Metaphase acrocentric associations in mentally retarded patients

Akeel A. Yasseen, *MSc(UK), PhD(UK),* ***Thekra A. Al-Musawi,*** *MSc.*

ABSTRACT

Objective: The metaphase of 21 severely mentally retarded patients were studies to assess the role of satellite association in the etiology of mental retardation.

Methods: Peripheral blood culture, chromosome harvesting and study scoring were conducted according to standard methods.

Results: Considered as a whole the results indicate that there is a significant increase in the frequency of satellite association among the mentally retarded.

Conclusion: The present study concluded that satellite association may play a role in the etiology of mental retardation and may explain to some extent the presence of normal karyotype in some mental retardation patients.

Keywords: Chromosome analysis, mental retardation, satellite association.

Neurosciences 2001; Vol. 6 (4): 233-237

It is observed that certain chromosomes commonly tend to occupy positions in the mitotic cells close to one another. The most obviously associated chromosomes are numbers 13, 14, 15, 21 and 22 that are often seen linked together by their short arms. This phenomenon is called satellite association (SA). However, numbers of studies have shown that the acrocentric chromosomes of the human metaphase complement are involved in non-random associations[1] and there is correlation between SA tendency and the incidence of anomalies involving acrocentric chromosomes.[2]

It is well documented that acrocentric associations might be related to Robertsonian translocations,[3] and also might have influence on both mitotic and meiotic non-disjunction, which give rise to zygotes with a numerical chromosome abnormality.[4] Likewise, the tendency of acrocentric chromosomes to associate depends on the activity of the nucleolus organizing regions (NORs).[5] Further, it is suggested that only those NORs that were functionally active during the preceding interphase are stained by the specific silver techniques,[6] and also, that the amount of Ag-staining of the NORs on acrocentrics is correlated with the frequency of their participation in satellite associations.[7] These 2 characteristics, associative tendency and NORs activity, are inheritable in a stable Mendelian fashion.[8]

Furthermore, reports have suggested that satellite associations involving acrocentric chromosomes, and specifically associations involving chromosome 21, were seen more frequently in parents of Down's syndrome patients than in controls,[9] lending credence to the idea that satellite associations play a significant role in non-disjunction events leading to Down's syndrome.[10] The same observations were made by other authors[11] who found a highly significant increase in satellite associations involving chromosome 21 in the parent in which the non-disjunction was known to have occurred, although they also found a significantly increased association frequency for certain chromosomes, including

From the Department of Pathology and Forensic Medicine (Yasseen), Middle Euphrates Center for Cancer Researches (Al-Musawi), College of Medicine, Kufa University, Kufa, Iraq.

Received 26th August 2000. Accepted for publication in final form 10th January 2001.

Address correspondence and reprint request to: Dr Akeel A. Yasseen, Professor/Consultant Medical Genetics, Department of Pathology and Forensic Medicine, Middle Euphrates Center for Cancer Researches, College of Medicine, Kufa University, PO Box 18, Kufa, Iraq. Fax. +964 (1) 886 7170.

chromosome 21, in the parents in which the non-disjunction had not occurred. Other investigations, however, did not find any increase in satellite association in parents of Down's syndrome patients.[12] Interestingly, several investigators have demonstrated that the association, involving chromosome 21 was significantly higher in trisomy 21 individuals.[13,14] Though others reported the absolute frequency of acrocentric association was lower in trisomy 21 individuals than disomic controls, but the relative involvement of chromosome 21 (after correction for the trisomic state) was higher than in normal controls.[15] On the contrary, there is no increased association found in patients with trisomy 13. This phenomenon was interpreted as resulting from decreased activity of the NORs in cells with supernumerary acrocentric chromosome which lessened the tendency to build up large associations[16] and, further, these results seem to indicate different involvement of the acrocentric chromosomes in the nucleolus organization in trisomies 13 and 21.[17]

The present study was designed to investigate the phenomenon of satellite association not only in the Down's syndrome patients but also in all mentally retarded patients who were subjected to the present study in an attempt to assess the possible role of this phenomenon in the etiology of mental retardation.

Methods. Twenty one patients who were clinically diagnosed as severely mentally retarded, with a median age of 13.2 years (range 4-19) were included to score the frequency of satellite association. The patients were randomly selected. Corresponding normal control individuals were subjected for comparison.

