Murine Trisomy 16 Model of Down's Syndrome: Central Nervous System Electron Microscopic Observations

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PLIOPLYS, A. V. AND H. M. BEDFORD. Murine trisomy 16 model of Down's syndrome: Central nervous system electron microscopic observations. BRAIN RES BULL 22(2) 233-243, 1989.—Murine trisomy 16 is an excellent model for the human Down's syndrome (DS) (13). Electron microscopic (EM) observations were made of the cortical plate within the developing telencephalic vesicle at the gestational age of E17. The EM observations revealed: (A) microtubular profiles which were more coiled and curved in the trisomic condition; (B) poor cell-to-cell apposition and increased cellular membrane fragmentation in trisomy 16; (C) increased nuclear contour irregularity in trisomic neurons; (D) significant decrease in the cross-sectional area of neuronal nuclei in trisomy 16 \( p < 0.01 \). The microtubular observations lend credence to the hypothesis that abnormal cytoskeletal interactions may underlie the mental deficiency seen in DS and may predispose to the eventual development of Alzheimer's disease (AD) in DS individuals (39,40). The cellular membrane findings may be related to reported CNS membrane lipid abnormalities in DS (1,2). The nuclear morphologic observations may be related to the reported differences in chromatin and nuclear histone expression in AD (7,8). These results strengthen the role of the trisomy 16 mouse as a model for DS and potentially for AD.

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Down's syndrome Membranes Microtubules Nucleus Trisomy 16

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DOWN'S syndrome (DS) is the most common identified cause of mental retardation. Even though the trisomic 21st chromosome is a marker for DS, it is not clearly understood how this genetic material causes neurologic impairment (5).

The published literature suggests that cytoskeletal abnormalities may underlie the neurobiologic cause of the mental deficiency associated with DS. The cytoskeleton is composed of three major polymeric protein systems: microtubules, intermediate filaments and microfilaments, as well as interconnections that link these systems together. These cytoskeletal components are important in developing and maintaining cell shape, allowing cell motility, moving organelles and molecules throughout the cell, and may be involved at the synaptic level in the process of learning (15,25,27). With Golgi staining techniques, DS cerebral cortical neurons have been shown to have abnormalities in dendritic arborization and in dendritic spine shape and distribution (29,30,56,57), suggestive of an underlying cytoskeletal defect. Human neuropathologic results have shown a decreased number of cortical neurons in DS (48,61); this may be caused by slowed neurogenesis due to defective cytoskeletal interactions. Also, anomalous expression of the 210 Kdalton neurofilament subunit has recently been reported within the first few months of life in DS individuals (39).

The hypothesis of an underlying cytoskeletal defect in DS is strengthened by the association of DS with Alzheimer's disease (AD). Aging individuals with DS develop the neuropathological hallmarks of AD and a large proportion display evidence of decreasing mental abilities (9,28,60). Furthermore, cerebral cortical dendritic abnormalities similar to those found in DS have been described in AD (43,50,58). These morphologic changes seen in AD may be due to underlying abnormalities in neuronal microtubules (38).

Pathologically, AD is typified by the accumulation of paired helical filaments which share antigenic determinants with microtubules and neurofilaments (15,42). Abnormalities in microtubular biochemical assembly and ultrastructural appearance have been reported in AD (19,38). The microtubule-associated protein tau has also been shown to be aberrantly distributed in AD (23), and is incorporated into paired helical filaments of AD (18). It is possible that DS neuronal cytoskeletal components or their expression are intrinsically different from normals, thus predisposing DS individuals to the development of AD.

Other lines of research suggest a strong association between DS and AD: extra copies of 21st chromosome genetic material are found in nonfamilial AD (11); genetic polymorphisms in genes coded on the 21st chromosomes have been found in families with familial AD (49); the gene for beta amyloid, one of the abnormally stored materials in AD, has

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been mapped to the 21st chromosome (14). Furthermore, cytoskeletal abnormalities are common to both DS and AD. A microtubule-dependent event, lymphocyte capping in response to concanavalin A, have been shown to be defective in both DS and AD (12). The 210 Kdalton neurofilament subunit is redistributed to aberrant locations in AD neuronal perikarya (6,55) and is precociously expressed in DS (39).

