Journal of the Anatomical Society of India 62 (2013) 52–56

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage:www.elsevier.com/locate/jasi

Original article

Cytogenetic studies in children with learning disability

S. Manjula Thulasi*

Assistant Professor, Department of Anatomy, SMCSI Medical College, Karakonam, Thiruvananthapuram, Kerala

KEYWORDS

Learning disability, Karyotyping, Fragile-X syndrome, FMR-1 gene.

ABSTRACT

Aim: Five percent of the public school children in our population have learning disability (LD). The main objective of the present investigation is to analyze the phenotypic and cytogenetic features of children having LD. *Materials and methods*: Eighty-six children having LD from various parts of Kerala were selected for the present study. Phenotypic data were collected and recorded and cytogenetic analysis was carried out by using peripheral blood. *Observation*: Phenotypic and cytogenetic analysis in selected samples showed various features of LD. *Results*: Among the total samples selected for cytogenetic analysis, 26 showed fragile sites in their X chromosome and its percentage of expression is 4–45%. *Conclusion*: Learning disability can be identified by comparing the results of phenotypic and genotypic analyses.

1. Introduction

Learning disability (LD) is a neurodevelopmental disorder that affects the people's ability to interpret what they see and hear or to link information from different parts of their brain. Kirk and Becker introduced the term 'learning disability' in a meeting with parents of children having learning problems in Chicago.[1] Learning disability is not a single disorder, but it is composed of disabilities in seven areas, which are receptive and expressive language, basic reading skills, reading comprehension, written expression, mathematical calculation, and reasoning. The term learning disability does not include the children who have learning problems, which are primarily the result of visual, hearing motor handicaps, or of mental retardation and emotional disturbance. Approximately, one half of all children receiving special education services nationally or about 5% of the total public school population are identified as having LD.

Fragile X syndrome, the most common cause of LD, is a genetic disease which affects approximately 1 in 2500 females and 1 in 1250 males worldwide. Fragile X syndrome is an X-linked recessive disorder.[2] The major identification criteria of children with this disorder are large ears and head, long and narrow face, prominent jaw, and macroorchidism in males after puberty.[3,4] The gene responsible for fragile X syndrome is fragile X mental retardation gene (FMR-1). This disability is due to the mutation of FMR-1 gene in the X chromosome that controls the intellectual function of every human being. Lubs demonstrated the presence of fragile site in the long arm of X chromosome in children with LD.[5] The fragile site is a non-staining gap located at the long arm of X chromosome (Xq27.3) and designated as FRAXA. The analysis of the karyotype of a person suffering from fragile X syndrome reveals a slight break or fracture on the X chromosome in this region.

Cytogenetic diagnosis is reliable only in affected individuals, practically in affected males and great majority of affected females (>90%). Only 50% of the normal carrier females could be identified by cytogenetic analysis.[6] The present study includes the cytogenetic visualization of fragile sites as an initial screening tool of investigation in affected individuals of Kerala population.

*Corresponding author: Tel: +91 (0) 9744798408
E-mail address: thulasidasr@yahoo.co.in. (S. Manjula Thulasi)

2. Materials and methods

For the present investigation, 86 LD children in the age group of 7–15 years, who were attending various therapies in the Institute of Communicative and Cognitive Neurosciences, Thiruvananthapuram, were selected. Children were screened by conducting the standardized intelligence test. The phenotypic features such as length and breadth of ear, long and narrow face, poor eye contact, macroorchidism, prominent forehead, hyperactivity, obesity, shyness, gaze avoidance, stereotypic movement, strabismus, nystagmus, and language difficulty were selected and analyzed in the present study. The expression of fragile sites was noticed and the percentage of expression was studied.

For cytogenetic study, 5 ml of peripheral blood samples was collected from all the selected individuals having LD. Routine karyotyping was carried out using RPMI medium, and chromosome was prepared, using standard protocol. Samples with chromosomal abnormalities such as deletion, translocation, and ring chromosomes were discarded in the next step. The samples with typical features of fragile X syndrome were subjected to fragile X karyotyping using folic acid deficient culture medium TC 199 with low serum concentration (5%) and high pH. Around 50 unbanded metaphases were screened for each culture. For clear visualization of fragile sites, slides were again G-banded. The microphotographs were taken for each sample using the Jetner–Biolux research microscope.

3. Observation and results

The present investigation was conducted to correlate the phenotypic and genotypic features of different age groups of children with LD and to understand the reliability of cytogenetic investigation for the conformation of fragile X syndrome. Various phenotypic features were analyzed in the present investigation. The length and breadth of the ear were measured and the maximum ear length was recorded in the age group of 16 and minimum was reported in the age group of 10. The phenotypic features such as long and narrow face, prominent fore head, hyper activity, shyness, gaze avoidance, stereotypic movement, and poor eye contact were expressed in all age groups. One of the remarkable phenotypic features in males, the macroorchidism, was exhibited by >50% of the individuals. Another feature strabismus was not noticed in the samples of present study but most of them showed language difficulty. Obesity was noticed in all the age groups and their body weight ranged from 27 kg to 60 kg (Table 1).

