considering the prevalence of full mutation and premutation as well as their consequences on children's development and their latter phenotypes, the impact of FXS is thought to be much more enormous than what has earlier been thought.

There have been growing interests worldwide on the molec-

ular diagnosis and clinical experiences on FXS. At present, polymerase chain reaction (PCR) (2), methylation-specific PCR (MS-PCR) (3), and Southern blotting (4) are considered as the golden standard for the diagnosis of FXS. Several laboratories in South Korea recently embarked on the molecular studies of FXS, thus allowing much experience on FXS.

To the best of our knowledge, reports on FXS in Korea had been relatively few, compared to those of western countries. Furthermore, there has been no report that MS-PCR was used for the diagnosis of FXS in Korea. The purposes of this study were to present DNA analysis findings of our case series of FXS based on MS-PCR, PCR, and Southern blotting alongside developmental characteristics including psychological profiles and to review the literature on FXS in Korea.

MATERIALS AND METHODS

Case series

During the period of 26 months from March 2004 to May 2006, the DNA samples of 65 children (male:female, 52:13; age, 6.12 ± 4.00 yr-old) were received at the Department of Medical Genetics, Ajou University Medical Center

for the diagnosis of FXS. The main problems suggestive of FXS were developmental delay, cognitive dysfunction, and behavioral problems.

The reports of these 65 cases were reviewed in order to identify either full mutation or premutation of *FMR1*. Full mutation for boys was diagnosed by the presence of methylated CpG island in the promoter of the *FMR1* as well as the presence of large expansions (≥ 200) of CGG repeats of *FMR1* on the MS-PCR, PCR, and Southern blotting. Premutation of *FMR1* for boys was diagnosed by the presence of 55-200 repeats of CGG in *FMR1* and unmethylated CpG island on the MS-PCR, PCR, and Southern blotting. For girls, the status of full mutation or premutation of *FMR1* was determined according to the results of PCR and Southern blotting for *FMR1*. It was based on the definition by American College of Medical Genetics in 2001 where the lower bound of the premutation range was 55 CGG repeats, irrespective of the nucleotide composition of the repeat sequence (5).

The phenotypic characteristics, such as age at diagnosis of FXS, gender, developmental characteristics, the presence of dysmorphic features, and brain MRI findings, were collected through a retrospective review of the medical charts of the children with full mutation or premutation of *FMR1*.

The MS-PCR, PCR, and Southern blotting were used for *FMR1*, and the laboratory procedures were as follows.

Methylation-specific PCR analysis

The genomic DNA was extracted by the EZ DNA methylation kit (Zymo Research, Orange, CA, U.S.A.). For PCR amplification of the CpG island located upstream of the repeats, the following primers were used: FR690F: 5'-AAC GAC GAA CCG ACG ACG-3' and FR611R: 5'-CGT CGT CGC GTT GTC GTAC-3'. The primers were designed for the modified antisense strand and were specific for the amplification of the methylated C present in affected males and in the normal female *FMR1* gene on the inactive X chromosome. PCR was modified and carried out as described previously (3).

PCR amplification for CGG expansion region of *FMR1*

Using the FXS kit (Abbott Laboratories, Abbott Park, IL, U.S.A.), amplification of the CGG repeat region of normal and premutated alleles in the *FMR1* gene was carried out by PCR with a total volume of 10 μ L, containing 20 ng of genomic DNA according to the manufacturer's protocols. The PCR-amplified products were resolved by electrophoresis on 1.5% agarose gels. To establish the fragment sizes of these products, the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.) was used. The Genescan software (Applied Biosystems) was used for accurate size calling and quantification of peak areas. The repeat number of CGG of *FMR1* that could be analyzed by the ABI PRISM 3100 Genetic Analyzer was up to 230 repeats, whereas the electrophoresis on 1.5% agarose gels was

used to identify 230-900 repeats of CGG of *FMR1*.

