Robust Screening and Cascade Testing for Fragile X Expansions in a Large Multigenerational Family Identify Many Affected Individuals: An Experience in the Remote Area of Indonesia

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Abstract: Fragile X Syndrome (FXS) is the most common known inherited form of intellectual disability (ID), caused by a CGG repeat expansion of *the FMR1* gene. The aimed of the study was to screen *FMR1* mutation among the ID population followed by cascade testing in a remote area. A PCR-based method was used to screen *FMR1* expanded alleles using dried blood spot cards in Flores Island, one of the very remote areas in East Indonesia. The screening included 130 males and 81 females from three schools of children with ID. The screening identified three individuals with expanded alleles including two full mutation males and one premutation male. No expanded allele was detected in females. A second blood sample for confirmatory diagnosis was done using Southern blot. Cascade testing in a remote area of Indonesia found a multigenerational family with a large number of cases with FXS. FXS screening of ID populations followed by cascade testing in positive FXS family in a remote area with challenging accessibility is recommended.

Keywords: Dried blood spot testing, screening, fragile X syndrome.

INTRODUCTION

Fragile X Syndrome (FXS) is the most common inherited cause of intellectual disability (ID) and the most common single-gene disorder associated with Autism Spectrum Disorder (ASD). FXS is caused by a mutation in the Fragile X Mental Retardation 1 (*FMR1*) gene located on the X long arm of chromosome X at position Xq27.3 [1, 2]. A CGG (Cytosine-Guanine-Guanine) repeats is located in the promoter of the *FMR1* gene; normal individual have approximately 5 to 44 CGG repeats, whereas premutation alleles have 55-200 repeats and full mutation alleles have more than 200 CGG repeats associated with methylation that silences the gene so neither *FMR1* mRNA or *FMR1* protein, FMRP, are produced. Alleles with 45-54 CGG repeat are classified as 'grey zone' where instability is observed, and expansion to a full mutation can occur over 2 generations [3, 4]. An AGG interruption after every 10 or so CGG repeats leads to more stability in the CGG repeat when it is passed on by a woman with the premutation to the next generation [5, 6]. The absence of FMRP leads to FXS characterized by moderate to severe ID which is accompanied by physical features including the long face, prominent ears, macroorchidism, and behavioural features such as hyperactivity, shyness, irritability aggression and perseveration [7].

The spectrum of involvement in FXS and the *FMR1*-associated disorders is very broad and in premutation individuals, this spectrum includes fragile X-associated tremor ataxia syndrome (FXTAS), fragile X- associated primary ovarian insufficiency (FXPOI), immune disorders and emotional difficulties [8-13].

The prevalence for both males and females varies in different populations being about 1 in 2600-5000 for the full mutation and 1 in 130-800 for premutation alleles [14, 15]. The prevalence of FXS was found to be similar in a female and male cohort of the Indonesian ID population (1.7-1.9%) [16, 17]. In ID population from Central Java, Indonesia, a study reported 1.7-1.9% prevalence of the full mutation [16, 18, 19].

Indonesia comprises 17,000 islands with a population of 237.6 million people in the archipelago from Sabang to Merauke (west to east). The most densely populated area is Java island where 58% of

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the Indonesian population lives [20]. Our previous screening study was performed on Java island, which has access to health facilities [21]. However, approximately 40% of the Indonesian population lives in remote areas including Flores island where access to health services is quite limited. The conventional technique for diagnosis of FXS, a combination of Polymerase Chain Reaction (PCR) and Southern Blot testing is time-consuming, laborious, expensive, and complicated. A simple screening PCR-based assay to diagnose FXS has already been established [22, 23] and applied in several studies across many countries [4, 21, 23-25]. This is the first screening of *FMR1* CGG expansions in Indonesia outside Java island using blood spots as a first-line test. This study was aimed to screen for *FMR1* CGG expansions in the ID population to identify new FXS cases through cascade testing in a remote area.

