

Short Report

Fragile X syndrome: clinical, cytogenetic and molecular screening among autism spectrum disorder children in Indonesia

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Fragile X testing is a priority in the evaluation of autism spectrum disorders (ASD) cases because identification of the *FMR1* mutation leads to new treatment options. This study is focused on determining the prevalence of the *FMR1* gene mutation among ASD cases in Indonesia. DSM-IV-TR criteria were administered to diagnose ASD; symptom severity was classified using the Childhood Autism Rating Scale. Cytogenetic analysis, polymerase chain reaction, and Southern blot for *FMR1* gene analysis were carried out to confirm the diagnosis of fragile X syndrome. The fragile X site and *FMR1* full mutation allele were identified in 3 out of 65 (4.6%) and 4 out of 65 (6.15%) children aged 3–17 years (57 boys and 8 girls), respectively. The Fragile X laboratory workup is essential in the evaluation of patients with ASD. Molecular analysis is most accurate, while cytogenetic documentation of the fragile X site can also be useful if molecular testing is not available.

Conflict of interest

The authors declare that there is no conflict of interest.

**TI Winarni^a, A Utari^{a,b},
FEP Mundhofir^a,
RJ Hagerman^c and SMH
Faradz^a**

^aDivision of Human Genetics, Center for Biomedical Research, ^bDepartment of Pediatrics, National Hospital, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia, and ^cMedical Investigation of Neurodevelopmental Disorders (MIND) Institute and Department of Pediatrics, University of California-Davis, Medical Center, Sacramento, CA, USA

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Corresponding author: Sultana M. H. Faradz, MD, PhD, Division of Human Genetics, Center for Biomedical Research (CEBIOR), Faculty of Medicine, Diponegoro University, Jl. Dr. Soetomo no. 14, Semarang, Central Java 50231, Indonesia.
Tel.: +62247460017;
fax: +62248454714
e-mail: sultana@indosat.net.id;
smhfaradz@yahoo.com

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Autism is a neurobehavioral disorder characterized by language delay; social and communication impairment; and repetitive and stereotypic behavior. The prevalence of autism among children in United State increased enormously from 0.6 to 6.7 per 1000 from 1990s to 2000s (1, 2). The overall prevalence of autism spectrum disorders (ASD) is currently 11.3 per 1000 (1 in 88) in United States (3). Boys are affected four times more frequently than girls (3).

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability (ID) and ASD. FXS is usually caused by a full mutation in the Fragile

X Mental Retardation 1 gene (*FMR1*) leading to the gene silencing and the absence of fragile X mental retardation protein (FMRP). The prevalence of the full mutation allele is 1 in 2500–3600 (4, 5), but the penetrance of ID is lower at 1 per 3600–4000 males and 1 per 8000 females (6, 7) because of mosaicism in males and X inactivation in females.

Anxiety and social deficits are core clinical features of FXS and they are also associated with autism in both males and females (8–10). Autism is diagnosed in 30% of boys with FXS and another 30% are diagnosed with pervasive developmental disorder not otherwise

specified (PDD NOS) (11). Those with FXS and autism have lower cognitive, adaptive, motor and language abilities compared to those with FXS without autism (12–15).

FXS is the most common single gene disorder associated with autism and it causes 2–6% of autism cases (16, 17). Therefore, all individuals with ASD should be tested for the *FMR1* mutation (18). ASD and FXS have similar symptoms and shared molecular mechanisms because FMRP regulates translation of many other genes also associated with autism and involved with synaptic plasticity (19, 20). Therefore the absence of FMRP leads to abnormal spine maturation and morphology, imbalances between excitatory and inhibitory pathways, and the reduction of brain serotonin synthesis which are also seen in other forms of autism (19–21). Furthermore, advanced studies in FXS have improved understanding of the neurochemical basis of autism and the use of targeted treatments which have benefited those with FXS and potential to benefit those with idiopathic autism (22–25).

Methods

Sixty-five children aged 3–17 who were diagnosed with ASD using the criteria in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM IV-TR) for ASD by experienced pediatricians were recruited (26). The DSM IV-TR required deficits in (1) social interaction; (2) communication; and (3) restricted repetitive and stereotyped patterns of behavior, interests, and activities, to be present prior to 3 years old for a diagnosis of ASD.

The severity of autism symptoms was classified using the Childhood Autism Rating Scale (CARS) (27), a behavior rating scale to classify autism severity (28). A threshold score for autism was 30, a score of 30–36.5 was considered mild-moderate and 37 or greater was considered severe autism. This research was approved by the Institutional Review Board of the Faculty of Medicine, Diponegoro University and the Kariadi Hospital Semarang, Indonesia, all participants signed an approved consent form to participate in this study.

Heparinized peripheral blood vein was collected for cytogenetic analysis of the fragile site at Xq27.3 as done previously (29). DNA was extracted from ethylenediaminetetraacetic acid blood using modified salting out method (30).

The fragile site was identified using solid staining followed by Giemsa staining for fragile X site confirmation. The *FMR1* gene was analyzed using a polymerase chain reaction-based method to determine the CGG repeat length in the promotor region as described previously (31). Males with no bands and females with only one band were assumed to have alleles consisting of high premutation or full mutation alleles, therefore, Southern blot analysis was utilized to confirm the diagnosis (31).

Table 1. Childhood Autism Rating Scale (CARS) categorical diagnosis of all children ($n = 65$)

CARS categories	Male	Female	Total
Mildly to moderately autistic	20	5	25
Severely autistic	37	3	40

Results

The results of the assessment of clinical features in the 65 children are summarized in Table 1. One child was diagnosed with PDD NOS and all other children had autism. The fragile X site located at Xq27.3 was identified in 3 out of 65 (4.5%) children (see Fig. 1). Four out of 65 (61.5%) children had expanded alleles of *FMR1* gene in the full mutation range. These alleles were confirmed by Southern blot.