To directly investigate cytoskeletal expression in DS an electron microscopic (EM) study would be of value. An EM investigation of cerebral cortical tissue taken from five children with unspecified mental deficiency revealed neuronal microtubules in disarray (4,43). An EM DS investigation has not been reported.

It has not been possible for the author to obtain adequate DS central nervous system (CNS) tissue for an EM study. Therefore, fetal murine trisomy 16 CNS was used. Murine trisomy 16 is an excellent model of DS (13, 17, 45). The human 21st chromosome and the mouse 16th both code for the free radical scavenging enzyme, superoxide dismutase-1 (SOD-1), the purine biosynthetic enzyme phosphoribosylglycinamidine synthetase (PRGS), the protooncogene ETS-2 and the interferon alpha and beta receptors. Most recently the gene coding for beta amyloid, one of the abnormal proteins which accumulate in AD, has been mapped to the 21st chromosome (14). Significantly, this same gene has been identified on the mouse 16th chromosome (46). In both cases it is the distal part of the chromosome that codes for SOD-1 and PRGS. Phenotypic features of human DS and murine trisomy 16 are also similar including flat facies, shortened neck, ear abnormalities, congenital heart disease (endocardial cushion defects and aortic arch abnormalities), fetal edema, thymic hypoplasia and decreased T-lymphocyte and antibody responsiveness. Both conditions have high rates of fetal wastage. The electrical membrane properties of cultured dorsal root ganglial neurons from DS and trisomy 16 are similar (36,51). Brain development is likewise similar with decreased brain size, reduced neuronal numbers and decreased brain levels of catecholaminergic, cholinergic and serotonergic markers (3,13,37,48,54,61).

An EM study of murine trisomy 16 CNS has not been previously reported. In undertaking this study, primary emphasis was placed on cytoskeletal expression. Observations were also made on the ultrastructural appearance of neuronal membranes and nuclei. In DS, CNS membrane lipid abnormalities have been reported (1,2) and these may be related to the observed membrane electrophysiologic abnormalities in DS and trisomy 16. Nuclei were also investigated since chromatin and the nuclear proteins—histones—have been reported to be abnormally expressed in AD (7,8).

for the 16th chromosome. Since the trisomy 16 mice do not survive to birth, all of the experiments were performed using fetuses obtained from timed pregnancies. The day that the vaginal plug was found was denoted as gestation day E0.

The identification of trisomic fetuses for this study has been for the most part on the basis of morphologic appearance. The difference in appearance between normals and trisomic fetuses is so striking that misidentification is difficult. The smaller length, flattened facies, abnormal ears, and striking hygroma of the neck have been uniformly present in all the trisomic conceptuses. The morphologic appearance of the trisomic fetuses is illustrated elsewhere (41).

To make sure that the phenotypic identification was correct chromosome analyses were performed on cultured fibroblasts taken from two phenotypic normal and three trisomic fetuses. The karyotyping was kindly performed by Dr. V. Markovic of the Genetics Laboratory, Surrey Place Centre. The karyotypes uniformly confirmed the phenotypic expression. Trisomic karyotypes are illustrated elsewhere (41).

The pregnant mice were sacrificed by cervical dislocation. After removal from the uterine sack, the normal and trisomic fetuses were rapidly decapitated and the parietal telencephalic vesicles dissected. Only fetuses which were clearly living were used. Since only 10 to 15% of the conceptuses were trisomic, the trisomic tissue was always prepared first. The CNS tissue was sectioned with a razor blade into 1 mm thick slices and placed into ice-cold 4% paraformaldehyde, 0.2% glutaraldehyde in phosphate buffer for 24 hr. It was not possible to perform intracardiac perfusion of the fetuses because of the cardiac defects in the trisomic mice. These defects would have produced perfusion dynamics different from these in normals thus compromising the results.