Among the samples selected for fragile X karyotyping, 26 showed fragile site in their X chromosome, 10 were females, and remaining were males. The fragile site seen in all these samples expressed a highly constricted area in the long arm of X chromosome at 27.3. The percentage of expression was also calculated on the basis of the number of cells that expressed fragile site in their mitotic chromosome and it ranged from 4% to 45% (Table 2, plate 1).

4. Discussion

In 1943, Martin and Bell identified several children with various intellectual problems who belonged to the same extended English family.[7] The name of the syndrome (Martin–Bell syndrome or fragile X syndrome) is derived from the characteristic chromosomal foliate sensitive fragile site at Xq 27.3, named FRAXA, which is a constriction or a non-staining gap at the distal end of the long arm of X chromosome. People with fragile X syndrome experience a slow development, emotional problems, and hyperactivity. A delay in learning language is often the first sign which prompts suspicion of the syndrome.[8] Noticeable signs of a child with fragile X syndrome include hand flapping, hand biting, poor eye contact, chewing on their clothes, repetitive speech patterns, rocking, and preservation.[9] Fragile X syndrome is an X-linked recessive disorder, which affects males more than females.

Fragile site cannot be visualized by routine karyotyping and the present investigation induced special culture medium TC199 with low folic acid concentration. Inhibitors such as methotrexate and 5-flurodeoxyuridine of the thymidine synthetase enzyme involved in the synthesis of dTTP in cells have been found to be effective inducers of FRAXA.[10,11] The expression of fragile site using the foliate deficient culture medium is highly reliable because it clearly demarcates the fragile site only after it is G-banded. G-banding is necessary for the accurate diagnosis of fragile X syndrome.[12] If the individual expresses fragile site in >4% of the cells, the metaphase of the lymphocyte culture is considered as fragile X positive.[13,14]

Acknowledgments

The author is thankful to Dr. Raveendran Ankathil, Former Additional Professor, Department of Cytogenetics, RCC Thiruvananthapuram, Dr. Suresh PA, Director, Institute of Communicative and Cognitive Neurosciences, Thiruvananthapuram, Dr. Vijaya Lekshmi L, Professor of Biochemistry and HOD, Applied Nutrition, Medical College, Thiruvananthapuram, for their kind guidance and support.

Table 1 – Phenotypic features of children with learning disability.

Phenotypic features		Age group 08		Age group 09		Age group 10		Age group 11		Age group 12		Age group 13		Age group 14		Age group 15		Age group 16	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Ear size	Length (cm)	5.7	5.6	5.5	5.8	5.8	5.6	5.9	5.9	6.0	5.9	6.0	5.9	6.1	6.1	6.0	6.0	6.2	6.1
	Breadth (cm)	2.3	2.3	2.3	2.6	2.4	2.5	2.3	2.4	2.6	2.5	2.6	2.6	2.6	2.6	2.8	2.9	2.9	2.9.
Long and narrow face		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Poor eye contact		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Macro-orchidism		+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Prominent forehead		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hyperactivity		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Obesity (weight in kg)		27	26.8	30.1	30.5	33	34.8	34	34.6	38.8	39.4	42	45	48	50.1	54.6	52.9	60	54
Shyness		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gaze avoidance		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stereotypic movement		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Strabismus		-	-	+	+	+	-	-	+	+	-	+	+	+	+	-	+	-	+
Nystagmus		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Language (difficulty %)		50	55	60	55	60	60	60	60	65	60	70	65	70	65	70	65	70	65

+: Present; −: Absent.

Table 2 – Genotypic features of children with learning disability.

Genotypic features	Age group 08		Age group 09		Age group 10		Age group 11		Age group 12		Age group 13		Age group 14		Age group 15		Age group 16	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Fragile X positive or negative	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Percentage of expression	30	28	22	4	13	15	12	24	45	13	18	12	10	18	30	27	18	22