MATERIALS AND METHODS

Blood spots were collected from 211 children (130 males and 81 females) from three schools for children with ID in Sikka, Ngada and Ende District of Flores Island, East Nusa Tenggara Province, Indonesia and were screened for the presence of an *FMR1* expanded allele. Informed consent was obtained from the parent or legal guardian and the study was approved by the Institutional Review Board (IRB) of the MIND Institute, UC Davis, California, USA (IRB 200311677-9) and Faculty of Medicine, Diponegoro University, Semarang, Indonesia (85/EC/FK/RSDK/2008). If an expanded allele was identified, family members who agreed to participate in the study and who signed a consent form were screened.

Finger-prick blood sampling was collected on FTA cards (Whatman, Inc). The FTA cards were shipped to the UC Davis MIND Institute where DNA was isolated from a 2 mm blood spot disk using the QIAxtractor Reagent Pack (Qiagen, Valencia, CA) on the QIAxtractor (Qiagen, Valencia CA) following the manufacturer instructions. Details are as described in Tassone *et al*. [23].

The blood spot PCR screening approach performed on the isolated DNA was as follows: first-round PCR screening was used to size normal an expanded using c and f primers (by Fast Start approach, Roche Diagnostics, Indianapolis, IN). Male samples with no band on the first-round or female samples with a single band underwent a second PCR screening assay using a CGG chimeric primer [22, 26]. Details are as described in Tassone *et al*. [23]. The PCR products were visualized using the ABI 3730 Capillary Electrophoresis (CE) Genetic Analyzer (Applied Biosystem, Foster City, CA). Results from the CE were analyzed via ABI Peak Scanner software (Applied Biosystems, Foster City, CA) [23]. Using the CGG-chimeric primer, serial peaks were visualized on CE when an expanded allele was present.

From the individuals identified with an expanded allele, 3 ml peripheral blood vein using EDTA tube was subsequently collected and DNA was isolated using the salting-out method in Center for Biomedical Research (CEBIOR), Faculty of Medicine Diponegoro University, Indonesia. DNA was shipped to the UC Davis MIND Institute to confirm the diagnosis. In all cases, expanded alleles identified through the screening were confirmed by standard *FMR1* diagnostic testing using a combination of Southern blot and PCR analysis as previously described [22, 27].

RESULTS

The CGG screening conducted in this study followed the workflow previously described in Tassone *et al*. [22] and identified two boys with full mutation and one boy with a premutation allele from 211 special need individuals (aged range 6-10 years) of three ID schools (Figure **1**). Cascade testing was performed in the family of the boys with expanded alleles.

Patient 1, a seven-year-old boy with a premutation of 77 CGG repeats was identified from the Sikka District. The child presented with a learning disability and some behaviour impairments such as shyness, irritability, and anxiety observed during the clinical and medical examination.

Patient 2, a 10-year-old boy with a full mutation allele of 300 CGG repeats was identified from Ende District. Cascade testing of the family was performed only in two family members. His mother was found to be a premutation carrier with normal and premutation alleles of 29 and 98 CGG repeats respectively and his younger sister had a normal allele of 30 CGG repeats and a full mutation allele of 620 CGG repeats.

Patient 3, from the Ngada District, was a seven-year-old boy full mutation size mosaic with full mutation alleles of 400, 510, 610 CGG repeats and a premutation allele of 88 CGG repeats. Cascade testing of the family of this full mutation boy led to the characterization and the testing of extended family