Sections were postfixed in buffered 2% OsO₄ for 1 hr and in buffered 2% OsO₄, 3% potassium ferrocyanide for another hr before embedding in epon. Silver sections were stained with 5% uranyl acetate and 0.1% lead citrate. A Phillips model EM 300 (Department of Pathology, Hospital for Sick Children) was used for EM observation and photography. A total of three litters were studied—one trisomic and three normals from each litter. The fetuses were of gestational age El7 (EO denotes the day that the vaginal plug was found). The observations were limited to the telencephalic vesicle cortical plate—a region in which postmitotic and postmigratory neurons, which will eventually form the cerebral cortex, are located (44). The cortical plate is primarily composed of immature neurons.

Nuclear circumference and area were determined planimetrically from EM photographs. Student’s t-test and the two-tailed t-test were used for statistical analysis.

METHOD

Strains of mice with a Robertsonian metacentric translocation of the 16th chromosome were obtained from the Jackson Laboratories, Bar Harbor, ME, which maintains these strains under contract to the National Institutes of Child Health and Human Development, Bethesda, MD. The strains that were used are Rb (6,16) 24 Lub and Rb (16,17) 7 Bnr. In order to obtain trisomic mice, two generations of breeding are required. The progeny of the first cross-breeding of the two Robertsonian translocation strains are double heterozygotes which carry a balanced translocation of the 16th chromosome. The double heterozygotes when sexually mature are bred with normal C57 mice. Approximately 15% of the conceptuses from this mating are trisomic for the 16th chromosome. Since the trisomy 16 mice do not survive to birth, all of the experiments were performed using fetuses obtained from timed pregnancies. The day that the vaginal plug was found was denoted as gestation day E0.

In the normal fetal mouse the EM revealed tissue of excellent quality (Fig. 1). In the cortical plate cell membranes and membrane apposition were well preserved, and subcellular components such as nuclei, nucleoli, mitochondria, ribosomes and lysosomes are easily identifiable. Microtubular profiles were also readily discernable.

In the trisomy 16 cortical plate, a uniform finding was that the integrity of cell-to-cell appositions and membranes were not as well preserved as in normals (Figs. 2, 3 and 6). Due to poor cell-to-cell apposition space between cells was significantly increased in trisomy 16 (Fig. 2). In areas where dendritic profiles were cross-cut it is evident that trisomic cellular membranes are much more fragmented than normals.

RESULTS

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FIG. 1. Electron microscopic photograph of normal, E17 mouse telencephalic vesicle cortical plate neurons. The quality of the tissue is excellent with excellent cell membrane apposition. Subcellular structures such as nuclei, nucleoli, mitochondria, ribosomes and lysosomal apparatuses are easily identifiable. There is little extracellular space evident. Microtubular profiles are also readily discernable. The scale bar indicates 2 μm.
FIG. 2. Electron microscopic photograph of trisomy 16, E17 mouse telencephalic vesicle cortical plate neurons. Cellular membranes and cell-to-cell appositions are not as intact as in the normal mouse (Fig. 1). Asterisks label areas of significantly increased extracellular space. Subcellular organelles are easily identifiable. In several areas (arrows) microtubular profiles are evident and appear more coiled than in the normal mouse. The scale bar indicates 2 μm.
FIG. 3. Higher power electron microscopic photographs of normal (A) and trisomy 16 (B) E17 mouse telencephalic vesicle cortical plate. In both images dendritic profiles are cross-sectioned. It is evident that in the trisomic mouse there is poor preservation of cell membrane integrity and cell-to-cell apposition. Microtubular profiles in cross-cut profile can be seen in both photographs. Within intact dendrites the trisomic microtubules do not differ significantly from normals in appearance, numerical density or in spacing. The scale bar indicates 0.5 μm.
FIG. 4. Electron microscopic photographs of normal (A) and trisomy 16 (B) E17 mouse telencephalic vesicle cortical plate. A and B were taken from littersmates. Microtubules within dendritic processes are evident. In trisomy 16 the microtubules are more curved and coiled than in the normal. The scale bar indicates 0.5 μm.
FIG. 5. Electron microscopic photographs of normal (A) and trisomy 16 (B) E17 mouse telencephalic vesicle cortical plate. A and B were taken from litter mates of a different litter from that illustrated in Fig. 4. In trisomy 16 the microtubules are more curved and coiled than in the normal. The scale bar indicates 0.5 μm.
FIG. 6. Electron microscopic photograph of trisomy 16, E17 mouse telencephalic vesicle cortical plate neurons revealing the general appearance of neuronal nuclei. In conjunction with the nuclear profiles illustrated in Fig. 2, when compared to normal nuclei (Fig. 1), trisomic nuclei appear to be smaller in size and more irregular in contour. As in Fig. 2, cell-to-cell apposition is not as well preserved as in normals. The scale bar indicates 2 μm.
These findings cannot be explained purely on the basis of lar stacks of folded cytomembranes, as described in AD prepared first, before that of the normal littermates. Consistently, the normal tissue was of excellent quality as illustrated in Fig. 1 and the trisomic tissue revealed poor cell-to-cell apposition and increased membrane fragmentation. These findings cannot be explained purely on the basis of technical factors.