Southern blot analysis

Total genomic DNA was extracted from peripheral blood leukocytes by the salt-precipitation method, using Puregene kits (Gentra Systems, Inc., Minneapolis, MN, U.S.A.) and stored in a buffer, containing 10 mM Tris-HCl and 1 mM disodium EDTA, pH 8.0, at 4°C. Genomic DNA (15 μ g) was digested with both EcoR I (100 U; New England Biolabs, Ipswich, MA, U.S.A.), and methylation-sensitive enzyme Eag I (50 U; New England Biolabs). Procedures were carried out, according to the procedures described in the Biotin Luminescent Detection kit (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, U.S.A.) (4).

Review of the literature

The PubMed database (January 1969 to January 2007) and the KoreaMed (<http://www.koreamed.org/SearchBasic.php>; January 1969 to January 2007) were searched to identify Korean reports on FXS with the key words, FXS and Korea.

RESULTS

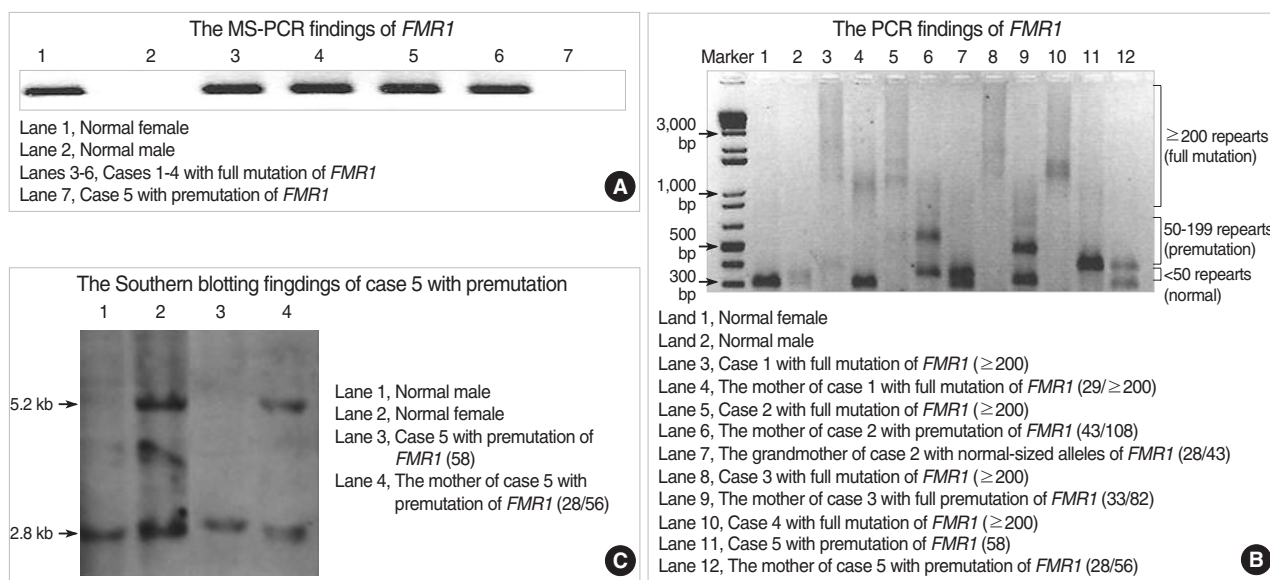
Case series

Among 65 children, who were referred for the evaluation of developmental delay, cognitive dysfunction, and behavioral problems, there were 4 boys with full mutation and one boy with premutation of *FMR1*. Table 1 shows the findings of molecular studies and clinical characteristics for 5 boys. Based on DNA analysis, this yielded a 6.15% positive rate of full mutation of *FMR1*. The mean age at the diagnosis of full mutation or premutation of *FMR1* was 4.28 yr (range, 1.2-9.9 yr old). Four boys (case 1-4) showed methylated status in the CpG island of the *FMR1* and had 200 or more CGG repeats of *FMR1* (Fig. 1). Case 1 showed methylated status on the MS-PCR. The PCR using ABI PRISM 3100 Genetic Analyzer and Genescan did not show any peak, which indicated more than 230 repeats of CGG. The electrophoresis using 1.5% agarose gels showed full mutation in the range of 200-900. Although Southern blotting could not be done, because there was not enough DNA sample left for the procedure, these findings indicated that case 1 had full mutation of *FMR1* with methylation. Case 5 did not show methylation on the MS-PCR, and the PCR analysis using ABI PRISM 3100 Genetic Analyzer and Genescan showed 58 repeats of CGG of *FMR1*, which indicated premutation according to our operant definition in which premutation of *FMR1* for boys was diagnosed by the presence of 55-200 repeats of CGG in *FMR1* and unmethylated CpG island.