+: Present

Fig. 1

Fig. 2

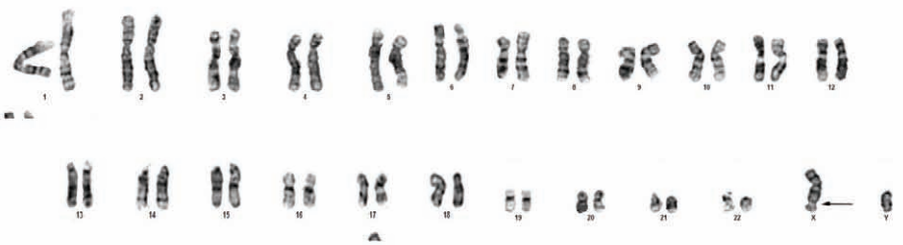

Fig. 3

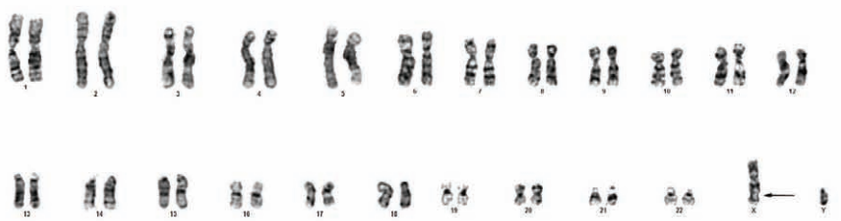

Fig. 4

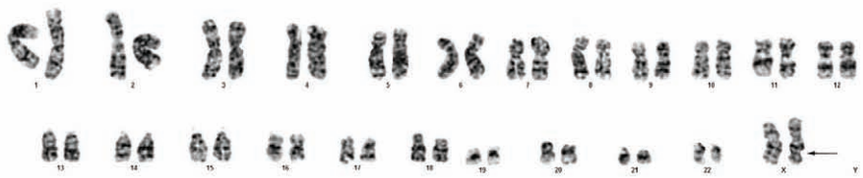

Fig. 5

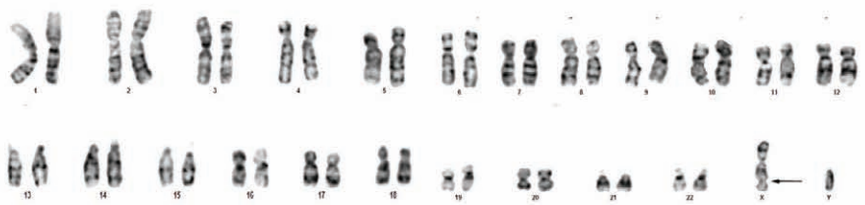

Fig. 6

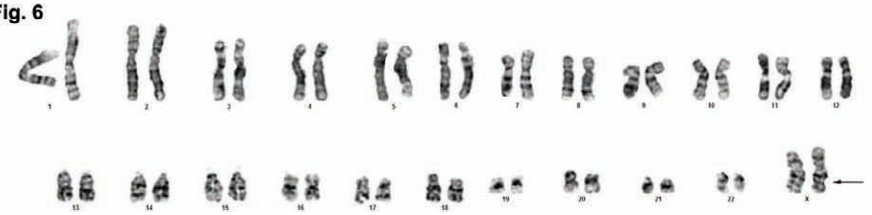

Plate 1 – Figs. 1 –3 and 5 are fragile karyotype of males, Figs. 4 and 6 are fragile karyotype of females. The arrow indicates the fragile site.

REFERENCES

1. Kirk SA, Becker W. *Conference on Children with Minimal Brain Impairment* Urbana: University of Illinois Press, 1963.
2. McKusick VA. *Mendelian Inheritance in Man: Catalogs of Autosomal Dominant, Autosomal Recessive and X-linked Phenotypes* 6th edn, Baltimore: John Hopkins University Press, 1983:1070–2.
3. Fryns JP. X-linked mental retardation and the fragile X syndrome: a clinical approach. In: *The Fragile X Syndrome* Davies KE, ed., Oxford: Oxford University Press, 1989.
4. Hagerman RJ. Physical and behavioural phenotype. In: *Fragile-X Syndrome: Diagnosis, Treatment and Research* Hagerman RJ, Chronister A, eds., 2nd edn, Baltimore: The Johns Hopkins University Press, 1996:3–87.
5. Lubs HA. A marker X chromosome. *Am J Hum Genet* 1969;21:231–44.
6. Tommerup N. Cytogenetics of the fragile site at Xq27. In: *The Fragile X Syndrome* Davies KE, ed., Oxford: Oxford University Press, 1988:103–35.
7. Martin JP, Bell JA. A Pedigree of mental defect showing sex-linkage. *J Neurol Psychiatry* 1943;6:154–7.
8. Sutherland GR, Gedeon A, Kornman L, et al. Parental diagnosis of fragile X syndrome by direct detection of the unstable DNA sequence. *New Engl J Med* 1991;325:1720–2.
9. Fryns JP. X-linked mental retardation and the fragile X syndrome a clinical approach. In: *The Fragile X Syndrome* Davies KE, ed., Oxford: Oxford University Press, 1988:1–39.
10. Glover TW. FUdR induction of the X chromosome fragile site: evidence for the mechanism of folic acid and thymidine inhibition. *Am J Hum Genet* 1981;33:234–42.
11. Mattei MG, Mattei JF, Vidal I, et al. Expression in lymphocyte and fibroblast culture of the fragile X chromosome: a new technical approach. *Hum Genet* 1981;59:166–9.
12. Webb GC. Detection of the fragile X chromosome and other fragile sites. *Clin Genet* 1985;27:520–1.
13. Jacobs PA, Glover TW, Mayer M, et al. X-linked mental retardation: a study of 7 families. *Am J Med Genet* 1980;7:471–89.
14. Jacky PB, Ahuja YR, Anyane-Yeboa K, et al. Guidelines for the preparation and analysis of the fragile X chromosome in lymphocytes. *Am J Med Genet* 1991;38:400–3.