All children with ASD who carried *FMR1* full mutation alleles were impaired in social interaction and communication domains, and had at least two out of four subdomains in the restricted repetitive and stereotyped patterns of behavior, interests, and activities domain. The phenotypic expression of ASD using the CARS addressed the severity of autism which ranged from mildly to severely autistic (see Tables 1 and 2).

Discussion

A genetic workup is critical to establish the etiology of ASD and determine appropriate treatments, particularly targeted treatments in addition to behavioral interventions and genetic counseling (32). This study confirms the higher rate of FXS (6.15%) compare to those of previous small population studies (33, 34).

Cytogenetic techniques have been used to diagnose FXS through the expression of the fragile X site until the late 1990s (35), and have been abandoned due to low sensitivity compared to molecular techniques (36). However, in developing countries where advanced laboratory equipments and services are very minimal and unaffordable, cytogenetic techniques are still an option to accomplish the FXS diagnosis (37). However, only 75% of children who carried the full mutation allele exhibit the fragile X site in this study (see Table 2). Although the sensitivity is lower than that of DNA testing, the fragile X site expression can still identify a positive result when DNA testing is not available.

Over the past three decades, ASD has been a concern to those diagnosed with FXS because the additional diagnosis of ASD is associated with a greater severity of developmental delay (14), motor and language impairments (15), and social deficits (38). Other genetic disorders are also known to contribute to ASD such as Prader-Willi syndrome, Angelman syndrome, Tuberous Sclerosis, and Smith-Lemli-Opitz syndrome (39–41), but FXS is the most common known genetic cause of autism, likely because FMRP controls the translation of many genes important for synaptic plasticity and associated with autism (42).

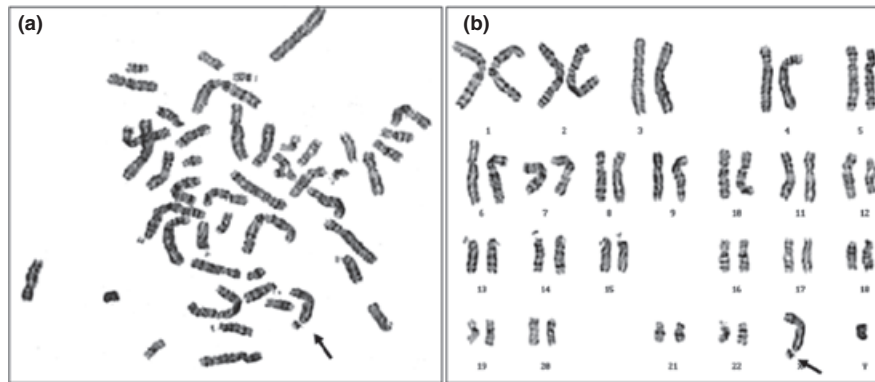


Fig. 1. Representative of Giemsa-banding fragile X site (Xq27.3) of case 1. Black arrows indicate fragile site in the long arm of X chromosome both in metaphase (a) and 46,XY fax(X)(q27.3) karyotype (b).

Table 2. Cytogenetic, molecular status, and clinical characteristics of four autism cases caused by fragile X syndrome

Fragile X syndrome	Case 1	Case 2	Case 3	Case 4
Sex	Male	Male	Male	Male
Age (year)	12	9	6	4
Fragile X site (Xq27.3)	17%	34%	0%	24%
<i>FMR1</i> allele	Full mutation	Full mutation	Full mutation	Full mutation
DSM IV score				
1. Social interaction	4	4	4	2
2. Communication	4	4	4	2
3. Repetitive, stereotypic and restricted patterns of behavior and interests	3	2	3	2
CARS categories	Severely autistic	Severely autistic	Mildly to moderate autistic	Mildly to moderate autistic

CARS, Childhood Autism Rating Scale.

Impairment of social communication and interaction domains have been found to be dominant in four FXS children with ASD compared to those of repetitive, stereotypic and restricted patterns of behavior and interests domain (see Table 2). These characteristics are consistent with previous studies (38, 43). The degree of autism symptoms severity varies from mild to severe in this study (see Table 2). A recent study by Smith et al. indicated that individuals who were diagnosed FXS with ASD displayed greater communication and social reciprocity impairments than individuals with FXS without autism (44). Our study confirms that the social and communication deficits are most predictive of ASD in those with FXS.

Individuals with idiopathic autism compared to those of complex autism, such as FXS, is considered better prognosis (32). However, as FXS leads the way to targeted treatments with initial studies demonstrating efficacy in a subgroup of FXS with mGluR5 antagonists (45), a GABA B agonist, and arbaclofen (46), it is likely that those with FXS may have the best prognosis with new targeted treatments. However, initial studies have demonstrated that the mouse models of idiopathic autism (23) and some human studies in autism may also respond to mGluR5 antagonists and GABA agonists (22, 47). A randomized, double-blind, placebo-controlled

phase 2 study of STX209 (arbaclofen and R-baclofen) in ASD was completed but not yet published to address social withdrawal (www.clinicaltrials.gov or www.seasidetherapeutics.com). It is likely that further studies to address efficacy of targeted treatments in both FXS and autism will be beneficial. Discovering the cause of ASD is an important step not only to establish appropriate intervention and treatment, but also to carry out genetic counseling that will inform the recurrent risk of FXS and fragile X-associated disorders in the family.

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