

Dendritic Anomalies in Disorders Associated with Mental Retardation

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Dendritic abnormalities are the most consistent anatomical correlates of mental retardation (MR). Earliest descriptions included dendritic spine dysgenesis, which was first associated with unclassified MR, but can also be found in genetic syndromes associated with MR. Genetic disorders with well-defined dendritic anomalies involving branches and/or spines include Down, Rett and fragile-X syndromes. Cytoarchitectonic analyses also suggest dendritic pathology in Williams and Rubinstein-Taybi syndromes. Dendritic abnormalities appear to have syndrome-specific pathogenesis and evolution, which correlate to some extent with their cognitive profile. The significance of dendritic pathology in synaptic circuitry and the role of animal models in the study of MR-associated dendritic abnormalities are also discussed. Finally, a model of genotype to neurologic phenotype pathway in MR, centered in dendritic abnormalities, is postulated.

Introduction

Since the early 1970s, the application of dendritic labeling techniques has shown that abnormalities of this postsynaptic neuronal process are common in genetic and environmental conditions associated with mental retardation (MR) [reviewed by Huttenlocher and Kaufmann (Huttenlocher, 1991; Kaufmann, 1996)]. Although these studies have been quite informative, their limitations in terms of number of examined disorders, sample size and tissue preservation have precluded definitive conclusions about the nature and specificity of dendritic anomalies in MR. The recent identification of the genetic bases of several prevalent MR-associated disorders, combined with the accompanying generation of animal models by transgenic technology, makes the elucidation of the neuronal phenotype of these disorders even more compelling.

For the purposes of this review, we will define as 'MR-associated disorders' those conditions characterized by a non-progressive global cognitive deficit (e.g. Down syndrome, DS). We will focus on genetic syndromes, because they provide the potential for understanding the pathogenetic mechanisms of dendritic anomalies, and will also make some reference to childhood degenerative disorders that during their evolution display a cognitive profile that resembles MR (Kaufmann, 1996). The review includes a survey of disorders associated with dendritic anomalies, analyses of the relationships between dendritic pathology, cognitive phenotype and synaptic abnormalities, and the characterization of dendritic pathology in animal models relevant to MR. We conclude with a proposal for pathways linking specific gene mutations and dendritic phenotype.

Dendritic Spine Dysgenesis and Unclassified MR

The pioneering studies with Golgi impregnations by Huttenlocher (Huttenlocher, 1970, 1974) and Purpura (Purpura, 1975a,b) established the foundations for the assessment of

dendrites in MR. The importance of these investigations is underscored by the fact that standard neuropathological methods, which had provided critical information about the biological bases of many neurological diseases (e.g. Parkinson disease) (Hornykiewicz, 1963), had failed to disclose specific abnormalities in many cases of MR (Freitag and Lindenberg, 1967; Crome and Stern, 1972; Jellinger, 1972). The 'rediscovered' Golgi method demonstrated two fundamental abnormalities in the cerebral cortex: reduction in number and length of dendritic branches and the aberrant morphology and number of dendritic spines (Huttenlocher, 1970, 1974; Purpura, 1974, 1975a,b). Most of the evaluations focused on pyramidal neurons, which constitute the majority of the neurons in human neocortex (Crosby *et al.*, 1962). In these cells, both apical and basilar branches were noted to be shorter or less complex in the brains of persons with unclassified MR. The most consistent and intriguing findings involved the shape and length of dendritic spines. The latter were not only sparse but also long and thin and, when compared with controls of different ages, resembled immature dendritic spine patterns (Purpura, 1974). Following these initial descriptions, this type of dendritic anomaly – referred to as spine 'dysgenesis' – was reported by Marin-Padilla in chromosomopathies associated with MR (see Figure 1) (Marin-Padilla, 1972, 1974, 1976). Recent investigations in children and adolescents with unclassified MR have confirmed reduced density and spine dysgenesis involving apical dendrites of the prefrontal cortex (von Bossanyi and Becher, 1990). However, the significance of dendritic spine abnormalities in unclassified MR is not yet clear. Changes in dendritic shafts are not necessarily present in every case. Huttenlocher, in his 1991 review on the subject, emphasized that in brains from adolescents with moderate to profound MR dendrites branches appeared comparable to normal controls (Huttenlocher, 1974, 1991). Williams *et al.* also found this to be the case in a qualitative study of autistic individuals with MR (Williams *et al.*, 1980). The extent to which age or degree of MR plays a role in dendritic arborization reduction in MR is still unknown. These initial investigations thus provided intriguing leads that MR is associated with dendritic abnormalities, but did not furnish definitive information with respect to their significance.

A strength of these pioneering studies resides in the combination of post-mortem and biopsy material. The latter, by eliminating the effects of post-mortem degradation, not only allowed optimal conditions for the Golgi material but also provided the opportunity for high-quality ultrastructural analyses. Purpura and collaborators (Purpura *et al.*, 1982) reported that varicosities in dendrites of both pyramidal and non-pyramidal neurons were associated with the spine changes described above and with disruption in microtubule organization. By 3-D reconstructions of these microtubular disarrays, these authors demonstrated that microtubular changes underlie