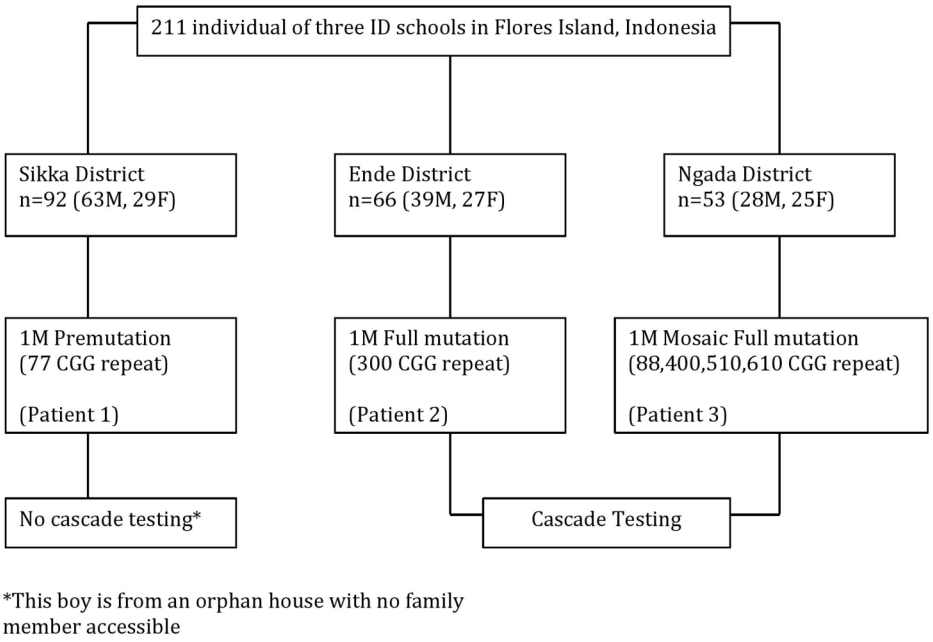

Figure 1: Flowchart of blood spot screening from special schools in Flores Islands, Indonesia. (n= number of subjects; M=Male; F=Female).

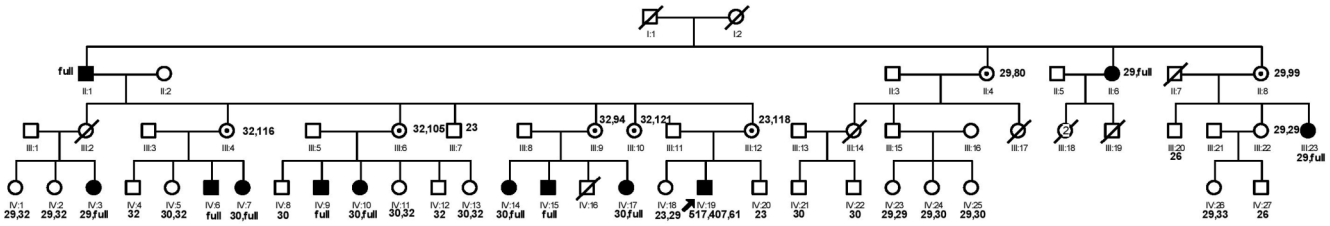

Figure 2: A pedigree of family 3 demonstrating the multigenerational family identifies with Fragile X CGG repeat number.

members from three generations. Forty-six blood spots were collected from family members and nineteen expanded alleles (including proband) were identified (Figure **2**).

Peripheral blood samples were collected to confirm the presence of *FMR1* expanded alleles and DNA testing using Southern Blot and PCR analysis and confirmed the presence of an expanded allele in all 19 individuals including. DNA testing identified 10 full mutation individuals (6 females, 4 males) and 8 females and 1 male with a premutation allele. Results for Southern blot analysis are shown in Figure **3**.

DISCUSSION

The ID is the highest risk group for population-based screening for *FMR1* gene mutations. The identification of a mosaic full mutation boy (patient 3), accompanied by cascade testing, identified a large number of individuals with FXS who were not in the school that we screened but they were members of a large multigenerational family. Such large families are common in Indonesia and many other developing countries. The oldest ancestors tested in this family included one male premutation carrier (CGG= 134 (II:1) (see Figure **3** lane 17) and three premutation females. Among nine mother-offspring pairings, the smallest allele that expanded to a full mutation allele contained 94 CGG repeat (III:9). From II:4, a premutation individual of 80 CGG repeats, the screening was performed only from one out of three offspring and no expanded allele was observed. Female premutation carriers are at risk to have offspring with FXS, with an allele of 56 CGG repeat with no AGG interruptions, being the smallest, so far reported, to expand to a full mutation [28]. It has been suggested that the presence of AGG interruptions at positions 10 and 20 in the normal population [5] within the maternal *FMR1* allele plays a crucial role in maintaining the stability of the repeat and can better predict the risk of expansion risk during transmission from a premutation mother to a full mutation allele in the offspring [6]. Cascade testing can

