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Satellite Associations in Down Syndrome

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ABSTRACT The association of acrocentric chromosomes by their satellites, referred as satellite associations (SAs), is implicated as a cause for non-disjunction and hence an etiological factor for Down syndrome. The present study compares the frequency of SAs observed in 30 children with Down syndrome and their parents to that seen in 16 healthy children and their parents. Silver (Ag-NOR) stained metaphases showed statistically significant increase in SAs in children with DS, compared to controls. Satellite associations were found to be also significantly increased in either parent with DS offspring. Two patients with Robertsonian translocation showed decreased SAs. This study supports the hypothesis that an increased tendency for satellite associations is an indicator for non-disjunction.

INTRODUCTION

Constitutional autosomal abnormalities can arise from mitotic errors during pre-mitotic cell divisions of the primitive germ cells in either parent, or from errors in meiosis in either parent or as post-zygotic mitotic errors early in the development of the embryo (McFadden and Friedman 1997). Non-disjunction is the failure of two members of a chromosome pair to disjoin during meiosis I or two chromatids of a chromosome to disjoin during meiosis II or mitosis, so that both members pass to one daughter cell and the other daughter cell receives neither resulting in trisomy and monosomy respectively (Nussbaum et al. 2001). The probability of non-disjunction and translocations during meiosis and mitosis has been thought to be increased due to the association of acrocentric chromosomes by their satellites, referred to as satellite associations (SA) (Ferguson-Smith and Handmaker 1961; Ohno et al. 1961).

The satellite stalks otherwise called as nucleolar organizer regions (NORs) localized on the short arm of acrocentrics have been suggested to be responsible for such abnormal meiotic events leading to certain chromosomal disorders and/or abortions (Ray and Pearson 1979). An increased tendency for satellite associations was reported in metaphases obtained from cultured lymphocytes of DS patients and their parents compared to control individuals and their parents (Hansson 1979; Kovaleva et al. 1993; Jyothy et al. 2000). However, a few investigators considered SAs to play insignificant role in the etiology of non-disjunction (Zankl and Nagl 1980; Jacobs and Mayer 1981). This study was carried out to analyze the frequency of SAs in DS patients and their parents in comparison with control individuals, to evaluate if SAs could be an early indicator for non-disjunction.

MATERIALS AND METHODS

Acrocentric associations in Ag-NOR banded metaphases were studied in 30 Down syndrome children and their parents. Their ages ranged from 1 day to 10 years. These children with provisional diagnosis of Down syndrome were referred for cytogenetic analysis to the Department of Genetics, University of Madras, from all over the city during 1995 to 1999. Clinical documentation of all cases pertaining to neonatal problems, mental and physical development was carried out with the help of the respective pediatrician. Sixteen healthy children, belonging to both sex and their parents formed the control set. These children belonged to age group of 7 days to 5 years. The study was carried out with informed consent of the parents and was

approved by the Internal Ethical committee (Yashwanth 2001).

Whole blood cultures were set up and processed following the protocol of Hungerford (1965). Subsequently slides were prepared and GTG-banded by a modified method of Seabright (1971). For AgNOR-banding the protocol of Howell and Black (1980) was adopted. Placed two drops of 2% gelatin followed by four drops of 50% silver nitrate (Sigma) solution. After mixing the solutions well the slide was then covered with a cover glass and kept over a slide warmer set to 70°C till the solution turned a golden yellow color. The cover glass was removed and the slide was washed thoroughly in distilled water followed by staining for 5 minutes in 2% Giemsa solution.

Twenty-five well spread and well banded metaphases from each individual was analysed under oil immersion (100x) lens. About 50 to 100 silver stained metaphases were analyzed for acrocentric associations. Representative metaphases were photographed using Nikon photomicroscope (Labophot–2), using NOVA (125 ASA) black and white film and standard nomenclature was followed for designation of chromosomal abnormalities (Mitelman 1995).

RESULTS

Analysis of GTG-banded metaphases revealed trisomy 21 in 28 children. A Robertsonian translocation involving chromosomes 14 and 21 was observed in two children. The parents of all the DS children exhibited a normal karyotype. The control set consisting of 16 healthy children and their parents also showed a normal karyotype.

An increased frequency of associations in Ag-NOR banded metaphases was observed in DS patients as compared with healthy children. This increase was found to be statistically highly significant ($Z = 3.787$; $P < 0.001$; Table 1). Chromosome 21 and 14 were mostly involved in the SAs among the DS patients and their parents when compared to control individuals and their parents (data not shown). Upon statistical comparison, significantly increased tendencies for acrocentric associations on silver (Ag-NOR) stained metaphases were observed in both fathers ($Z = 2.93$; $P < 0.01$) and mothers ($Z = 2.96$; $P < 0.01$) of DS children (Table 1).

Ag-NOR banded metaphases showed a decrease in satellite associations in DS patients with translocation and their parents when compared to the patients with free trisomy and their parents, but slightly higher than controls (Table 1). Figure 1A shows GTG-banded metaphase (partial) from a mother of a DS child showing all ten acrocentric chromosomes to be involved in association. Ag-NOR banded metaphase depicting acrocentric association is shown in Figure 1B.

DISCUSSION

Acrocentric chromosomes possess satellites attached through stalks or secondary constrictions where the nucleolar organizer regions (NORs) are localized. NORs contain multiple copies of genes coding for 18s and 28s ribosomal RNA. The proximity of NORs is a consequence of nucleolar fusion and results in clustering of the short arms of acrocentrics known as satellite associations (SA) (Ferguson-Smith and Handmaker 1961), which have been postulated to increase the risk of non-disjunction (Ohno et al. 1961).