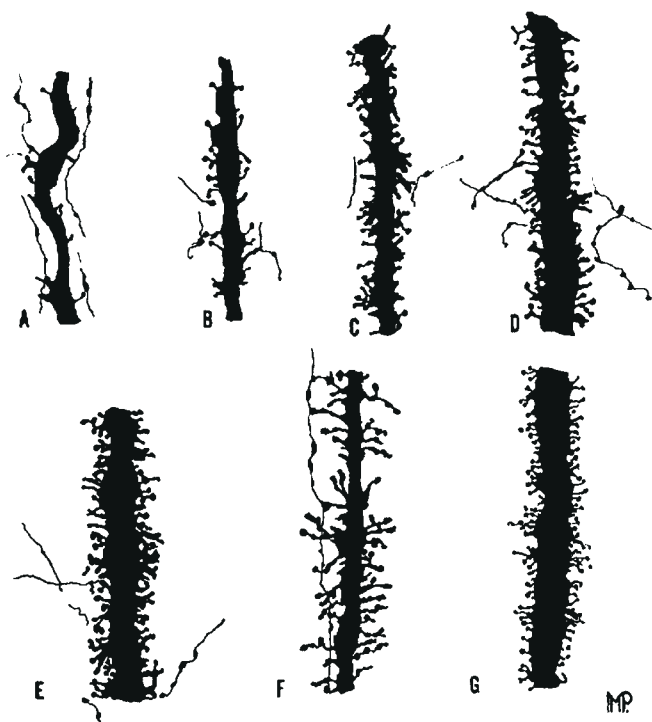


Figure 1. Dendritic spine abnormalities in Patau syndrome and DS. Drawings are from Golgi preparations depicting comparable segments of apical dendrites from layer V pyramidal neurons (motor cortex). (A–E) Different developmental stages in normal subjects (5th gestational month, 7th gestational month, neonatal period, 2nd postnatal month, 8th postnatal month, respectively). (F) A newborn with 13–15 trisomy. (G) An 18-month-old infant with DS (trisomy 21). Note the progressive increase in spine density, associated with a reduction in spine length, during normal development. Spines in Patau syndrome are not only sparse, but also longer than expected for a neonate. On the other hand, the infant with DS had shorter and thinner rather than long spines. Reprinted with permission (Marin-Padilla, 1972).

dendritic irregularities and that they represent aberrant cross-linking of cytoskeletal elements (Bodick *et al.*, 1982). Even though these data on unclassified MR provided a strong base for understanding the role of dendritic pathology in MR, the lack of definition of etiology made the delineation of pathogenetic mechanisms difficult. Many cases classified as ‘MR of unknown cause’ two or three decades ago, can now be defined in respect to etiology. For instance, the study by Williams and colleagues (Williams *et al.*, 1980) included a girl with features compatible with Rett syndrome (RS) (Huttenlocher, 1991). The recent identification of the gene responsible for a large proportion of RS cases (Amir *et al.*, 1999) now permits accurate diagnosis of this condition.

Genetic Disorders with Definitive Dendritic Involvement: DS, RS, Fragile-X Syndrome

Most of the studies of dendritic pathology in syndromes associated with MR have focused on pyramidal neurons in the cerebral cortex. The most comprehensively examined genetic disorders are DS and RS. DS, the most common genetic condition associated with MR (Moser, 1995), is characterized by abnormal physical and neurologic growth. In addition to malformations involving mainly the heart and gastrointestinal tract, brain growth is delayed in DS. Brain size and weight are reduced at birth, gyral pattern is immature, neocortical laminar formation is irregular and myelination of cortical fibers is delayed (Kemper,

1988; Wisniewski, 1990; Golden and Hyman, 1994; Wisniewski and Kida, 1994). Several neocortical regions have been examined by Golgi impregnations and have revealed changes similar to those reported in unclassified MR. The initial study of an 18-month-old infant with DS by Marin-Padilla, the earliest to analyze dendritic spines in MR, demonstrated spines that were sparse, small and had short stalks in the motor cortex (Marin-Padilla, 1972). This dendritic spine abnormality contrasted with features displayed in a newborn with Patau syndrome (D, 13–15 trisomy), which demonstrated the typical elements of spine dysgenesis (Purpura, 1974), decreased density, and long and tortuous profile (Marin-Padilla, 1972, 1974) (see Figure 1). A later and more detailed follow-up study by Marin-Padilla (Marin-Padilla, 1976) showed that the spine changes in DS are not specific. Short spines were intermingled with the unusually long spines described by Purpura (Purpura, 1974). Interestingly, the spine dysgenesis was associated with dendritic vacuolation and neuronal necrosis, suggesting a degenerative process in DS (Marin-Padilla, 1976). At least three other investigations have examined dendritic spines in DS neocortex and hippocampus. Suetsugu and Mehraein (Suetsugu and Mehraein, 1980) counted spines in the cingulate cortex and hippocampus of seven DS subjects without Alzheimer disease (AD)-like pathology. They found that, compared with normal controls, spines were significantly reduced in the middle and distal segments of apical dendrites. That these findings in DS are specific is suggested by the fact that similar analyses in a group of individuals with unclassified MR did not show a decrease in dendritic spines (Suetsugu and Mehraein, 1980). The possibility that dendritic spine dysgenesis in DS represents an early postnatal degenerative process was raised by the study by Takashima and colleagues (Takashima *et al.*, 1981), one of the largest studies in respect to sample size, which analyzed neurons of the visual cortex in fetuses, neonates and infants with DS. A decrease in dendritic spines was observed only in newborns and older subjects with DS (Takashima *et al.*, 1981). Another study which supports the unique nature of spine pathology in DS is that of Ferrer and Gullotta (Ferrer and Gullotta, 1990). They examined CA1–CA3 pyramidal neurons of the hippocampus in two older patients with DS but without AD features. Spine counts were compared with normal controls and two DS subjects with associated AD pathology. The number of spines was reduced in all DS subjects, even though the changes were more severe in those DS individuals with AD particularly in CA1.

Dendritic length has also been evaluated in DS neocortex. In the previously cited study by Takashima and collaborators (Takashima *et al.*, 1981), 14 fetuses and infants with DS were compared with normal age-matched controls. They found that shorter basilar dendrites were present only in those DS subjects who were older than 4 months. In a subsequent quantitative study of multiple dendritic branch parameters in eight infants and children with DS they showed that dendritic branching (measured as intersections using the Sholl’s concentric circle) and length, in both apical and basilar dendrites, was greater than in controls in DS infants less than 6 months old (Becker *et al.*, 1986). Subsequent to this age, there was a steady decrease in these measures to below normal in subjects older than 2 years. These changes seemed more dramatic in apical dendrites of layer III neurons (Becker *et al.*, 1986). Additional cross-sectional studies have demonstrated marked reductions in dendritic branching, length and spine density in aged DS subjects (Takashima *et al.*, 1989) [reviewed by Becker *et al.* (Becker *et al.*, 1991)]. In these older individuals degenerative neuronal

changes, such as those described by Marin-Padilla (Marin-Padilla, 1976), were also associated with dendritic abnormalities. In the last decade, these findings have been corroborated by two other reports on pyramidal (Schulz and Scholz, 1992) and non-pyramidal neurons (Prinz *et al.*, 1997) from the parietal and motor cortices, respectively. Normal or increased branching in DS infants (Schulz and Scholz, 1992; Prinz *et al.*, 1997) contrasts with reduced dendrites and degenerative changes in older DS children (Schulz and Scholz, 1992).