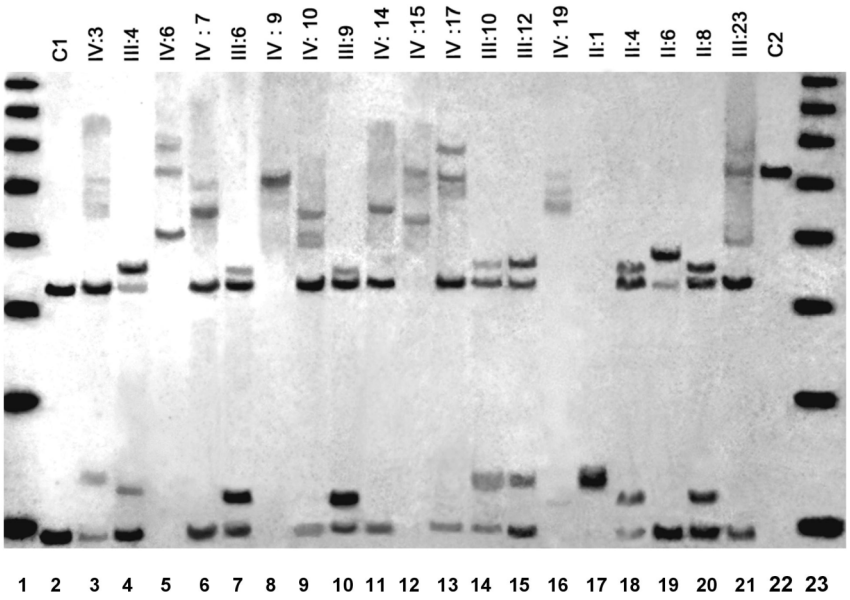

Figure 3: Southern Blot result of individuals with an expanded allele in family 3.

Diagnosis of FXS was confirmed by Southern Blot analysis in nineteen subjects identified through the blood spot screening. DNA was digested with Eco RI and Nru I and StB 12.3 probes was used [22]. DNA size marker (1 Kb) is shown in lane 1 and lane 23.

Lane 2: normal female control showing a normal unmethylated band (2.8Kb) and normal methylated band (5.2Kb).

Full mutation females: IV-3, IV-7, IV-10, IV-14, IV-17, III-23.

Full mutation males: IV-6, IV-9, IV-15 and IV-19.

Premutation females: III-4, III-6, III-9, III-10, III-12, II-4, II-6, II-8.

Male premutation: II-1.

Lane 22: full mutation male control.

bring some benefits to the families including access to services and to targeted treatments for children with FXS [29], reproductive options to the carrier women and knowledge of carrier status may lead to lifestyle changes and treatments to reduce the risk of late neurodegenerative or psychopathological disorders in carriers [25, 30]. In addition, the social and emotional impact of screening in a given culture must be considered [31].

Premutation carriers are more common than those with the full mutation and carriers can sometimes demonstrate ID, ASD or learning problems including ADHD [32, 33]. Premutation carriers can also demonstrate other medical or neurological problems including FXTAS, FXPOI, in addition to FXS-like physical, behavioural and cognitive effects, alterations in brain function [34] and neurological, immunological and psychiatric issues [12, 32, 35-40].

During the screening, a boy with learning and behavioural disabilities found had a premutation allele of 77 CGG repeats (patient 1), similar to previous reports of premutation developmental problems [41, 42]. Although those with premutation are usually intellectually and behaviorally unaffected, a subgroup of children experience attention-deficit hyperactivity disorder (ADHD), anxiety, autism spectrum disorder (ASD), seizures, learning difficulties or even ID [32, 35, 37, 42-46]. A mild to a moderate deficit of FMRP can occur in some premutation carriers, although those with the premutation are at greater risk for environmental toxicity or they may also experience a second genetic hit leading to their more involved phenotype [33, 43, 47-50]. On the contrary, Myers *et al*. found that premutation children were not having developmental problems [51].

