

The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes

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Abstract Autism is characterized by a broad spectrum of clinical manifestations including qualitative impairments in social interactions and communication, and repetitive and stereotyped patterns of behavior. Abnormal acceleration of brain growth in early childhood, signs of slower growth of neurons, and minicolumn developmental abnormalities suggest multiregional alterations. The aim of this study was to detect the patterns of focal qualitative developmental defects and to identify brain regions that are prone to developmental alterations in autism. Formalin-fixed brain hemispheres of 13 autistic (4–60 years of age) and 14 age-matched control subjects were embedded in celloidin and cut into 200- μ m-thick coronal sections, which were stained with cresyl violet and used for neuropathological evaluation. Thickening of the subependymal cell layer in two brains and subependymal nodular dysplasia in one brain is

indicative of active neurogenesis in two autistic children. Subcortical, periventricular, hippocampal and cerebellar heterotopias detected in the brains of four autistic subjects (31%) reflect abnormal neuronal migration. Multifocal cerebral dysplasia resulted in local distortion of the cytoarchitecture of the neocortex in four brains (31%), of the entorhinal cortex in two brains (15%), of the cornu Ammonis in four brains and of the dentate gyrus in two brains. Cerebellar flocculonodular dysplasia detected in six subjects (46%), focal dysplasia in the vermis in one case, and hypoplasia in one subject indicate local failure of cerebellar development in 62% of autistic subjects. Detection of flocculonodular dysplasia in only one control subject and of a broad spectrum of focal qualitative neuropathological developmental changes in 12 of 13 examined brains of autistic subjects (92%) reflects multiregional dysregulation

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of neurogenesis, neuronal migration and maturation in autism, which may contribute to the heterogeneity of the clinical phenotype.

Keywords Autism · Developmental neuropathology · Subependymal nodular dysplasia · Heterotopia · Dysplasia

Introduction

Autism is characterized by a broad spectrum of clinical manifestations, including (a) qualitative impairments in reciprocal social interactions, (b) qualitative impairments in verbal and nonverbal communication, (c) restricted repetitive and stereotyped patterns of behavior, interests and activities and (d) onset prior to the age of 3 years [1]. In most cases, the etiology is unknown, and patients are diagnosed with idiopathic or non-syndromic autism [10, 43]. About 70% of individuals with idiopathic autism have essential autism, defined by the absence of physical abnormalities, but in 30%, complex autism with dysmorphic features such as microcephaly and/or a structural brain malformation is diagnosed [79]. In 5–10% of cases, autism is diagnosed in association with other disorders such as fragile X syndrome, Rett syndrome, Down syndrome, and tuberous sclerosis [94, 105]. Intellectual impairments, defined as intelligence quotient (IQ) scores less than 70, were reported in 44.6% of children diagnosed with autism [28]. Epilepsy is observed in up to 33% of individuals with autism [106].

The phenotypic heterogeneity is a major obstacle in all areas of autism research [83] and may be the result of a contribution of non-overlapping gene effects. The genetic fractionation of social impairment, communication difficulties and rigid and repetitive behaviors suggests that different features of autism are caused by different genes associated with different brain regions and are related to different cognitive impairments and functional abnormalities [48].

In spite of the broad spectrum of clinical manifestations and striking inter-individual differences, studies of thousands of children have resulted in establishing the clinical diagnostic criteria of pervasive developmental disorder [1]; however, corresponding neuropathological diagnostic criteria do not exist. One of the reasons for the disproportionate progress in clinical and neuropathological studies is the limited tissue resources available for post-mortem studies. Between 1980 and 2003, only 58 brains of individuals with autism were examined [85]. Due to the diversity of research aims, of protocols for tissue preservation and of methods of sampling and examination, and the small number of brains examined in an individual

project, the pattern of neuropathological changes emerging from these studies remains incomplete and inconsistent.

The hypothesis that autism is associated with neuropathological changes was explored in the first reports published between 1980 and 1993 [7, 21, 22, 27, 42, 50, 51, 82, 90]. Since then, implementation of broader diagnostic terms such as Autism Spectrum Disorder (ASD), examination of larger cohorts, applications of stereology, and functional and structural magnetic resonance imaging (MRI) have resulted in the detection of several major types of pathology, most likely contributing to the clinical phenotype. An emerging concept of autism-related brain pathology integrates evidence of (a) abnormal acceleration of brain growth in early childhood [89], (b) minicolumn pathology [13, 14], (c) curtailed neuronal development [7, 108] and brain structure-specific delays of neuronal growth [111] with indications of abnormalities in brain cytoarchitecture [4, 7], metabolic modifications with abnormal amyloid protein precursor (APP) processing [5, 101], enhanced oxidative stress [17] and enhanced turnover of cell organelles with pigment accumulation and glial activation [68].