Table 1. The molecular diagnostic findings and clinical characteristics of the cases with full mutation or premutation of *FMR1*

Case	Sex/ age (yr)	MS-PCR	PCR	Southern blotting	Interpretation	PCR findings of maternal <i>FMR1</i>	Birth weight (g)/ gestational period (week)	Psychological evaluation	Brain MRI
1	M/1.2	Methylated	≥230	Not done	Full mutation	29/≥230	4,250/38	MDI<50 PDI<50	NL
2	M/1.9	Methylated	≥230	≥200	Full mutation	43/108	2,900/38	MDI<50 PDI<50	NL
3	M/4.8	Methylated	≥230	≥200	Full mutation	33/82	3,300/37	VIQ 61/PIQ 63	NL
4	M/9.9	Methylated	≥230	Not done	Full mutation	Not done	3,400/40	IQ: failed SQ 41.4	NL
5	M/4.1	Unmethylated	58	50-200	Premutation	28/56	2,600/40	MDI<50 PDI<50	NL

MS-PCR, methylation-specific polymerase chain reaction; MDI PDI, the mental and psychomotor developmental indices of the Bayley scales of infant development-II; VIQ, verbal intelligence quotient; PIQ, performance intelligence quotient; SQ, social quotient; NL, normal findings.

**Fig. 1.** The molecular diagnostic findings of fragile X syndrome.

Among the 5 cases, the mothers in 4 cases (case 1, 2, 3 and 5) also underwent PCR for *FMR1*, and the findings are shown in Table 1. The mother of case 1 showed full mutation (29/≥200) of *FMR1*. Case 2 who showed full mutation of *FMR1* had his mother with premutation (43/108 repeats of CGG) of *FMR1*. His maternal grandmother had normal-sized alleles (28/43 repeats of CGG; Fig. 1-[2]-lane 7), therefore, the premutated *FMR1* of the mother of case 2 could be transmitted from her father who had died from a motor vehicle accident 10 yr ago. The premutated *FMR1* of the mother was transmitted to her son (case 2), which expanded to full mutation of *FMR1*. Case 3 with full mutation of *FMR1* had the mother with premutation (33/82 repeats of CGG). The mother of case 5 who had premutation of *FMR1* (58 repeats of CGG) showed premutation (28/56 repeats of CGG) of *FMR1*.

The clinical characteristics of 4 boys with FXS and the boy with premutation of *FMR1* are shown in Table 1. As shown in the table, they all revealed significant cognitive dysfunction. Their birth weight and gestational period were within the normal range (3,290 ± 625 g; 38.6 ± 1.34 weeks). Some dysmorphic features were recognized such as large ears, pes planovalgus, high arched palate, macroglossia, and large hands with plenty of palmar creases. Five children all showed varying degrees of autistic behaviors and significant delay in language development. Brain MRI did not show remarkable structural abnormalities in all 5 cases. The boy with premutation (case 5) showed moderate mental retardation with SQ 48.4, severe drooling, microcephaly, short attention span, and gaze avoidance.