This finding shows the role of *FMR1* gene mutation screening in the ID population is very crucial, particularly in countries with a lack of awareness of genetic cause among healthcare provider and government. The etiological assessment is essential to tailor treatment, discuss the prognosis, calculate the recurrence risk, and avoid unnecessary testing, thus leads the opportunity to improve health and functional

outcomes. This screening approach could be implemented in a remote area of Indonesia, the largest archipelagic country, to identify new cases of FXS. Consequently, better prevention (reproductive options, reducing the risk of late-onset of the neuropsychiatric problem among premutation carriers), early intervention (including targeted treatments among FXS), and long-term clinical follow-up can be done among identified individuals. It is indeed extremely important that long-term clinical follow-up is offered to individuals who test positive for *FMR1* mutations and to the extended family members identified as results of subsequent cascade testing. *The limitation of the study*. This study was a focus on a genotyping screening and so behavioural and emotional abnormalities are not specifically reported.

In conclusion, blood spot sampling is a suitable sampling method to screen *FMR1* gene mutation of the ID population in a remote area, where the facilities, knowledge, awareness are lacking, and accessibility is a major problem. Furthermore, cascade testing has been proved to enable us to identify more cases.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

ABBREVIATIONS

ADHD = Attention-Deficit Hyperactivity Disorder

ASD = Autism Spectrum Disorder

CE = Capillary Electrophoresis

CEBIOR = Center for Biomedical Research

CGG = Cytosine-Guanine-Guanine

FMR1 = Fragile X Mental Retardation 1

FMRP = Fragile X Mental Retardation Protein

FXPOI = fragile X- associated primary ovarian insufficiency

FXS = Fragile X Syndrome

FXTAS = fragile X-associated tremor ataxia syndrome

ID = intellectual disability

IRB = Institutional Review Board

PCR = Polymerase Chain Reaction

REFERENCES

[1] Fu Y-H, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, *et al*. Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the Sherman paradox. Cell 1991; 67(6): 1047-1058. https://doi.org/10.1016/0092-8674(91)90283-5

[2] Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, *et al*. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 1991; 65(5): 905-914. https://doi.org/10.1016/0092-8674(91)90397-H

[3] Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, *et al*. 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(3): 200-205. https://doi.org/10.1097/00125817-200105000-00010

[4] Fernandez-Carvajal I, Walichiewicz P, Xiaosen X, Pan R, Hagerman PJ, Tassone F. Screening for expanded alleles of the FMR1 gene in blood spots from newborn males in a Spanish population. J Mol Diagn 2009; 11(4): 324-329. https://doi.org/10.2353/jmoldx.2009.080173

[5] Eichler EE, Holden JJ, Popovich BW, Reiss AL, Snow K, Thibodeau SN, *et al*. Length of uninterrupted CGG repeats determines instability in the FMR1 gene. Nat Genet 1994; 8(1): 88-94. https://doi.org/10.1038/ng0994-88

[6] Yrigollen CM, Durbin-Johnson B, Gane L, Nelson DL, Hagerman R, Hagerman PJ, *et al*. AGG interruptions within the maternal FMR1 gene reduce the risk of offspring with fragile X syndrome. Genet Med 2012; 14(8): 729-736. https://doi.org/10.1038/gim.2012.34

[7] Hagerman RJ, Hagerman PJ. Fragile X syndrome: diagnosis, treatment, and research. 3rd ed. Baltimore: Johns Hopkins University Press 2002; xii: p. 540.

[8] Sherman SL. Premature ovarian failure in the fragile X syndrome. Am J Med Genet (Semin Med Genet) 2000; 97(3): 189-194. https://doi.org/10.1002/1096-8628(200023)97:3<189::AID-AJMG1036>3.0.CO;2-J

[9] Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, *et al*. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology 2001; 57: 127-130. https://doi.org/10.1212/WNL.57.1.127

[10] Hunter JE, Rohr JK, Sherman SL. Co-occurring diagnoses among FMR1 premutation allele carriers. Clin Genet 2010; 77(4): 374-381. https://doi.org/10.1111/j.1399-0004.2009.01317.x