The chromosome preparation was carried out by the method of Moorhead et al (1960).[18] Peripheral blood lymphocytes were cultured in RPMI-1640 culture media supplemented with 20% fetal calf serum, L-glutamine, 2% phatohemaglutinin, 50 IU/ml penicillin and 100 IU/ml streptomycin. Then, culture was incubated for 72 hours at 37°C. Colchicine was added for 2 hours at a final concentration of 0.004/(w/v). The cells were treated with 0.075M kcl for 30 minutes and fixed in methanol: glacial acetic acid (3:1). After that, the cell suspension was dropped on cold damped slides from a height of 30 cm. The slides were dried in a stream of cold air. Finally, chromosome staining was carried out with 10/(v/v) Giemsa in phosphate buffer saline (PH 6.8) for 10 minutes. Indeed, the cultures of both groups (patients and normal control) were harvested at 72 hours in order to avoid any differences in the frequency of satellite association as a result of culture time.[19] Further, it was found that there is no correlation between association frequency and sex, racial, or age.[15] Accordingly, the above factors were excluded in the present study.

Table 1 - Percentage of cells containing association for 21 mentally retarded patients as compared with normal controls.

Number of Individuals	Percentage Cells Association	
	Patients	Control
1	64	20
2	60	12
3	72	24
4	88	36
5	68	28
6	60	28
7	68	16
8	80	32
9	56	24
10	64	20
11	84	4
12	72	32
13	68	24
14	88	36
15	68	36
16	72	16
17	72	36
18	88	32
19	52	24
20	72	8
21	76	40
P	0.72	0.25
* P < 0.01 (Z-test)		

Satellite association scoring. The association was considered to be present if the satellited ends of 2 (or more) acrocentric chromosomes lay within 1.2 um of each other. So all 3 types of associations (D/D; D/G; G/G) were scored in 25 adequate metaphase spreads for each subject.

Results. The association data obtained from the analysis of somatic metaphase nuclei are summarized in Tables 1, 2 and 3. Table 1 shows a higher percentage of mitosis containing association in the retarded patients (0.71) than in the control individual (0.25), Z-test shows the difference is highly significant ($Z=15.33$, $P<0.01$). The mean number of association per cell is 0.984, as compared with control group (0.278). So the difference is statistically highly significant ($t+16.41$, $P<0.001$) and the mean number of associated chromosome is higher in the retarded patients than that of control individuals giving a significant difference ($t=16.61$, $P<001$) (Table 2). Table 2 also shows that the mean number of small association is higher in the retarded group than in the control giving rise to a significant difference ($t=13.099$, $P<0.001$). While, in the respect of large association the number is significantly higher in the retarded patients ($t=11.95$, $P<0.001$). The mean number of cells with one association is higher in the retarded group than those in the control giving a significant difference ($t=6.705$, $P<0.001$), as with cells of 2 association which is significantly higher in

Table 2 - The satellite association for the 21 mentally retarded patients as compared with normal control.

Satellite Association	Association/ cell mean ± SD	Associated chromosome per cell mean ± SD	No of small association mean ± SD	No of large association mean ± SD	No of cells with one association mean ± SD	No of cells with 2 associations mean ± SD	No of cells with more than 2 associations mean ± SD
Patients	0.984 ± 0.168	2.232 ± 0.405	19.047 ± 3.153	5.571 ± 2.111	11.238 ± 3.048	6.333 ± 2.394	0.285 ± 0.560
Controls	0.278 ± 0.115	0.554 ± 0.234	6.904 ± 2.861	0.047 ± 0.218	5.619 ± 2.355	0.666 ± 0.856	0
t cal. = P< 0.001	16.41	16.61	13.099	11.95	6.705	10.24	

SD = standard deviation, No = Number, df = 40

Table 3 - The satellite association for the 21 mentally retarded patients.