Table 2. Review of the literature on fragile X syndrome published in Korea

Authors	Year of publication	Diagnostic methods	Study subjects	Main findings
Yim	This study	MS-PCR, PCR & Southern blotting	65 children referred for the diagnosis of fragile X syndrome	4 cases of full mutation (6.15%) and 1 case of premutation
Hur (10)	2003	PCR & Southern blotting	83 women with idiopathic premature ovarian failure	3 cases of premutation (3.61%)
Song (18)	2002	PCR	1,000 low risk women	Mean number of CGG: 26.9 repeats
Kwon (13)	2001	PCR & Southern blotting	101 children with mental retardation	1 case of full mutation (0.99%)
Kim (12)	2001	PCR & Southern blotting	Prenatal diagnosis for a mother with premutation	Fetus with full mutation
Park (16)	1999	PCR	699 neonates	3 cases with premutation (0.43%)
Kang (11)	1999	Southern blotting	66 autistic children	1 case of size mosaicism (full mutation/premutation; 1.64%) 5 cases of premutation
Hong (8)	1999	Cytogenetic study	604 child psychiatry patients	5 children with fragile X syndrome (0.83%)
Choi (6)	1999	Cytogenetic, PCR & Southern blotting	212 children with mental retardation	6 cases of full mutation (2.83%)
Hong (9)	1998	Southern blotting	122 children with PDD	2 cases of full mutation (1.64%)
Chung (7)	1997	PCR & Southern blotting	7 children	3 cases of full mutation 4 cases of premutation
Lee (14)	1995	PCR & Southern blotting	94 cases referred for diagnosis of fragile X syndrome	8 case of full mutation (8.51%) 9 cases of premutation
Shin (17)	1994	Cytogenetic study	259 children with mental retardation	2 cases of fragile X syndrome (0.77%)
Moon (15)	1993	Cytogenetic study	153 boys with mental retardation of unknown etiology	Positive for fra(X) in 10 boys (6.54%)

PCR, polymerase chain reaction; PDD, pervasive developmental disorder.

Review of the literature

Thirteen articles on FXS published in Korea since 1993 were reviewed (6-18) and its summary is presented in Table 2. The positive rate of FXS was in the range of 0.77-8.51%, depending on the study groups and the method of diagnosis (6, 8, 9, 11, 13-15, 17). The prevalence survey for 699 neonates showed 0.43% of premutation of *FMR1* (16). There was a case report of prenatal diagnosis for the fetus from the mother of known premutation of *FMR1*, which showed that the fetus had full mutation of *FMR1* (12). Song et al. reported the mean CGG repeat number (26.9 repeats) of *FMR1* in Korean women (18), and Hur et al. detected premutation of *FMR1* in 3.6% of women with idiopathic premature ovarian failure (10).

DISCUSSION

Here, we presented our case series of FXS with a review of the Korean literature on FXS. To the best of our knowledge, this is the first report on the use of MS-PCR for the diagnosis of FXS. In FXS, there are two special subclasses of mosai-

cism based on the size and methylation status. Size mosaics occur in those patients with both full mutation and premutation, a pattern that is detected in about 25% of male and in less than 10% of female patients. 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% (19, 20). Therefore, the use of MS-PCR in the molecular diagnosis of FXS should be complemented with the PCR and Southern blotting due to methylation mosaicism as well as size mosaicism of the *FMR1*.

FXS has been found in many populations throughout the world. Many studies on the prevalence of FXS were carried out in the past by cytogenetic analysis (21), and similar studies are now being undertaken using DNA analysis. Nevertheless, there is relatively a few published on DNA-based FXS screening among different ethnic groups. The prevalence of FXS, based on DNA analysis of population samples, has been reported as 1/4,000 males and 1/6,000-8,000 females in western countries (22). Crawford et al. reported a higher point estimate of FXS for African-American males (1/2,545; 95% confidence interval [CI], 1/5,208-1/1,289) than reported previously, although CI include the prevalence for Caucasians

estimated from this (1/3,717; 95% CI, 1/7,692-1/1,869) (23). With regard to the prevalence of FXS, there is always the possibility that founder effects (the founding group having an overrepresentation of relatively unstable alleles in the intermediate or premutation range) could result in some populations having a higher prevalence of FXS.

Through the molecular diagnosis, Goldman *et al.* found FXS in 6.1% of 148 mentally retarded, black South African males (24), which is similar to our result. In Asia, Arinami *et al.* reported 8.6% of the positive rate of FXS among 152 male inmates population with unknown causes of mental retardation (25). In 1995, Lee *et al.* reported 8 cases of FXS (8.5%), and 9 cases of premutation (9.6%) out of 94 cases referred for the diagnosis of FXS in Korea, using the molecular diagnosis (14). According to the literature review, although there has yet been population-based prevalence study published in Korea, the positive rate of FXS appears to be in the range of 0.77-8.51% (6, 8, 9, 11, 13-15, 17), depending on the study subjects and the diagnostic methods. Therefore, this information does not seem to allow any conclusion on the prevalence of FXS among Korean population.