[11] Lachiewicz A, Dawson D, Spiridigliozzi G, Cuccaro M, Lachiewicz M, McConkie-Rosell A. Indicators of anxiety and depression in women with the fragile X premutation:

assessment of a clinical sample. J Intellect Disabil Res 2010; 54(7): 597-610. https://doi.org/10.1111/j.1365-2788.2010.01290.x

[12] Winarni TI, Chonchaiya W, Sumekar TA, Ashwood P, Morales GM, Tassone F, *et al*. Immune-mediated disorders among women carriers of fragile X premutation alleles. Am J Med Genet Part A 2012; 158A(10): 2473-2481. https://doi.org/10.1002/ajmg.a.35569

[13] Tassone F, Hagerman PJ, Hagerman RJ. Fragile x premutation. J Neurodev Disord 2014; 6(1): 22. https://doi.org/10.1186/1866-1955-6-22

[14] Fernandez-Carvajal I, Lopez Posadas B, Pan R, Raske C, Hagerman PJ, Tassone F. Expansion of an FMR1 grey-zone allele to a full mutation in two generations. J Mol Diagn 2009; 11(4): 306-310. https://doi.org/10.2353/jmoldx.2009.080174

[15] Song FJ, Barton P, Sleightholme V, Yao GL, Fry-Smith A. Screening for fragile X syndrome: a literature review and modelling study. Health Technol Assess 2003; 7(16): 1-106. https://doi.org/10.3310/hta7160

[16] Faradz SMH, Buckley M, Tang L-P, Leigh D, Holden JJA. Molecular screening for fragile X syndrome among Indonesian children with developmental disability. Am J Med Genet A 1999; 83(4): 350-351. https://doi.org/10.1002/(SICI)1096-8628(19990402)83:4<350::AID-AJMG26>3.0.CO;2-G

[17] Mundhofir FEP, Winarni TI, Nillesen W, van Bon BWM, Schepens M, Ruiterkamp-Versteeg M, *et al*. Prevalence of fragile X syndrome in males and females in Indonesia. World Journal of Medical Genetics 2012; 2(3): 15. https://doi.org/10.5496/wjmg.v2.i3.15

[18] Mundhofir FE, Winarni TI, Nillesen W, Bon BWV, Schepens M, Ruiterkamp-Versteeg M, *et al*. Prevalence of fragile X syndrome in males and females in Indonesia. World J Med Genet 2012; 2(3): 15-22. https://doi.org/10.5496/wjmg.v2.i3.15

[19] Winarni TI, Mundhofir FE, Faradz SM. A Cohort Study of Intellectual Disability Focusing on Fragile X Syndrome in Indonesia 2016; 2016: 7. https://doi.org/10.14710/jbtr.v2i1.536

[20] Indonesian Central Bureau of Statistic. Population of Indonesia by province 1971, 1980, 1990, 1995, 2000, 2010. http: //www.bps.go.id; 2011.

[21] Winarni TI, Utari A, Mundhofir FE, Tong T, Durbin-Johnson B, Faradz SM, *et al*. Identification of expanded alleles of the FMR1 gene among high-risk population in Indonesia by using blood spot screening. Genet Test Mol Biomarkers 2012; 16(3): 162-166. https://doi.org/10.1089/gtmb.2011.0089

[22] Tassone F, Pan R, Amiri K, Taylor AK, Hagerman PJ. A rapid polymerase chain reaction-based screening method for identification of all expanded alleles of the fragile X (FMR1) gene in newborn and high-risk populations. J Mol Diagn 2008; 10(1): 43-49. https://doi.org/10.2353/jmoldx.2008.070073

[23] Tassone F, Iong KP, Tong TH, Lo J, Gane LW, Berry-Kravis E, *et al*. FMR1 CGG allele size and prevalence ascertained through newborn screening in the United States. Genome Med 2012; 4(12): 100. https://doi.org/10.1186/gm401

[24] Yuhas J, Walichiewicz P, Pan R, Zhang W, Casillas EM, Hagerman RJ, *et al*. High-risk fragile x screening in Guatemala: use of a new blood spot polymerase chain reaction technique. Genet Test Mol Biomarkers 2009; 13(6): 855-859. https://doi.org/10.1089/gtmb.2009.0108