No of patients	Sex	Age (years)	No of cells examined	% cells association	Association /cell	Associated chromosome per cell	No of small association	No of large association	No of cells with one association	No of cells with 2 associations	No of cells with more than 2 associations
1	F	9	25	64	0.84	1.84	17	4	11	5	0
2	M	17	25	60	0.96	2.04	21	3	6	9	0
3	M	17	25	72	1.04	3.36	19	7	10	8	0
4	M	14	25	88	1.12	2.76	19	9	17	4	1
5	F	8	25	68	1.12	2.64	18	10	8	7	2
6	M	19	25	60	0.84	1.84	17	4	9	6	0
7	M	18	25	68	0.96	2.12	19	5	10	7	0
8	M	12	25	80	1	2.28	18	7	15	5	0
9	M	10	25	56	0.72	1.68	14	4	10	4	0
10	M	10	25	64	1.08	2.48	22	5	6	9	1
11	M	16	25	84	1.28	2.92	25	7	10	11	0
12	F	8	25	72	0.96	2.2	20	4	12	6	0
13	M	16	25	68	0.84	1.76	11	2	13	4	0
14	M	18	25	88	1.32	3	24	9	11	11	0
15	M	15	25	68	0.92	2.12	18	5	11	6	0
16	M	18	25	72	0.88	2.04	16	6	14	4	0
17	M	19	25	72	0.84	1.92	15	6	17	2	0
18	F	4	25	88	1.2	2.64	25	5	14	8	0
19	M	5	25	52	0.68	1.56	14	3	9	4	0
20	M	5	25	72	1.08	2.44	20	7	10	7	1
21	M	19	25	76	1	2.24	20	5	13	1	1

No = Number, F = Female, M = Male, % = percentage

the retarded patients (t=10.24, P<0.001) (Table 2). Table 2 also shows that the mean number of cells with more than 2 associations is slightly higher in the retarded group (0.235) than in the control (0).

The most interesting finding is that, in 2 patients with Down's syndrome (Numbers 4 and 18), the association frequency is almost much more higher as compared with other retarded patients in the same group (Table 3).

Discussion. The results of the mitotic investigations were often inconsistent, some workers reporting random association and others non-random involvement of the acrocentric chromosomes.[20] Nevertheless, the frequency of a specific acrocentric association has been considered as a predisposing factor to meiotic and mitotic non-disjunction and the acrocentric chromosome associations do not occur randomly.[4,21]

In the present study, we have found a significant increase in the frequency of SA in 21 mentally retarded patients as compared to normal control individuals. Interestingly, the association frequency was high in both cases of Down's syndrome (trisomy 21 mosaicism and translocation case). Indeed, reports in the literature on a possible relationship between association frequency and the occurence of Down's syndrome were conflicting.[12,22] However, one possibility of this higher association frequency may be due to the existence of compensation mechanism in the acrocentric chromosomes in which the degree of compensation might depend on the activity of the NOR of the supernumerary or missing acrocentric chromosome. The compensation mechanism was found to keep the number of associations constant despite a different number of acrocentric chromosomes per mitosis as per the explanation of Hansson 1975 who detected that in carriers of DD, DG, and GG translocation, the number of associations remained constant because the homologues of the translocation chromosomes showed a higher association tendency.[23]

Another possibility is that acrocentric association might be related to Robertsonian translocation or aneuploidy condition in which the special association behavior may depend on the same factors, which have caused the fusion of the 2 acrocentric chromosomes or the non-disjunction. Many authors[13,17,24] reported similar findings. So far, several studies were concluded that homology at the molecular level has the advantage of the reconciling the non-random distribution of Robertsonian translocation.[20] Because, the tendency for specific acrocentric chromosomes to be in Robertsonian translocation could result from the homology at a molecular level and the breakpoints are preferentially located within the satellite DNA consisting of the short arms of acrocentric chromosomes.[25]

However, our own observation, presented here, confirms the relationship between associative tendency and occurrence of Down's syndrome. Clearly, the higher frequency of association occurred in all mentally retarded patients. This may be related to their condition and, therefore; this phenomenon may play a role in the etiology of mental retardation.