In FXS, the transition from unmethylated moderate expansions of the CGG repeat (premutations) to methylated large expansions (full mutations) occurs only through maternal transmission. The timing of this transition was the subject of much speculation. Based on the observation that males with full mutation have premutation in sperm, a postzygotic transition was proposed as a preferred model. The risk of such transition is highly correlated with the size of the maternal premutation, being very low for small PM alleles (≤ 60 repeats) and 100% for alleles above 100 repeats. The American College of Medical Genetics (ACMG) in 2001 defined the lower bound of the premutation range at 55 CGG repeats, irrespective of nucleotide composition of the repeat sequence (5). The positive rate of premutation alleles >54 repeats in the general population is estimated at 1/259 females and 1/813 males (26). Smaller CGG repeats (~ 45 -54 repeats) can also be associated with repeat length instability upon transmission, with the possibility of expanding to a full mutation. It has been reported that the smallest premutation alleles that expanded to a full mutation (>200 repeats) in one generation contained 59 repeats with no AGG interruptions within the *FMR1* CGG repeat (27). In terms of premutation, besides the number of CGG repeats, the number of AGG interruptions within the CGG tract also plays a role in repeat-length stability (27). There is some evidence to indicate that the instability in both meiosis and mitosis depends critically on the length of pure CGG tracts within the 3' end of the array. The majority of *FMR1* alleles have the CGG tract interrupted by regularly interspersed AGG triplets; every 10th, 11th, or 12th CGG repeat unit. 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 keep the pure 3' CGG tract below the 34 threshold. In

vitro studies 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 (19). From the practical point of view, the distinction of normal-size alleles, intermediate-size alleles, and lower end of premutation seems to be not always clear, and it may be due to technical sensitivity as well as the presence of family history and repeat instability.

Although patients with FXS suffer from profound to borderline mental retardation, the presence of a methylated full mutation is routinely associated with their cognitive dysfunction. Nevertheless, no correlation is observed between the degree of MR and the size of the full mutation. The expansion size does not necessarily have an influence on the severity of the clinical phenotype in the males, presumably because hypermethylation of the *FMR1* gene at a particular repeat-size threshold produces an 'all or none' effect. To the best of our knowledge, it is not clear whether the normal size alleles of *FMR1* can produce functional FMRP or not, in the mosaics of full mutation/normal size alleles of *FMR1*. Further study on the assessment of FMRP level is required. In our case series of FXS, there was one mother with full mutation and 3 mothers with premutation of *FMR1*. Although the psychological evaluations for those mothers were not done, these mothers were regular high school or college graduates and denied any learning problems. Case 3 reported to have three maternal cousins with mental retardation. It is generally known that about 50% of heterozygous females with full mutation have some degree of learning difficulties due to inactivation of one of the X chromosomes (19).

In our case series, there was one boy with premutation who showed severe clinical presentations indistinguishable from boys with full mutation. He had premutation with 58 CGG repeats, which was thought to be relatively low copy number. Besides the clinical effects of premutation in adults such as premature ovarian failure and fragile-X associated tremor ataxia syndrome (FXTAS), several studies show the presence of neurobehavioral symptoms in children (28). Esch identified several boys with premutation with learning problems, developmental delay and/or autistic features, and she identified four times more premutations in the group of boys younger than 16 yr old with developmental problems who were referred for *FMR1* testing. Although larger and more longitudinal standardized studies are needed, these data provide further evidence that fragile X premutations may affect neurocognitive and behavioral functioning in children (29). Tassone *et al.* found lowered FMRP levels in some children with premutation who presented with mental retardation and/or autism, suggesting a spectrum of clinical severity associated with related protein deficit (30). Similar observation has also been reported in 5 children with diagnosis of autistic spectrum disorder and premutation (31). However, whether his clinical features including mental retardation

and behavioral problems were related with his premutation of *FMR1* or not is inconclusive at this point.

In our case series, the mean age at the diagnosis of FXS or premutation of *FMR1* was 4.28 yr (range; 1.2-9.9 yr old). Most of Down syndrome cases are identified at birth on the basis of physical features; however, FXS is not at all obvious at birth. Usually, parents begin to notice behavioral problems or delayed attainment of developmental milestones. Sometimes, these signs are subtle and it may take a long time for a physician or other professional to acknowledge that a problem exists. Early identification of FXS remains important not only for genetic counseling and prenatal testing, but also for parents and children to explain their difficulties, thereby facilitating the access to educational and clinical services.