In contrast to DS, reductions in dendritic arborizations are present throughout life in RS (Armstrong *et al.*, 1995). RS is a developmental disorder that affects females almost exclusively, with a large proportion of cases linked to mutations in the X chromosome gene *MeCP2* (Amir *et al.*, 1999). The latter codes for a transcriptional regulator protein, linked to transcriptional repression of methylated gene sequences (Stratling and Yu, 1999). *MeCP2* mutations in RS would lead to altered functional domains of the protein (Amir *et al.*, 1999; Wan *et al.*, 1999). RS is characterized by an apparently normal perinatal period, followed by physical and neurological developmental arrest. Between ages 2 and 10 years (stages II and III) there is a regression of language and motor skills, seizures, and appearance of characteristic stereotypic movements. Severe neurologic impairment, including MR, stabilizes by late childhood (Naidu *et al.*, 1995; Naidu, 1997). Initial neuroanatomic evaluations showed that cortical structure, including neuronal number and lamination, are relatively preserved in RS (Jellinger and Seitelberger, 1986; Jellinger *et al.*, 1988). Cytoarchitectonic studies by Bauman and colleagues (Bauman *et al.*, 1995) showed that while neuronal size is reduced there is an increase in neuronal cell packing density. Consistent with this increased neuronal packing, Armstrong and collaborators (Armstrong *et al.*, 1995) demonstrated that basilar and, in some instances, apical branches of pyramidal cells from frontal, motor and inferior temporal cortices were significantly reduced when compared with normal controls. Relative preservation of posterior cortices is suggested by both volumetric neuroimaging (Reiss *et al.*, 1993) and these post-mortem dendritic (Armstrong *et al.*, 1995) studies. Basilar dendrites were more affected than apical ones, particularly in layer V neurons (Armstrong *et al.*, 1995). A more recent investigation by the same group provided additional evidence for the specificity of the dendritic abnormalities in RS cerebral cortex. When compared with neurons from individuals with DS, dendritic arborizations from RS subjects showed the greatest reductions again in premotor, motor and inferior temporal cortices (Armstrong *et al.*, 1998). These data underscore the severe nature of the dendritic tree disturbances in RS neocortex. Belichenko and collaborators (Belichenko *et al.*, 1994), using the lipophilic dye labeling technique and confocal microscopy, were able to delineate the 3-D structure of dendrites from prefrontal, motor and middle temporal areas. They demonstrated that apical dendrites were asymmetric and reduced, and that dendritic spines were markedly decreased, with some segments devoid of these elements. Apparently these spine changes did not correspond to spine dysgenesis, since illustrations in this publication show rather short but otherwise unremarkable spines (Belichenko *et al.*, 1994). Dendrites of hippocampal neurons have also been analyzed in RS. Armstrong *et al.* (Armstrong *et al.*, 1995) showed reduced dendritic arborizations confined to neurons of layers II and IV of the subiculum, and none in CA1. These data are somewhat at variance with cytoarchitectonic evaluations that reported higher neuronal density throughout

the pyramidal layer (including CA1) and, to a lesser extent, in the subiculum (Bauman *et al.*, 1995).

Fragile-X syndrome (FraX) is the second most genetically determined form of MR [reviewed by Moser and Kaufmann and Reiss (Moser, 1995; Kaufmann and Reiss, 1999)]. Despite its high frequency, FraX has been studied less extensively with neuropathological techniques than DS or RS. Only two publications have addressed the issue of neuronal abnormalities. Hinton and colleagues (Hinton *et al.*, 1991) extended the data on one subject previously reported by Rudelli *et al.* (Rudelli *et al.*, 1985). Neocortical analyses of the three mildly to moderately mentally retarded adult FraX subjects showed that neuronal density in the posterior cingulate and anterior temporal regions was similar to controls. However, Golgi preparations showed long tortuous dendritic spines with prominent heads. The less than optimal quality of the Golgi impregnations precluded an analysis of the dendritic branch or spine density (Hinton *et al.*, 1991). No subsequent studies have been reported in FraX subjects, although dendritic labeling by Golgi techniques has been applied to the mouse model of this condition, as described in a following section.

Genetic Disorders with Probable Dendritic Involvement: Williams Syndrome, Rubinstein-Taybi Syndrome

Cytoarchitectonical techniques have helped to delineate cortical development in a large number of developmental disorders [reviewed by Kaufmann (Kaufmann, 1996)]. These approaches have been particularly helpful in conditions affecting neuronal proliferation and migration, in which disturbances in laminar organization and neuronal number typically occur [reviewed by Barth and Kaufmann and Galaburda (Barth, 1987; Kaufmann and Galaburda, 1989)]. Cytoarchitectonic studies have also shown aberrant cytodifferentiation in neuronal migration disorders, such as the presence of neurons of abnormal size and orientation [reviewed by Rorke and by Crino and Eberwine (Rorke, 1994; Crino and Eberwine, 1997)]. Similar findings have been reported by Logdberg and Brun (Logdberg and Brun, 1993) in unclassified MR. In addition to these parameters, quantitative cytoarchitectonics can provide information about neuronal cell body size and cell packing density. In RS, for instance, there is a reduction in neuronal soma size that is associated with an increase in cell packing density (Bauman *et al.*, 1995). Similar features have been reported by the same investigators in the hippocampus and other limbic-related regions in autism (Bauman and Kemper, 1985) [reviewed by Kemper and Bauman (Kemper and Bauman, 1998)]. The significance of increased neuronal density has been underscored by parallel studies using the Golgi method that showed a correlation between cell packing and reduction in dendritic arborizations in these two conditions [discussed for RS (Raymond *et al.*, 1996; Kaufmann *et al.*, 1998) and for autism (Kemper and Bauman, 1998)]. These data support the concept that the finding of increased neuronal density by cytoarchitectonic analyses can be interpreted as signifying a likely reduction in length and complexity of dendritic trees.