References

1. Rodman TC, Flehinger BJ, Rohlf FJ. Metaphase chromosome associations: Colcemide distorts the pattern. Cytogenet Cell Genet 1980; 27: 98-110.
2. Hansson A. Satellite associations in human metaphase: A comparative study of normal individuals, patients with syndrome of their parents. Heredit 1979; 90: 59-67.
3. Ohno S, Trujillo JM, Kaplan WD, Kinsita R. Nucleolus-organizers in the causation of chromosomal anomalies in may. Lancet 1961; 2: 123-126.
4. Ferguson-Smith MA, Handmarker WD. Observations on the satellited human chromosomes. Lancet 1961; 1: 638-640.
5. Zankl H, Mayer C, Zang KD. Association frequency and silver staining of nucleolus organizing regions in hyperthyroid patients. Hum Genet 1980; 54: 111-114.
6. Miller DA, Dev VG, Tantravahi R, Miller OJ. Suppression of human nucleolus organizer activity in mouse-human somatic cell hybrid. Cell Exp 1977; 101: 235-243.
7. Miller DA, Tantravahi R, Dev VG, Miller OJ. Frequency of satellite associations of human chromosomes is correlated with the amount of Ag-staining of the nucleolus organize region. Am J Hum Genet 1977; 29: 490-502.
8. Zakharov AF, Davudov AZ, Benjush VA, Egolina NA. Genetic determination of NOR activity in human lymphocytes from twins. Hum Genet 1982; 60: 24-29.
9. Hansson A, Mikkelsen M. An increased tendency to satellite association of human chromosome 21: A factor in the etiology of Down's syndrome. IRCS Auat Pediat Psychiat 1974; 2: 1617.
10. Jacobs PA, Mayer M. The origin of human trisomy: A study of hetromorphisms and satellite association. Ann Hum Genet 1981; 45: 357-365.
11. Hansson A, Mikkelsen M. The origin of the extra chromosome 21 in Down syndrome: studies of fluorescent varients and satellite association in 26 informative families. Cytogenet Cell Genet 1978; 20: 194-203.
12. Taysi K. Satellite association: Giemsa banding studies in parents of Down's syndrome patients. Clin Genet 1975; 8: 319-323.
13. Rosenkranz W, Fleck S. On the relationship between the frequency of association and nucleolar constriction of individual acrocentric chromosomes. Humangenetik 1969; 23: 267-277.
14. Rosenkranz W, Holzer S. Satellite association. A possible cause of chromosome aberrations. Humangenetik 1972; 16: 147-150.
15. Yip MY, Fox DP. Variation in pattern and frequency of acrocentric association in normal and trisomy 21 individuals. Hum Genet 1981; 59: 14-22.
16. Zankl H, Michaelsen H, Zang KD. Quantitative studies on the arrangement of human metaphase chromosome VI. The association pattern of acrocentric chromosomes in patients with trisomy 13. Hum Genet 1979; 49: 185-189.
17. Zankl H, Zang KD. Quantitative studies on the arrangement of human metaphase chromosome VII. The association pattern of acrocentric chromosomes in carriers of Robertsonian translocation and in their relatives with normal karotypes. Hum Genet 1979; 52: 119-125.

18. Moorhead PS, Nowell PC, Melman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultures from human peripheral blood. Exp Cell Res 1960; 20: 613-616.
19. Mattevi MS, Salanzo FM. Effect of sex, age and cultivation time on number of satellite and acrocentric association in man. Hum Genet 1975; 29: 265-270.
20. Guichaoua MR, Devictor M, Hartung M, Luciani JM, Stahl A. Random acrocentric bivalent associations in human pachytene spermatocytes. Cytogenet Cell Genet 1986; 42: 191-197.
21. Mattei JF, Ayme S, Mattei MG, Gouvernet J, Giraud F. Quantitative and qualitative study of acrocentric associations in 109 normal subjects. Hum Genet 1976; 34: 185-194.
22. Cooke P, Curtis DJ. General and specific patterns of acrocentric association in parents of Mongol children. Hum Genet 1974; 23: 279-287.
23. Hansson A. Compensatory mechanism in the satellite association patterns of individuals with Robertsonian translocation. Heredit 1975; 8: 101-112.
24. Zellweger H, Abbo G, Cauny R. Satellite association and translocation mongolism. J Med Genet 1966; 3: 186-189.
25. Gosden JR, Lawrie SS, Gosden CM. Satellite DNA sequences in human acrocentric chromosomes: information from translocations and hetromorphisms. Am J Hum Genet 1981; 33: 243-251.