Trisomic microtubules had a different appearance from normals: they were more curved and coiled than their normal counterparts (Figs. 2, 4 and 5). These microtubular abnormalities are suggestive of reported microtubular disarray found in conditions associated with mental deficiency (4,43) but are much less severe. In particular, dendritic varicosities with malaligned microtubular profiles were not observed. In fields of dendritic cross-sections, within intact dendrites, the apparent numerical density and spacing of microtubules did not differ between normal and trisomy 16 mice (Fig. 3). Such abnormalities have been reported in human mental deficiency (4) and in AD (38). Paired helical filaments, the pathological hallmark of AD, were not observed in our trisomy 16 material.

An additional finding was that neuronal nuclear profiles in trisomy 16 were different from normal. Trisomic nuclei were smaller and were more irregular (Figs. 2 and 6). The nuclear sizes were determined planimetrically from the EM photographs. A total of 117 normal and 131 trisomic nuclei were analyzed. The mean area of the trisomic nuclei was 12.33 μm², with a standard deviation (SD) of 8.35 μm²; and for the normal nuclei 15.53 μm² with SD of 9.74 μm². Using a two-tail t-test for statistical comparison, trisomic nuclei were significantly smaller than normals (p<0.01).

From the EM pictures, the mean circumference of the trisomic nuclei was 3.66 μm with a SD of 1.76 μm and for the normal nuclei 3.14 μm with a SD of 1.51 μm. The circumference of the trisomic nuclei was significantly greater than normal (p<0.05). The significant increase in nuclear circumference in conjunction with decreased mean nuclear cross-sectional area indicates that the trisomic nuclei are indeed more irregular than normals, as visual inspection had suggested (Figs. 2 and 6).

Nuclear envelope invaginations have been reported to occur in neurons during development (24). To see whether the data concerning greater nuclear circumference in trisomy 16 could be due to an increased number of nuclear envelope invaginations, these were counted. A significant invagination was defined as one whose depth was greater than its width at the nuclear surface and whose depth was greater than 10% of the mean nuclear diameters. The incidence of nuclear invaginations did not differ significantly between the normal and trisomic nuclei, with 19% of normal nuclei and 18% of trisomic nuclei having invaginations. Only two normal and two trisomic nuclei had more than one invagination (two invaginations in each case). Thus, the increased nuclear circumference in trisomy 16 neurons was due to nuclear shape irregularity, not due to an excessive number of nuclear invaginations.

The numbers of nucleoli were also counted. There was no significant difference in the incidence of nucleoli—73% of the normal nuclei and 70% of the trisomic nuclei had nucleoli. The numerical distribution of nucleoli likewise did not differ significantly between normal and trisomic nuclei.