Preliminary studies of cortical cytoarchitecture have been performed in two genetic disorders associated with MR: Williams syndrome (WS) and Rubinstein-Taybi syndrome (R-TS). WS is caused by a submicroscopic deletion on chromosome 7q11.23, which includes the elastin gene and the *HPC-1/syntaxin 1A (STX1A)* gene that codes for a protein involved in the docking of synaptic vesicles [reviewed by Bellugi *et al.* and Botta *et al.* (Bellugi *et al.*, 1999a; Botta *et al.*, 1999)]. Patients with WS show a distinctive cognitive and social phenotype.

While they demonstrate relatively preserved language and face processing abilities, they are typically impaired in visuospatial domains. In addition, they are hypersociable, with engaging personality and excessive sociability with strangers (Bellugi *et al.*, 1999a,b). Neuropathological data on WS are limited to descriptions of associations with CNS malformations (Pober and Filiano, 1995) and AD-like changes (Golden *et al.*, 1995). A single study of one patient by Galaburda and colleagues (Galaburda *et al.*, 1994) reported several cytoarchitectural anomalies, which included a reduction in columnar organization throughout the cortex, abnormal neuronal orientation and a generalized increase in cell packing density. These findings appeared to be more severe in posterior cortical regions, where there was a decrease in volume (Galaburda *et al.*, 1994), as had previously been shown by quantitative neuroimaging (Jernigan *et al.*, 1993). As the authors of both publications point out, this topographic distribution of severity of abnormalities is in general agreement with the WS cognitive phenotype. In conclusion, preliminary data indicate that in WS there is selective cortical hypoplasia associated with cytoarchitectural features previously described in unclassified MR and DS (reduced laminar organization and abnormal neuronal orientation), and in autism and RS (increased neuronal cell packing density).

The second MR-related disorder in which cytoarchitectonics suggests dendritic abnormalities is R-TS. With an approximately similar incidence in the general population to RS (Moser, 1995), this condition is characterized by MR and a specific pattern of somatic abnormalities. The initial description by Rubinstein and Taybi (Rubinstein and Taybi, 1963) emphasized short stature, facial dysmorphism, broad thumbs and first toes, and MR. More recent work has demonstrated a wider spectrum of physical and neurologic abnormalities, which include deficits in expressive language and maladaptive behavior (Stevens *et al.*, 1990). The genetic defect in R-TS (16p13.3) has been reported to involve cyclic AMP response element binding protein (CREB)-binding protein (CBP) (Petrij *et al.*, 1995), a protein that is recruited by CREB to bind DNA and activates the basal transcription factor-enzyme complex (Kaufmann and Worley, 1999). Limited neuroimaging and neuropathologic investigations have shown an association between R-TS and several CNS malformations, such as agenesis of the corpus callosum (Stevens *et al.*, 1990), Dandy-Walker malformation (Bonnioli *et al.*, 1989) and cortical clefts (Sener, 1995). The most comprehensive neuropathologic evaluation of a R-TS brain was carried out by Pogacar and collaborators (Pogacar *et al.*, 1973). These authors reported one adult male case (33 years) with mild reduction in brain weight, preserved general cortical architecture, but decreased neuronal size and a marked increase (semi-quantitative) in cell packing density. As the latter findings closely resembled those reported in RS (Kaufmann *et al.*, 1998), a reduction in dendritic arborizations appears also to be a feature of R-TS.

Two other relatively frequent genetic disorders, neurofibromatosis-1 and tuberous sclerosis (Bourneville disease, BD), usually present with mild MR or learning disorders (Harrison *et al.*, 1999; Ozonoff, 1999), and display focal cytoarchitectonic abnormalities suggestive of dendritic pathology. Direct dendritic evaluations have been carried out only in BD. Cortical tubers, the hallmark lesion in BD, are foci of disrupted laminar cortical architecture containing large and disorganized cells. Ferrer and colleagues (Ferrer *et al.*, 1984) and Machado-Salas (Machado-Salas, 1984) demonstrated that tubers consist of maloriented pyramidal neurons, with simplified structure and aberrant dendritic branches and spines. Moreover, large numbers of

Table 1
Neocortical cytoarchitectonic and dendritic abnormalities in genetic disorders associated with MR^a

Disorder	Laminar disturbance	Increased packing density	Reduced dendritic length	Spine dysgenesis
DS	Y	N	Y	Y
Fragile-X syndrome	N	N	N	Y
Neurofibromatosis-1	Y (focal)	N	?	?
Patau syndrome	N	N	Y	Y
Tuberous sclerosis	Y (focal)	Y (focal)	Y (focal)	Y (focal)
Williams syndrome	Y	Y	?	?
Phenylketonuria	N	Y	Y	Y
RS	N	Y	Y	Y
Rubinstein-Taybi syndrome	N	Y	?	?

^aThe conditions have been listed according to estimated incidence, as an adaptation of the article by Moser (Moser, 1995).

stellate neurons and astrocytes are also present in these clusters. Machado-Salas (Machado-Salas, 1984) even suggested neuroglial formations with specialized contacts. Huttenlocher and Heydemann (Huttenlocher and Heydemann, 1984) confirmed most of these findings and examined the neocortex intervening between tubers. Despite its normal architecture and dendritic morphology, this adjacent cortex shows a decrease in dendritic branch length. These authors emphasized the similarity between these dendritic reductions and those found in several forms of MR. Table 1 summarizes the dendritic and cytoarchitectonic abnormalities found in genetic disorders with definitive and probable dendritic involvement, respectively.

Dendritic Abnormalities and Cognitive Profile

Proof of dendritic pathology as a distinctive feature and substrate of MR requires the demonstration of its consistency across multiple conditions associated with MR. The data reviewed above suggest that either reductions in dendritic branch complexity or length or changes in dendritic spines are consistent features in genetic MR. More limited information is available about non-genetic causes of MR; nevertheless, a few studies demonstrate cytoarchitectonic and dendritic abnormalities similar to those in genetic disorders [reviewed by DeLong (DeLong, 1993)]. Two investigations have shown reduction in dendritic arborizations (Cordero *et al.*, 1993; Benitez-Bribiesca *et al.*, 1999) and spine dysgenesis (Benitez-Bribiesca *et al.*, 1999), in moderate to severe protein-calorie malnutrition. Iodine deficiency leads to MR, deaf-mutism and muscle hypertonia, in addition to the somatic changes associated with cretinism (DeLong, 1993). Neuropathological evaluations have shown that, in addition to moderate brain weight decreases, there is a reduction in neuronal density and aberrant neuronal orientation, and a decrease in dendritic branching involving both pyramidal and non-pyramidal neurons (DeLong, 1993; Yan *et al.*, 1989). Studies of fetuses from iodine-deficient mothers do not display such abnormalities (Liu *et al.*, 1989), suggesting that late phases of neuronal development, particularly differentiation, are affected most.