[25] Sorensen PL, Gane LW, Yarborough M, Hagerman RJ, Tassone F. Newborn screening and cascade testing for FMR1 mutations. Am J Med Genet A 2013; 161A(1): 59-69. https://doi.org/10.1002/ajmg.a.35680

[26] Chen L, Hadd A, Sah S, Filipovic-Sadic S, Krosting J, Sekinger E, *et al*. An Information-Rich CGG Repeat Primed PCR That Detects the Full Range of Fragile X Expanded Alleles and Minimizes the Need for Southern Blot Analysis. The Journal of Molecular Diagnostics 2010; 12(5): 589-600. https://doi.org/10.2353/jmoldx.2010.090227

[27] Filipovic-Sadic S, Sah S, Chen L, Krosting J, Sekinger E, Zhang W, *et al*. A novel FMR1 PCR method for the routine detection of low abundance expanded alleles and full mutations in fragile X syndrome. Clin Chem 2010; 56(3): 399-408. https://doi.org/10.1373/clinchem.2009.136101

[28] Fernandez-Carvajal I, Lopez Posadas B, Pan R, Raske C, Hagerman PJ, Tassone F. Expansion of an FMR1 Grey-Zone Allele to a Full Mutation in Two Generations. J Mol Diagn 2009; 11(4): 306-310. https://doi.org/10.2353/jmoldx.2009.080174

[29] Hagerman RJ, Berry-Kravis E, Hazlett HC, Bailey DB, Jr., Moine H, Kooy RF, *et al*. Fragile X syndrome. Nat Rev Dis Primers 2017; 3: 17065. https://doi.org/10.1038/nrdp.2017.65

[30] McConkie-Rosell A, Abrams L, Finucane B, Cronister A, Gane LW, Coffey SM, *et al*. Recommendations from multi-disciplinary focus groups on cascade testing and genetic counseling for fragile X-associated disorders. J Genet Couns 2007; 16(5): 593-606. https://doi.org/10.1007/s10897-007-9099-y

[31] Finucane B, Abrams L, Cronister A, Archibald AD, Bennett RL, McConkie-Rosell A. Genetic counseling and testing for FMR1 gene mutations: practice guidelines of the national society of genetic counselors. J Genet Couns 2012; 21(6): 752-760. https://doi.org/10.1007/s10897-012-9524-8

[32] Farzin F, Perry H, Hessl D, Loesch D, Cohen J, Bacalman S, *et al*. Autism spectrum disorders and attention-deficit/ hyperactivity disorder in boys with the fragile X premutation. J Dev Behav Pediatr 2006; 27(2 Suppl): S137-144. https://doi.org/10.1097/00004703-200604002-00012

[33] Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, *et al*. Clinical involvement and protein expression in individuals with the FMR1 premutation. Am J Med Genet 2000; 91(2): 144-152. https://doi.org/10.1002/(SICI)1096-8628(20000313)91:2<144::AID-AJMG14>3.0.CO;2-V

[34] Hessl D, Wang JM, Schneider A, Koldewyn K, Le L, Iwahashi C, *et al*. Decreased fragile X mental retardation protein expression underlies amygdala dysfunction in carriers of the fragile X premutation. Biol Psychiatry 2011; 70(9): 859-865. https://doi.org/10.1016/j.biopsych.2011.05.033

[35] Chonchaiya W, Au J, Schneider A, Hessl D, Harris SW, Laird M, *et al*. Increased prevalence of seizures in boys who were probands with the FMR1 premutation and co-morbid autism spectrum disorder. Hum Genet 2012; 131(4): 581-589. https://doi.org/10.1007/s00439-011-1106-6

[36] Chonchaiya W, Nguyen DV, Au J, Campos L, Berry-Kravis EM, Lohse K, *et al*. Clinical involvement in daughters of men with fragile X-associated tremor ataxia syndrome. Clin Genet 2010; 78(1): 39-46. https://doi.org/10.1111/j.1399-0004.2010.01448.x