Discussion

The cytoskeletal EM observations in trisomy 16 revealed microtubular profiles which were more curved and coiled than in normals. These results are analogous to the reported microtubular disarray found in human conditions associated with mental deficiency (4,43), but are much less severe. Dendritic varicosities and malaligned microtubular profiles were not observed. The apparent numerical density and spacing of microtubules did not differ between normal and trisomy 16 mice as has been reported in human conditions associated with mental retardation and in AD (4,38). As discussed in the introduction, the ultrastructural microtubular differences in trisomy 16 may be analogous to the proposed cytoskeletal abnormalities in DS which may in turn predispose DS individuals to develop AD.

The EM also revealed increased cellular membrane fragmentation in trisomy 16 CNS neurons. During the processes of fixation, embedding and/or sectioning, trisomic neuronal membranes became fragmented and lost cell-to-cell apposition. CNS lipid abnormalities have been reported in DS. In particular, phosphatidylethanolamine content is decreased and the cholesterol/phospholipid ratio is increased (1,2). Possibly similar membrane lipid abnormalities occur in trisomy 16 which may account for the observed ultrastructural membrane changes. CNS membrane constituent investigations have not been reported in trisomy 16. However, both DS and trisomy 16 cultured dorsal root ganglia cells display abnormal electrophysiologic properties (36,51) which may be on the basis of abnormal membrane constituents. Alternatively, anomalous expression of cerebrovascular amyloid protein (CVAP; beta amyloid) may account for these membrane findings. In normal rodents, CVAP is widely expressed throughout the CNS as a cell surface receptor (53). CVAP is coded in the mouse 16th chromosome and may be anomalously expressed in trisomy 16. CVAP may play an important role in cell membrane integrity and in maintaining cell-to-cell contacts (20,53).

CNS nuclear morphologic differences in DS and in the trisomy 16 mouse have not been previously investigated. Within circulating leukocytes, however, nuclear differences in DS individuals have been noted. In DS the nuclei of polymorphonuclear neutrophil cells have fewer lobes than normal (21, 32, 47, 52, 59). In males with DS the neutrophil lobe count is 2.11, 2.19 in DS females, 2.62 in control males and 2.61 in control females (33). Furthermore, Davidson et al. (10), reported that in normal females, 14 out of 500 neutrophils have an appendage described as a "drumstick." In DS females the incidence of "drumsticks" is very low—3.8 per 500 cells (34,35).

Our results indicate that trisomy 16 CNS neurons have nuclei that are smaller and more irregular in shape than normals—results which are at variance with the decreased segmentation of circulating polymorphonuclear neutrophils in DS. Release of immature, poorly segmented neutrophils into the circulation is a natural response to bacterial infection and is termed "a shift to the left." Possibly the reported neutrophil nuclear findings in DS are related to the well-documented immune system abnormalities and increased incidence of infections in DS and not to an affect on nuclear structure by the additional chromosomal material.

The trisomy 16 CNS nuclear observations may be a reflection of the additionally stored chromosomal material in each nucleus. Alternatively, abnormalities in chromatin and nuclear proteins—histones—have been described in AD. For instance, histones have been shown to decrease with age and increase in AD. In addition, trisomy 16 may also be analogous to Down's syndrome.
eukaryotic gene expression it is necessary for the genome to be accessible to RNA polymerase systems. The H1 histones can condense DNA and make it inaccessible for transcription (22). CNS chromatin in AD is in a much higher state of compaction (7), is less accessible to the enzyme micrococcal nuclease (26) and has increased H1 linker histones on dinucleosomes (8). Possibly, trisomy 16 nuclei have similar histone abnormalities and that the observed nuclear morphologic differences are due to differences in these nuclear proteins.

The EM cytoskeletal, cellular membrane and nuclear contour observations strengthen the role of the trisomy 16 mouse as a model for DS and potentially for AD.

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REFERENCES