The finding of a statistically significant increase in the frequency of acrocentric

Table 1: Frequency of acrocentric associations analyzed by AgNOR-banding in DS children, their parents and control set

Group	*No. of individuals*	*Malformed Children*			*Mother*			*Father*		
		N	*n*	*%*	*N*	*n*	*%*	*N*	*n*	*%*
Trisomy 21	28	1390	1083	77.9	1450	1010	69.7	1390	905	65.1
Translocation	2	80	58	72.5	100	57	57.0	85	49	57.6
Total	30	1470	1141	77.6	1550	1067	68.8	1475	954	64.7
Control	16	765	538	70.3	730	457	62.6	760	443	58.3
		$Z = 3.787$; $p<0.001$			$Z = 2.93$; $p<0.01$	$Z = 2.96$; $p<0.01$				

N = Total number of cells analyzed
n = Number of cells with association

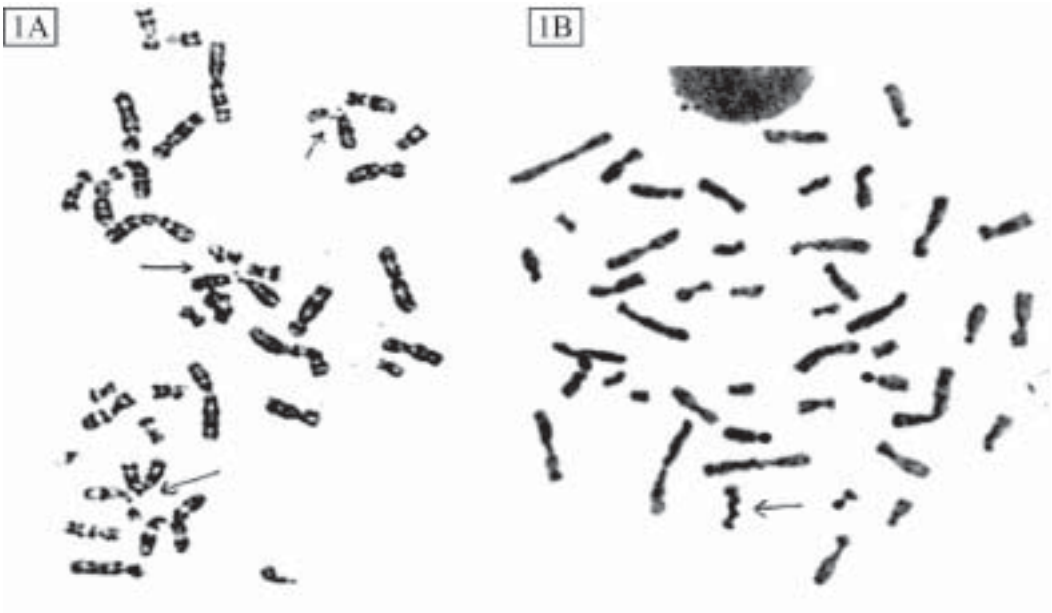

Fig. 1A - GTG-banded metaphase (partial) from a mother of a DS child showing all ten acrocentric chromosomes in association.
Fig. 1B - Ag-NOR banded metaphase exhibiting an acrocentric association.

associations in DS children and their parents was in agreement with previous reports (Hansson 1979; Jyothy et al. 2000). The mothers of DS children have a high SA tendency, especially for chromosome 21 compared with control individuals. The fathers of DS patients' also exhibited increased SA tendency. Kovaleva et al. (1993) studied 373 individuals comprising of DS children, their parents and controls, and observed an increased frequency of chromosome 21 associations in the whole group of parents (including errors at both meiotic divisions). An earlier study from this laboratory had also shown a significantly increased frequency of acrocentric associations among seven DS children and their parents than in normal children and their parents (Punitha 1998). Though, Zankl and Nagl (1980) could not observe significant differences in SAs between the normal and trisomic cells of a mosaic DS patient, they however, observed the association of chromosome 21 to be significantly higher in trisomic cells, while all other acrocen-trics showed somewhat decreased association.

In this study, two Down syndrome patients with translocation did not show a significant rise in satellite associations. Neither did their parents show higher SAs. The reason for such low number of SAs in the Robertsonian translocation group is expected, since the NORs of two of the fused chromosomes are lost, at least with the rob(13q14q) and rob(14q21q) translocations (Gardner and Sutherland 2004). An individual with Robertsonian translocation has eight NORs instead of usual 10 and not all the NORs are active as evidenced by Ag-NOR staining; most individuals have four to seven per cell that are functioning (Varley 1977). Nikolis and Kekic (1988) analyzed families with *de novo* 21/21 translocation offspring and reported that the total NOR activity of the cell also decreases, but the NOR activity of its chromosomes increases when the Ag-NOR number of a cell decreases, suggesting a compensatory mechanism that regulates NOR activity at the cellular level.

Meta-analysis of the available literature on the frequency and pattern of associations involving acrocentric chromosomes in parents of DS individuals confirm NOR activity to be an important factor in the etiology of trisomy 21 (Kovaleva 1991). In the present study, satellite

associations are higher in Down syndrome children and their parents than the control set and this supports the hypothesis that increased satellite associations increase the risk of non-disjunction. Multiple cellular factors could affect the satellite associations, in addition to active NORs and NOR heteromorphisms, promoting non-disjunction causing Down syndrome.

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