Are dendritic abnormalities a specific feature of MR? Huttenlocher (Huttenlocher, 1991) and others (Williams *et al.*, 1978) in their studies of dendrites have emphasized the existence of confounding variables, such as cardiac malformations in chromosopathies. Even after these factors are taken into account, the description of similar changes in several metabolic disorders that affect primarily the cerebral cortex and present with a degenerative-dementing course (Della Giustina, *et al.*,

1981; Takashima *et al.*, 1985) support the concept that marked dendritic abnormalities are an index of major neuronal disruption. However, there are some differences between the findings in metabolic-degenerative disorders and those of genetic MR. First, neuronal migration defect or severe lamination disturbance are relatively common in the progressive metabolic disorders (Barth, 1987) but absent in the non-progressive genetic disorders such as DS. Second, in many metabolic disorders there is accumulation of storage material in neuronal somata and proximal dendrites and axons that leads to distinctive Golgi impregnation patterns (Williams *et al.*, 1977; Purpura, 1978; Takashima *et al.*, 1985). The dendritic aberrations in storage disorders such as gangliosidoses and neuronal ceroid lipofuscinoses are associated with abnormalities of the axon and the presynaptic domain, and include anomalous spatial configuration of cortical neurons (Purpura and Suzuki, 1976). Phenylketonuria (PKU) shows a yet different pattern. This aminoacidopathy, which leads to MR when untreated, has been characterized from the neuropathologic standpoint to be associated with changes in the white matter. There is no storage. However, Bauman and Kemper (Bauman and Kemper, 1982) with the aid of Golgi techniques have shown that gray matter pathology, particularly dendritic changes, is even more pronounced than that in the white matter. They found reductions in dendritic arborizations and spine dysgenesis that were similar to those reported in unclassified MR and DS. It appears therefore that metabolic-degenerative disorders show qualitative and quantitative alterations of dendritic morphology that correlate with cognitive impairment as in MR. However, these changes are dynamic and share with MR only the diffuse and severe magnitude of the dendritic pathology (Kaufmann, 1992).

If severe dendritic changes are a reflection of generalized cortical dysfunction, milder cognitive impairment should be associated with milder dendritic abnormalities. Two studies have partially addressed this issue. The first compared cortical areas with significant dendritic reductions in RS, in which there is profound cognitive impairment, with comparable samples from DS patients. All three premotor, motor and visual cortices in RS had reduced dendritic length compared with DS, with the greatest reductions affecting the frontal regions (Armstrong *et al.*, 1998). As cognitive impairment is, in general, greater in RS than DS, these comparisons suggest a direct relationship between dendritic pathology and cognitive deficit. A more direct assessment was carried out by Yan *et al.* (Yan *et al.*, 1989), who demonstrated a parallel between dendritic decreases and degree of MR in adult cretinism. Unfortunately, virtually no data about dendritic anomalies are available in individuals with learning disabilities. If severity and extent of dendritic pathology correlates with cognitive function, it is expected that learning disabled subjects would show mild dendritic abnormalities. In support of this hypothesis are the findings in three cases with FraX, with IQ in the 40–60 range. Hinton and collaborators (Hinton *et al.*, 1991) reported the presence of dendritic spine abnormalities without cytoarchitectonic changes.

If dendritic abnormalities are a signature of global cognitive dysfunction, do they play a role in the selective cognitive deficits observed in many syndromes associated with MR? Neuropathologic and neuroimaging studies have already shown some correlations between cortical regional volume and specific impairment. For instance, in DS there is a reduction in temporal and frontal lobe volumes that, in general terms, is in agreement with the selective impairment in language in these patients (Kemper, 1988; Jernigan *et al.* 1993). Similar associations have

been demonstrated for visuospatial impairment and parieto-occipital volumes in WS (Jernigan *et al.*, 1993). These topographical surveys, which require the evaluation of a large number of cortical regions, can be correlated with neuroimaging approaches, particularly when large blocks of cortex are measured. A more difficult task is the quantitative or semi-quantitative study of cytoarchitectonics or Golgi-based dendritic evaluations of multiple cortical areas. To our knowledge, complete qualitative cytoarchitectonic surveys have only been reported for DS [multiple subjects (Kemper, 1988)], RS [three patients (Bauman *et al.*, 1995)] and WS [one individual (Galaburda *et al.*, 1994)]. Only the latter study attempted to relate pattern of cortical structure and cognitive profile, and no cytoarchitectonic investigation has yet reported measures of multiple cortical regions. In terms of dendritic arborizations, a single study of RS evaluated several cortical regions and neuronal populations within each area (Armstrong *et al.*, 1995). Areas related to preparation (area 6, frontal premotor) and execution (area 4, frontal motor) of movements were affected in RS, whereas the occipital visual cortex (area 17) was relatively preserved. These findings are consistent with the RS phenotype, in which there is severe motor impairment (hypotonia, poor hand use, stereotypic movements, gait disturbances) with relative sparing of visual function (Naidu, 1997). A follow-up study confirmed and expanded the initial observations, but also showed preservation of the superior temporal cortex (Armstrong *et al.*, 1998), a region involved in language processing. As language delay and regression are typical features of RS (Naidu, 1997), these later studies do not support the postulate that dendritic abnormalities reflect cortical dysfunction. More studies are needed to evaluate the relationship between dendritic abnormalities and cognitive profile in MR.