In our case series, brain MRI findings were not remarkable. Although brain weight and gross anatomy in FXS have been reported to be normal, there is, however, mounting evidence of morphological abnormalities of the brain in the FXS. Several structural MRI studies have reported reduced cerebellar vermis size and enlarged fourth ventricle in FXS individuals. An increase in caudate nucleus volume has been found in children with FXS (32, 33).

Several limitations are noted in our current study, mainly due to its retrospective nature. Regardless of these limitations, however, it is believed that this case series might be able to add some valuable information to the body of knowledge of FXS in Korea.

In summary, we presented four cases of FXS and one case of premutation with their clinical characteristics with a review of the literature.

REFERENCES

- Phalen JA. Fragile X syndrome. *Pediatr Rev* 2005; 26: 181-2.
- O'Connell CD, Atha DH, Jakupciak JP, Amos JA, Richie K. Standardization of PCR amplification for fragile X trinucleotide repeat measurements. *Clin Genet* 2002; 61: 13-20.
- Panagopoulos I, Lassen C, Kristoffersson U, Aman P. A methylation PCR approach for detection of fragile X syndrome. *Hum Mutat* 1999; 14: 71-9.
- Gold B, Radu D, Balanko A, Chiang CS. Diagnosis of Fragile X syndrome by Southern blot hybridization using a chemiluminescent probe: a laboratory protocol. *Mol Diagn* 2000; 5: 169-78.
- Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, Hirsch B, Jacky P, McDowell GA, Popovich B, Watson M, Wolff DJ. Technical standards and guidelines for fragile X: the first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. *Genet Med* 2001; 3: 200-5.
- Choi YM, Hwang DY, Jun JK, Choe J, Park SH, Noh MK, Oh SK, Ku SY, Suh CS, Kim SH, Yang SW, Cho SC, Moon SY, Lee JY. Incidence of Fragile X Syndrome in Korean Patients with Mental Retardation. *Korean J Obstet Gynecol* 1999; 42: 2458-64.
- Chung HJ, Cha KE, Lee SH. Fragile X Syndrome : Clinical Characteristics and EEG Findings. *J Korean Pediatr Soc* 1997; 40: 1110-9.
- Hong KM, Kim JH, Moon SY, Oh SK. Chromosomal abnormalities in child psychiatric patients. *J Korean Med Sci* 1999; 14: 377-85.
- Hong SD, Lee S, Oh MR, Jin DK. DNA Testing for Fragile X Syndrome in School for Emotionally Severely Handicapped Children in Korea. *J Genet Med* 1998; 2: 83-6.
- Hur CY, Choi YM, Park SH, Yoon BK, Lee KS, Na YJ, Lee BS, Rheu CH, Lee HJ, Seol HW, Oh SK, Ku SY, Suh CS, Kim SH, Kim JG, Moon SY. Fragile X Premutation in Patients with Idiopathic Premature Ovarian Failure. *Korean J Obstet Gynecol* 2003; 46: 978-83.
- Kang KM, Kwak DI, Lee MS. Molecular Genetic Study for *FMR1* Gene in Autistic Children. *J Korean Neuropsychiatr Assoc* 1999; 38: 1479-87.
- Kim GJ, Kim SY, Hwang BC, Park CY, Choi YD, Whang YJ. Prenatal Diagnosis of Fragile X Syndrome using Amniotic Fluid DNA. *Korean J Obstet Gynecol* 2001; 44: 558-65.
- Kwon SH, Lee KS, Hyun MC, Song KE, Kim JK. Molecular screening for fragile x syndrome in mentally handicapped children in Korea. *J Korean Med Sci* 2001; 16: 271-5.
- Lee SH, Kim UK, Kwak IP, Kim JW, Lee WS, Kim SB, Cha KY. Molecular Genetics in Fragile X syndrome (1). *Korean J Obstet Gynecol* 1995; 38: 2293-302.
- Moon HR, Moon SY. Fragile site X chromosomes in mentally retarded boys. *J Korean Med Sci* 1993; 8: 192-6.
- Park W, Lee KJ, Choi EY. A Study on Prevalence of Premutation Sized *FMR1* Gene Using Polymerase Chain Reaction. *J Korean Child Neurol Soc* 1999; 7: 42-7.
- Shin SK, Yoo HW. Etiological Classification of Mentally Retarded Children Enrolled in a Special Educational Institution. *J Korean Pediatr Soc* 1994; 37: 1437-48.
- Song KC, Kim GJ, Whang YJ, Choi SR, Lee SP, Whang BC, Lee ED. Allele distribution of *FMR1* gene in Korean women. *Korean J Obstet Gynecol* 2002; 45: 990-3.
- Pembrey ME, Barnicoat AJ, Carmichael B, Bobrow M, Turner G. An assessment of screening strategies for fragile X syndrome in the UK. *Health Technol Assess* 2001; 5: 1-95.
- Han XD, Powell BR, Phalin JL, Chehab FF. Mosaicism for a full mutation, premutation, and deletion of the CGG repeats results in 22% *FMRP* and elevated *FMR1* mRNA levels in a high-functioning fragile X male. *Am J Med Genet A* 2006; 140: 1463-71.
- Sabaratham M, Laver S, Butler L, Pembrey M. Fragile-X syndrome in North East Essex: towards systematic screening: clinical selection. *J Intellect Disabil Res* 1994; 38 (Pt 1): 27-35.
- Turner G, Webb T, Wake S, Robinson H. Prevalence of fragile X syndrome. *Am J Med Genet* 1996; 64: 196-7.
- Crawford DC, Meadows KL, Newman JL, Taft LF, Scott E, Leslie M, Shubek L, Holmgreen P, Yeargin-Allsopp M, Boyle C, Sherman SL. Prevalence of the fragile X syndrome in African-Americans. *Am J Med Genet* 2002; 110: 226-33.
- Goldman A, Jenkins T, Krause A. Molecular evidence that fragile X syndrome occurs in the South African black population. *J Med*

