Identification of Chromosomal fragile sites in Down syndrome

Author(s): Sangeetha A, Parkash Chand, Vishnu Bhat B, Ramachandra Rao K, Nandha Kumar S

Vol. 15, No. 1 (2011-01 - 2011-06)

Sangeetha A, Parkash Chand, Vishnu Bhat B *, Ramachandra Rao K, Nandha Kumar S

Department of Anatomy and Paediatrics*, JIPMER, Puducherry, India

Abstract

Chromosomal aberrations play a role in growth and ageing in Down Syndrome (DS) and identification of these fragile sites depends on culture conditions. In the present study lym-phocyte cell culture was carried out using RPMI 1640 with folic acid and RPMI 1640 without folic acid, both with and without addition of 5-Azacytidine. Chromosomal fragile sites were seen in folate free RPMI 1640 media with 5-Azacytidine. Fragile sites were seen in 22 cases in the form of chromosome breaks/gaps,chromosome loss ,triradial/quadriradial configuration and ring/ dicentric chromosomes . Breakpoints were seen in A,B,C, and D group of Denver's classification of chromosomes. Hence chromoso-mal fragile sites were seen best with RPMI 1640without folic and with 5-Azacytidine.

Key Words: Down syndrome (DS), Chromosomal Fragile Sites (CFS)
Accepted October 27 2010

Introduction

Fragile sites are specific loci that show up during karyo-typing as a gap/s on a chromatid arm after culturing the cells under specific conditions [1]. These fragile sites are confined to almost all chromosomes except the Y chro-mosome. Identification of these fragile sites depends upon various cultural conditions, like with and without folic acid. Further, chromosomal fragile sites have been useful for mapping chromosomal regions of the genome that contain genetic loci important for the causation of dis-eases and ageing [2,3]. Down syndrome being the most common genetic cause of mental retardation and associ-ated with complications like ageing, the present study was designed to study the chromosomal fragile sites in Trisomy 21 under various culture conditions using 5-azacytidine which is commonly used as hybridoma re-agent in in-vitro cell culture studies [4]..

Materials and Methods

Forty children with clinical profile of Trisomy 21 and confirmed karyotypically in the age group below eleven years were investigated. Conventional lymphocyte culture was carried out using RPMI 1640 and other media like folate free RPMI 1640 with 5-Azacytidine a DNA methyl transferase inhibitor. Culture arrest was done at appropri-ate hour of incubation using colchicine and hypotonic treatment with Ohnuki solution. Metaphase spreads were pruned under Olympus BX51, Japan, bright/ epiflorescence microscope and freezed with automoated karyotyping workstation – Ikaros Metasystems, Carl Zeiss, Germany. AGT recommendations were followed for identification and interpretation of structural aberrations. The number of fragile sites identified using different culture media were compared.

Results

Lymphocyte cell culture done using RPMI 1640 without folic acid and with 5-Azacytidine showed folate sensitive fragile sites in twenty two (22) cases; of which 13 (thir-teen) were males and 09 (nine) were females. (Table 1).

Fragile sites were seen in the form of chromosome breaks in 13(thirteen) cases, chromosome loss in 03 (three) case, triradial/quadriradial configuration in 09 (nine) cases, ring/ dicentric chromosomes in 05(five) cases. (Table 3) (Fig 1)

The metaphase status and the number of fragile sites iden-tified under various culture conditions are shown in (Table 2).

Karyogram showed folate sensitive common fragile sites in A, B, C and D group of chromosomes. In A group frag-ile sites were seen in 1p32 ,1q32, 2p22, 2p23, 2p24, 2q32, 2q33, 3q26.3, in B group it was seen in 4q12, 4q25, 5q31 , in C group it was seen in 6p21, 6q22, and in D group 14q21, 14q31 were seen. (Table 4).