Another critical feature shown by several MR-associated disorders is the diminished maturational increment of cognition and sometimes-frank decline. This has been evaluated from the dendritic viewpoint in three of the main genetic syndromes: DS (Hayes and Batshaw, 1993), RS (Naidu, 1997) and FraX (Fisch *et al.*, 1999a). Studies of DS have suggested dendritic changes as a basis for cognitive decline, but there is as yet no evidence for such a relationship for the other two disorders. In cross-sectional analyses of dendritic growth (length) in DS (Takashima *et al.*, 1981; Becker *et al.*, 1986), dendritic arborizations appeared to be overproduced initially, with a tendency to regress towards the end of the first year, a temporal pattern that parallels cognitive evaluations. In contrast, Armstrong and collaborators (Armstrong *et al.*, 1995) did not find a relationship between dendritic length and age. It should be noted, however, that their regression analyses covered a wide age range (2.9–35 years), which could have masked the declining phase of cognition that occurs between ages 2 and 10 years.

Dendritic Abnormalities and Synaptic Circuitry and MR

The most obvious mechanism by which dendritic pathology in general, and reductions in dendritic arborizations in particular, could lead to neurologic impairment is a decrease in the cortical postsynaptic surface. The latter should correlate with a reduction in synaptic density, while spine dysgenesis may represent a preferential reduction in excitatory synapses. These assumptions have not yet been tested rigorously in genetic MR. To date no study has compared dendritic labeling and synapse analysis in MR, although some subjects have been independently examined by one or the other approach. Electron microscopic (EM) studies have been confined almost exclusively to DS, and with contra-

dictory results. There are reports of both decreased (Wisniewski *et al.*, 1985) and increased (Cragg, 1975) synaptic density as well as of arrest in synaptic development (increased primitive

synaptic contacts) (Petit *et al.*, 1984). Only a preliminary analysis by P.R. Huttenlocher (personal communication) found reduced synaptic density in the prefrontal cortex in RS. What are the factors causing these discrepancies? They could be related to the quality of the Golgi staining; long post-mortem interval, agonal changes and aldehyde fixation interfere with adequate dendritic impregnation (Williams *et al.*, 1978; Buell, 1982). Hinton and colleagues (Hinton *et al.*, 1991), in their study of FraX neocortex, stated that 'Golgi analysis was less than optimal because of incomplete dendritic stain impregnation', and observations about dendritic spine morphology are the only reliable data obtained in this investigation (Hinton *et al.*, 1991). In his review, Huttenlocher (Huttenlocher, 1991) emphasized age as an important factor. While dendritic labeling has been performed in young subjects, synaptic counts have involved older individuals. This is of particular significance, since some studies suggest catch-up dendritic growth in unclassified MR (Williams *et al.*, 1980). The potential of correlating EM and Golgi studies is suggested by Kaufmann *et al.* (Kaufmann *et al.*, 1995, 1997a,b), who demonstrated in RS a parallel between dendritic protein immunoreactivity, by immunoblotting and immunocytochemistry (Figure 2), and dendritic evaluations by Golgi (Armstrong *et al.*, 1995, 1998). Similar 'validation' of dendritic abnormalities (Kaufmann, 1992) has been carried out in HIV encephalopathy (Masliah *et al.*, 1997). Moreover, in RS dye labeling has also corroborated findings by Golgi impregnations (Belichenko *et al.*, 1994). These data suggest that combined

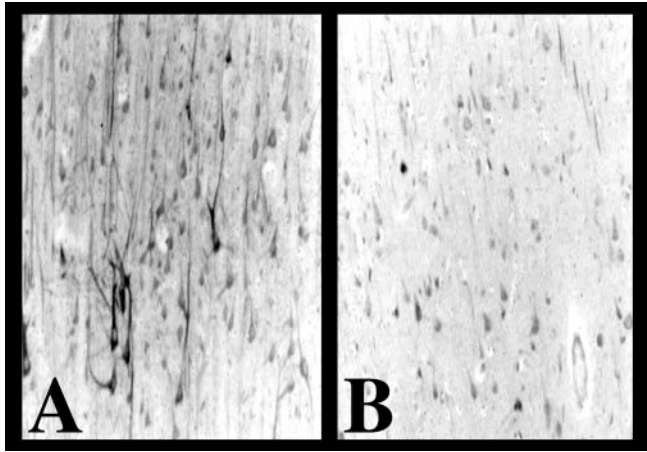


Figure 2. MAP-2 immunoreactivity in RS. The marked reduction in MAP-2 immunostaining in somatosensory cortex is also seen, with variable intensity, in other neocortical regions. (A) MAP-2 immunostaining of somas and, predominantly, dendrites in a control subject. (B) An equivalent section from an RS patient displays smaller neuronal somas and few MAP-2 immunoreactive dendrites (arrowheads). Reprinted with permission (Kaufmann *et al.*, 1998).