- Genet* 1998; 35: 878.
25. Arinami T, Kondo I, Nakajima S. *Frequency of the fragile X syndrome in Japanese mentally retarded males. Hum Genet* 1986; 73: 309-12.
 26. Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. *Prevalence of carriers of premutation-size alleles of the FMR1 gene--and implications for the population genetics of the fragile X syndrome. Am J Hum Genet* 1995; 57: 1006-18.
 27. Nolin SL, Brown WT, Glicksman A, Houck GE Jr, Gargano AD, Sullivan A, Biancalana V, Brondum-Nielsen K, Hjalgrim H, Holinski-Feder E, Kooy F, Longshore J, Macpherson J, Mandel JL, Matthijs G, Rousseau F, Steinbach P, Vaisanen ML, von Koskull H, Sherman SL. *Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. Am J Hum Genet* 2003; 72: 454-64.
 28. Moore CJ, Daly EM, Schmitz N, Tassone F, Tysoe C, Hagerman RJ, Hagerman PJ, Morris RG, Murphy KC, Murphy DG. *A neuropsychological investigation of male premutation carriers of fragile X syndrome. Neuropsychologia* 2004; 42: 1934-47.
 29. Van Esch H. *The Fragile X premutation: new insights and clinical consequences. Eur J Med Genet* 2006; 49: 1-8.
 30. Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, Hagerman PJ. *Clinical involvement and protein expression in individuals with the FMR1 premutation. Am J Med Genet* 2000; 91: 144-52.
 31. Aziz M, Stathopulu E, Callias M, Taylor C, Turk J, Oostra B, Willemssen R, Patton M. *Clinical features of boys with fragile X premutations and intermediate alleles. Am J Med Genet B Neuropsychiatr Genet* 2003; 121: 119-27.
 32. Koukoui SD, Chaudhuri A. *Neuroanatomical, molecular genetic, and behavioral correlates of fragile X syndrome. Brain Res Rev* 2007; 53: 27-38.
 33. Lombroso PJ. *Genetics of childhood disorders: XLVIII. Learning and memory, Part 1: Fragile X syndrome update. J Am Acad Child Adolesc Psychiatry* 2003; 42: 372-5.