Table 1. Distribution of fragile sites in males and females

Gender	No. of cases with fragile sites	No. of cases without fragile sites	Total
Males	13	08	21
Females	09	10	19
	22	18	40

Table 2. Metaphase status and Fragile sites in various Culture media adopted

Type of Culture Media	Metaphase status	No. of Cases with fragile sites
RPMI 1640 With Folic Acid without 5-Azacytidine	Good mitotic index Metaphase spreads were abundant Chromosomes were of normal size. No aberrations seen	Nil
RPMI 1640 With Folic Acid with 5-Azacytidine	Less mitotic index Metaphase spreads were scanty Chromosomes were of condensed form No aberrations seen	Nil
RPMI 1640 Without Folic Acid without 5-Azacytidine	Good mitotic index Metaphase spreads were fairly abundant Chromosomes were of normal size. Aberrations were of 'breaks' form.	3
RPMI 1640 without Folic Acid with 5-Azacytidine	Good mitotic index Metaphase spreads were fairly abundant Chromosomes were of normal size. Aberrations were of 'breaks, loss, dicentrics, acentrics, ring, triradial/ quadriradial configurations' form.	**22**

Table 3. Distribution of types of fragile sites in cases

Chromosome breaks	Chromosome loss	Tri/Quadriradial Configurations	Ring/ Dicentric
13	03	09	05

Figure 1. Showing various types of Fragile sites seen

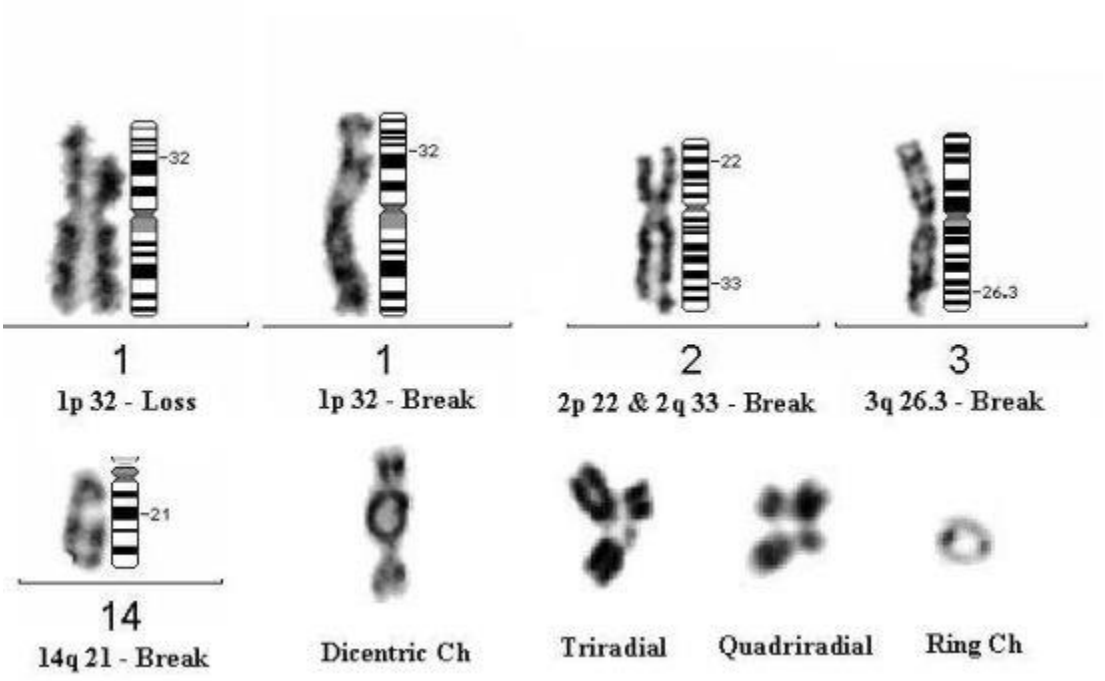

Table 4. Distribution of fragile sites in each group

Autosomes in groups and chromosomes in each involved					Sex Chromo-some
A	B	C	D	E,F&G	X&Y
1p32, 1q32	4q25, 4q12	6p21, 6q22	14q31, 14q21	Nil	Nil
2p22, 2p23, 2p24, 2q32, 2q33	5q31				
3q26.3					