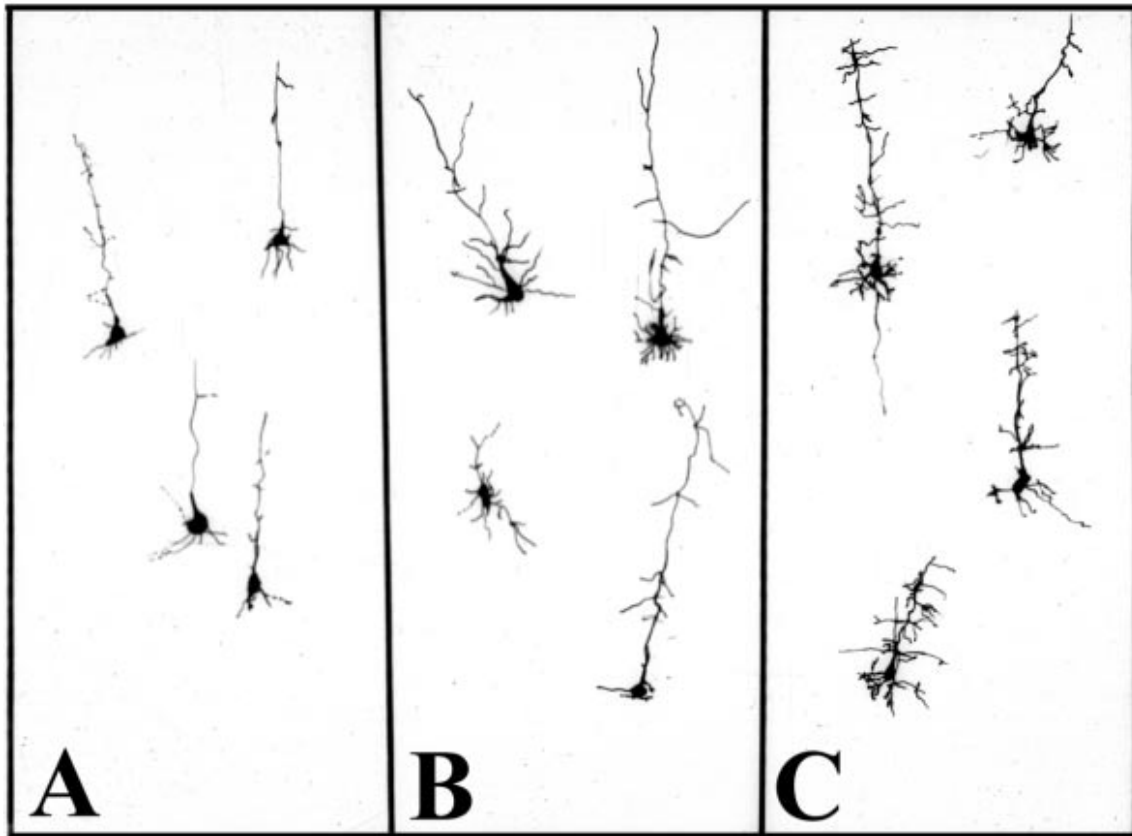


Figure 3. Dendritic anomalies in a model of MR. Camera lucida drawings of parietal cortex pyramidal neurons from juvenile (postnatal day 18) Sprague-Dawley rats. (A) Cells from animals that were deprived nutritionally and in terms of sensory stimulation, by changes in litter size. (B) The control situation. (C) Rats that were relatively enriched. Note the marked reduction in number and length of dendrites in deprived animals and the increased complexity of dendritic trees in enriched rats. Scale bar = 25 μ m. The experimental paradigm is explained in detail elsewhere (Adaro *et al.*, 1986). Reprinted with permission (Kaufmann *et al.*, 1994).

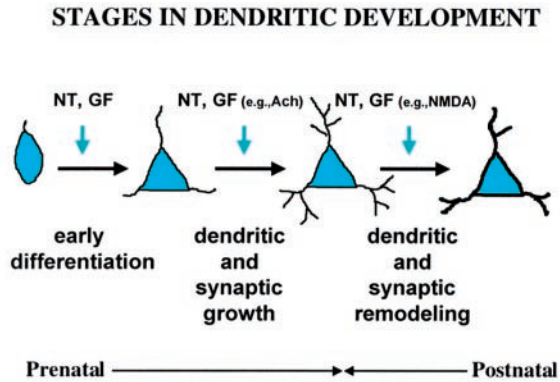


Fig. 4

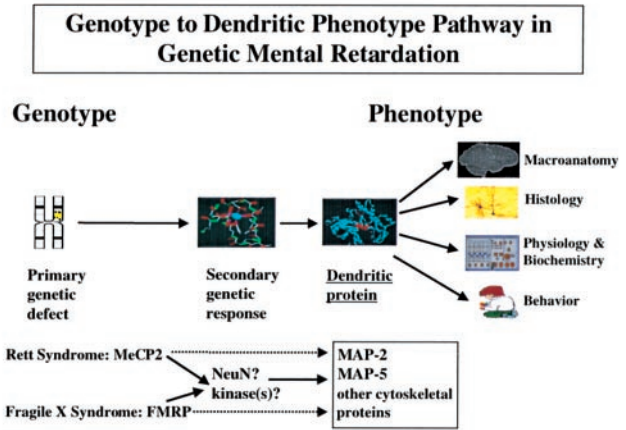


Fig. 5

Figure 4. Diagrammatic representation of dendritic development. Dendritic formation includes three distinctive stages, characterized by morphological and molecular features: early dendritic differentiation, dendritic expansion or growth, and dendritic remodeling and pruning. Several modulators that are displayed between parentheses have been identified to influence this process. In humans, a substantial portion of dendritic development occurs postnatally.

Figure 5. Hypothetical genotype–dendritic phenotype pathway. We postulate that a primary genetic defect will lead to changes in the expression of proteins that play a critical role in dendritic protein expression. Thus several mutations can converge at nodal points of signaling pathways that regulate the levels and conformation of key dendritic constituents. These changes in dendritic proteins will, in turn, have a spectrum of phenotypic features ranging from anatomical to behavioral. The diagram exemplifies two single gene disorders, RS and FraX, and proposes potential secondary gene targets. Most of the data supporting this model come from immunochemical studies carried out in RS (Kaufmann *et al.*, 1995, 1997a,b). Identification of critical points in dendritic signaling pathways may help in designing specific therapies for these MR-associated disorders. Abbreviations: NT, neurotransmitter; GF, growth factor.

dendritic labeling–EM studies will aid the evaluation of the impact of dendritic changes in MR.

Animal Models of MR and Dendritic Pathology

Study of dendritic abnormalities in animal models of MR minimizes confounding technical factors and permits evaluation of developmental patterns. There is an extensive literature on dendritic morphology and development in animal models relevant to MR [see the respective section in the review by Huttenlocher (Huttenlocher, 1991)]. Nonetheless, the validity of the animal models must be examined with care. Considerable efforts have been made in generating trisomic mice that model DS. Limited postnatal viability (Haydar *et al.*, 1996) and incomplete characterizations (Holtzman *et al.*, 1996) have precluded direct dendritic evaluations of these DS models. Other problems include unexpectedly mild behavioral phenotype, such as in the FraX mouse model (Fisch *et al.*, 1999b). This *FMR1* knockout mouse does, however, show spine dysgenesis (Comery *et al.*, 1997) that closely resembles that reported by Hinton and colleagues (Hinton *et al.*, 1991). Some experimental models of several non-genetic causes of MR have succeeded in reproducing the neuropathological and behavioral features of the human disorder. Examples of these are PKU (Nigam and Labar, 1979), protein-calorie malnutrition (Adaro *et al.*, 1986; Diaz-Cintra *et al.*, 1990), hypothyroidism (Ipina and Ruiz-Marcos, 1986; Nunez *et al.*, 1992) and fetal alcohol exposure (Hannigan and Berman, 2000). These experimental paradigms are important because they help overcome the limitations of human studies due to small samples (e.g. PKU and cretinism) and suboptimal tissue processing (e.g. FraX). Even more significantly, they provide information about the dynamics of the process and potential remediation. For instance, the study by Lacey (Lacey, 1985)