[37] Clifford S, Dissanayake C, Bui QM, Huggins R, Taylor AK, Loesch DZ. Autism spectrum phenotype in males and females with fragile X full mutation and premutation. J Autism Dev Disord 2007; 37(4): 738-747. https://doi.org/10.1007/s10803-006-0205-z

[38] Hamlin AA, Sukharev D, Campos L, Mu Y, Tassone F, Hessl D, *et al*. Hypertension in FMR1 premutation males with and without fragile X-associated tremor/ataxia syndrome (FXTAS). Am J Med Genet Part A 2012; 158A(6): 1304-1309. https://doi.org/10.1002/ajmg.a.35323

[39] Leehey MA, Legg W, Tassone F, Hagerman R. Fibromyalgia in fragile X mental retardation 1 gene premutation carriers. Rheumatology 2011; 50(12): 2233-2236. https://doi.org/10.1093/rheumatology/ker273

[40] Roberts JE, Bailey DB, Jr., Mankowski J, Ford A, Sideris J, Weisenfeld LA, *et al*. Mood and anxiety disorders in females with the FMR1 premutation. Am J Med Genet B Neuropsychiatr Genet 2009; 150B(1): 130-139. https://doi.org/10.1002/ajmg.b.30786

[41] Basuta K, Narcisa V, Chavez A, Kumar M, Gane L, Hagerman R, *et al*. Clinical phenotypes of a juvenile sibling pair carrying the fragile X premutation. Am J Med Genet A 2011; 155A(3): 519-525. https://doi.org/10.1002/ajmg.a.33446

[42] Aziz M, Stathopulu E, Callias M, Taylor C, Turk J, Oostra B, *et al*. Clinical features of boys with fragile X premutations and intermediate alleles. Am J Med Genet 2003; 121B(1): 119-127. https://doi.org/10.1002/ajmg.b.20030

[43] Goodlin-Jones B, Tassone F, Gane LW, Hagerman RJ. Autistic spectrum disorder and the fragile X premutation. J Dev Behav Pediatr 2004; 25(6): 392-398. https://doi.org/10.1097/00004703-200412000-00002

[44] Cornish K, Kogan C, Turk J, Manly T, James N, Mills A, *et al*. The emerging fragile X premutation phenotype: evidence from the domain of social cognition. Brain Cogn 2005; 57(1): 53-60. https://doi.org/10.1016/j.bandc.2004.08.020

[45] Moore CJ, Daly EM, Schmitz N, Tassone F, Tysoe C, Hagerman RJ, *et al*. A neuropsychological investigation of male premutation carriers of fragile X syndrome. Neuropsychologia 2004; 42(14): 1934-1947. https://doi.org/10.1016/j.neuropsychologia.2004.05.002

[46] Bailey DB, Jr., Raspa M, Olmsted M, Holiday DB. Co-occurring conditions associated with FMR1 gene variations: findings from a national parent survey. Am J Med Genet A 2008; 146A(16): 2060-2069. https://doi.org/10.1002/ajmg.a.32439

[47] Hagerman R, Hoem G, Hagerman P. Fragile X and autism: Intertwined at the molecular level leading to targeted treatments. Mol Autism 2010; 1(1): 12. https://doi.org/10.1186/2040-2392-1-12

[48] Garcia-Arocena D, Hagerman PJ. Advances in understanding the molecular basis of FXTAS. Hum Mol Genet 2010; 19(R1): R83-89. https://doi.org/10.1093/hmg/ddq166

[49] Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet 2000; 66(1): 6-15. https://doi.org/10.1086/302720

[50] Lozano R, Hagerman RJ, Duyzend M, Budimirovic DB, Eichler EE, Tassone F. Genomic studies in fragile X premutation carriers. J Neurodev Disord 2014; 6(1): 27. https://doi.org/10.1186/1866-1955-6-27

[51] Myers GF, Mazzocco MM, Maddalena A, Reiss AL. No widespread psychological effect of the fragile X premutation in childhood: evidence from a preliminary controlled study. J Dev Behav Pediatr 2001; 22(6): 353-359. https://doi.org/10.1097/00004703-200112000-00001

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