Discussion

In the present study, twenty two cases out of forty inves-tigated showed chromosomal fragile sites affecting A to D groups. This is in agreement with the findings of Suzue Kanata et al wherein the involvement of 3p (Group A) and chromosomes of Group C was noted(5). The fragile sites were designated as “hot points” and there was no involvement of X chromosome. Although, MEM folate free media adopted by Suzue Kanata et al showed fragile sites only in three cases out of nine investigated, RPMI 1640 without folic acid cultures in the current se-ries showed aberrations only in three cases out of forty cases investigated. The trials with RPMI 1640 with folic acid alone and RPMI 1640 with folic acid and with 5 – Azacytidine also did not reveal any fragile sites. There-fore it appears that folate free media either MEM or RPMI 1640 is essential for investigations pertaining to cultures with regard to identification of chromosomal fragile sites. By using 5 – Azacytidine and RPMI 1640 without folic acid media, the instances of fragile sites and their percentage were found to be more – thirteen out of twenty one males and nine out of nineteen females inves-tigated in the present series. While comparing the fre-quency of the incidence of fragile sites between MEM media and folate free RPMI 1640 media used , MEM had a percentage of 33.3% (3 out of 9 cases of Zuzue et al) and folate free RPMI 1640 had a percentage of 55% (22 cases out of 40 cases in the present series) among the cases investigated.

The involvement of 3p14 and Xq21.3 with the frequency of 12 % as stated by Ajit et al was not observed in the present series. Ajit et al had observed these fragile sites in RPMI 1640 media with folic acid and with 5-Azacytidine. Marilia et al has further observed the involvement of 3p14 in both the age groups ie younger and older (6). However, the involvement of 3q26.3 was observed in the current series. In the present series fragile sites affecting 3q26.3 were seen in 3 cases below 5 years of age and none in the older. In the older age group it was stated to be 5q.31, 6p21 and 9q12. But in the present series 1p32 and ring – 21 were observed in children above 5 years.

The involvement of 6p in older children with a frequency of 7.4% as referred by Marilia et al was not observed in the current series; but, 6p.21, 6q.22 and dicentric variety affecting 6th chromosome were observed affecting the younger age group between 8-18 months only. 6p21 which hosts the gene for microtubule associated tau like protein (MTBT2) which is responsible for ageing process in Down syndrome as suggested by Kidd et al (7,8).There was a single instance of a case of 8 months old baby with 6p21 involvement. Involvement of this gene for prema-ture aging process in the current series could not be ascer-tained as the case was an infant.

Fragile sites affecting 2q11 region, both in the older and younger age group which is often called as unstable sec-ondary constrictions was not observed in the present se-ries(9). This is often associated with typical or atypical phenotypic features affecting cardiovascular malforma-tions, growth retardation and mental retardation. Instead, 2p22, 2q32, 2q33 were observed in below 12 months age group in our series. It has been observed that folate free RPMI 1640 with 5-Azacytidine enhances the appearance of fragile sites and these fragile sites were seen in varying age groups.

Reference

1. Sutherland GR, Baker E. The clinical significance of fragile sites on human chromosomes.Clin Genet 2000; 58: 157-161.
2. Sutherland GR. Heritable fragile sites on Human chro-mosomes II. Distribution,Phenotypic Effects, and Cyto-genetics. American journal of Human Genetics 1979; 31: 136-148.
3. Saxena AK, Srivastava AK. Unexpected segregation of chromosome and common fragile site expression indu-ced by 5-azacytidine exposure in human lymphocytes of Downs syndrome patients. Biomedical Research 2007; 18 (1): 31-34.
4. Sutherland GR. The role of nucleotides in Human Frag-ile site expression. Mutation Research 1988; 200: 207-213.
5. Kanata S, Kadotani T, Watanabe Y, Kurosaki N, Ku-mada T, Toi O. Cytogenetic study of Fragile sites on Down’s syndrome. Proc Japan acad. 1988; 64: 249-251.
6. Smith MAC, Borsatto B. Down’s syndrome,ageing and fragile sites .Mechanisms of Ageing and Develop-ment 1998; 101: 167-173.
7. Kidd KK, Bowcock AM, Schmidtke J. Report of the DNA Committee and catalogs of cloned and mapped genes and DNAPolymorphisms;Cytogenet Cell Genet-ics 1989; 51: 622-947.
8. Kormann-Bortolotto MH, Smith MAC, Toniolo Neto J. Fragile sites, Alzheimer’s disease and ageing. Mech. Ageing Dev1992; 65: 9-15.
9. Lejeune J, Dutrillaux B, Lafourcade J, Berger R, Abonyl D, Rethore MO. Endoreduplication selective du bras long du chromosome 2 chez une femme et sa fille. Academic Science Paris 1968; 266: 24-26.

Correspondence to:
Vishnu B. Bhat
Department of Pediatrics
JIPMER (Institution of National Importance)
Pondicherry 605006 India

Curr Pediatr Res 2011 Volume 15 Issue 1 37