showed that dendritic spine maturational delay and increased spine density can be reversed by metabolic correction in PKU. On the other hand, Diaz-Cintra and collaborators (Diaz-Cintra *et al.*, 1994) found that dendritic disturbances caused by prenatal malnutrition (as in placental insufficiency) in the rat are not reversed by postnatal nutritional rehabilitation. These approaches are critically important for the field of genetic MR, since gene-based and other therapies are beginning to be tested. If dendritic abnormalities are the cause of MR, they should be evaluated as outcome measures in experimental treatments. An example is the positive influence of environmental stimulation on cognitive development of children with DS (Ludlow and Allen, 1979). The morphological effects of sensory enrichment in rodent models have reported by Greenough (Greenough, 1984) and ourselves (Adaro *et al.*, 1986) (Figure 3). These studies demonstrate that environmental stimulation leads to increases in length and complexity of dendritic arborizations in several cortical regions, even in adult life.

From Genotype to Dendritic Phenotype

Dendritic development is a sequential process in which generation, elongation, and retraction of dendritic branches and spines are the result of the interaction between intrinsic genetic programs and external modulators (e.g. neurotransmitters) [reviewed by Kaufmann (Kaufmann, 1999)]. Each morphologic stage is characterized by the expression of a set of dendritic proteins (Petit *et al.*, 1988; Kaufmann *et al.*, 1997a). For instance, early neuronal differentiation (extension of primary branches) is associated with the expression of the immature form of microtubule-associated protein 2 (MAP-2), also termed MAP-2c, and of MAP-5. Dendritic branching and terminal growth is linked to the adult form of MAP-2 (MAP-2a/b),

whereas late phases such as dendritic modeling are associated with high levels of the high-molecular-weight form of non-phosphorylated neurofilaments (see Figure 4) (Kaufmann *et al.*, 1997a). The role of neurotransmitters and growth factors in the progression of dendritic development is beginning to be disclosed (Figure 4). While acetylcholine seems to affect the dendritic expansion (Hohmann *et al.*, 1991), activation of some subtypes of glutamate receptors is linked to dendritic pruning (Kaufmann, 1999). On the other hand, neurotrophins modulate dendritic arborizations in a layer-specific manner (McAllister *et al.*, 1997).

We postulate that the genetic defect in MR associated with dendritic pathology disrupts signaling pathways and/or modulators of dendritic development. The three genetic MR syndromes in which single gene defects have been identified, FraX [reviewed by Kaufmann and Reiss (Kaufmann and Reiss, 1999)], RS (Amir *et al.*, 1999) and R-TS (Petrij *et al.*, 1995), share a defect in the regulation of gene expression. MeCP2 and CBP are involved in transcriptional regulation while fragile-X mental retardation protein (FMRP) modulates protein synthesis. The targets of these proteins are still unknown; however, they most likely influence the expression of regulatory proteins in signaling pathways rather than of components of dendrites directly. Preliminary analyses of transcripts potentially targeted by FMRP reveal that synthesis of MAP-2 in dendrites appears not be affected by the FMRP deficit (Steward *et al.*, 1998). While it has been shown that FMRP binds transcripts of itself as well as of other FMRP homologues, no evidence of binding to transcripts of cytoskeletal or other structural proteins has been presented (Ceman *et al.*, 1999). The large number of genes potentially regulated by either MeCP2 (Coy *et al.*, 1999) or CBP (Bading, 1999) suggests a complex mechanism analogous to the one we proposed for early genes (Kaufmann and Worley, 1999). In the latter, we postulated that early genes that regulate transcription have a 'double effect' by influencing the expression of target (e.g. structural protein) and regulatory genes (e.g. transcription factor). Despite the large number of potential targets, we hypothesize that FMRP, MeCP2 and CBP will ultimately affect common signaling pathways. In 'convergence' points, we postulate there are proteins that are essential for neuronal differentiation and maintenance, such as kinases and the exclusively neuronal transcription factor NeuN. These 'secondary' genes may then modulate the quality and quantity of dendritic proteins, manifested morphologically as dendritic branch and spine structure. Figure 5 depicts this model of dendritic formation and maintenance. Dendritic 'regression' in DS may be the consequence of a different mechanism. Among the genes overexpressed in trisomy 21 is the one coding for β amyloid protein. Amyloid deposition in the form of the A β peptide has been detected as early as at 21 gestational weeks in DS (Teller *et al.*, 1996), which coincides with initial cortical dendritic growth (Huttenlocher, 1999). Inflammatory responses associated with A β deposition in senile plaques in Alzheimer disease include production of cytokines (Dickson, 1997), which may operate as negative modulators of dendritic growth and maintenance in DS.

The identification of common (nodal) points in 'dendritic' cell signaling pathways may be of great importance, since the regulation of these proteins by neurotransmitters and growth factors provides the opportunity for therapeutic intervention. Identification of the nature and timing of negative modulators of dendritic growth opens the possibility of preventing deleterious processes. The protracted dendritic development that

characterizes the human cortex provides the opportunity for modifying its course, and the potential for interrupting or reversing abnormal synaptic development.

Concluding Comments

Dendritic abnormalities are associated with many forms of MR, and may be the only evident pathology in some of these disorders. On the other hand, evaluations of presynaptic elements in MR have not yet been carried out. More systematic studies of both pre- and postsynaptic components in multiple cortical regions, at different stages, are still needed to determine the impact of dendritic abnormalities on synaptic structure and function. As it has been demonstrated for neurodegenerative disorders, establishment of genotype to phenotype molecular pathways, as well as validation of putative animal models, depend on accurate characterizations of neuropathologic features (Borchelt *et al.*, 1998). Recent advances in developmental neurobiology and genetics of MR raise the possibility of more specific therapies for these disorders. A better characterization and understanding of dendritic abnormalities in MR will increase our capacity to accomplish this goal.

Notes

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