In spite of the conceptual limitations, “localizing” models are still the main approach to the identification of pathological changes as a component of the networks’ structural and functional abnormalities [81]. We hypothesize that dysregulation of neurogenesis, neuronal migration and maturation is also reflected in qualitative, focal, developmental alterations of brain microarchitecture. The aim of this study is to detect the pattern of focal, qualitative, developmental defects in autism brain, including their type, topography and severity, and to identify the structures and brain regions that are prone to developmental alterations in autism.

Materials and methods

The autistic cohort study consisted of 13 subjects (4–62 years of age), including 9 males (69%) and 4 females (31%), while the control cohort consisted of 14 subjects (4–64 years of age), including 9 males and 5 females (Table 1).

Clinical and genetic characteristics of the autistic subjects

The source of our clinical data was the medical records of the autistic subjects, which consisted of psychological, behavioral, neurological and psychiatric evaluation reports. All of the records were obtained after the subjects’ deaths. The Autism Diagnostic Interview-Revised (ADI-R) was administered to each donor family as a standardized assessment tool in order to confirm the diagnosis on a postmortem basis. Inclusion of the subject in this study was based on a summary of scores of four domains:

Table 1 Material examined

#	Group	Brain bank #	Sex	Age (years)	Cause of death	PMI (h)	H	Brain weight (g)
1	A	IBR425-02	M	4	Drowning	30	R	1,280
2	A	UMB-1627	F	5	Traumatic multiple injuries	13.2	R	1,390
3	A	B-6403	M	7	Drowning	25	R	1,610
4	A	B-5666	M	8	Rhabdomyosarcoma	22.2	R	1,570
5	A	B-5342	F	11	Seizure-related drowning	12.9	L	1,460
6	A	B-5535	M	13	Seizure-related	8	L	1,470
7	A	B-6115	F	17	Cardiac arrest related to cardiomyopathy	25	L	1,580
8	A	UMB-1638	F	21	Seizure-related respiratory failure	50	R	1,108
9	A	B-6337	M	22	Seizure-related	25	R	1,375
10	A	IBR93-01	M	23	Status epilepticus-related respiratory failure	14	R	1,610
11	A	B-6212	M	36	Cardiac arrest	24	R	1,480
12	A	B-6276	M	56	Cardiac arrest	3.35	R	1,570
13	A	B-7090	M	60	Pancreatic cancer	26.5	R	1,210
1	C	B-6736	F	4	Acute bronchopneumonia	17	R	1,530
2	C	UMB-1499	F	4	Lymphocytic myocarditis	21	R	1,222
3	C	UMB-4898	M	7	Drowning	12	R	1,240
4	C	UMB-1708	F	8	Traumatic multiple injuries	20	R	1,222
5	C	BTB-3638	M	14	Electrocution	20	R	1,464
6	C	UMB-1843	F	15	Traumatic multiple injuries	9	R	1,250
7	C	UMB-1846	F	20	Traumatic multiple injuries	9	R	1,340
8	C	UMB-1646	M	23	Ruptured spleen	6	R	1,520
9	C	UMB-4543	M	29	Traumatic multiple injuries	13	R	1,514
10	C	UMB-1576	M	32	Traumatic compressional asphyxia	24	R	1,364
11	C	BTB-3899	M	48	Atherosclerotic heart disease	24	L	1,412
12	C	IBR252-02	M	51	Myocardial infarct	18	L	1,450
13	C	BTB-3983	M	52	Heart atherosclerosis	13	R	1,430
14	C	B-6874	M	64	Cardiac arrest	28	R	1,250

PMI postmortem interval, H hemisphere, R right, L left

(a) qualitative abnormalities in reciprocal social interaction; (b) qualitative abnormalities in verbal and nonverbal communication; (c) restricted, repetitive and stereotyped patterns of behavior; and (d) abnormality of development evident at or before 36 months [69]. All 13 autistic subjects met ADI-R criteria for autism. For some subjects, the intellectual evaluation was available and was based on the Wechsler Intelligence Scale for Children III and the Woodcock-Johnson Tests of Achievement-Revised (Table 2). Eight subjects were diagnosed with intellectual disability, usually in the range from mild to severe (61%). Six of 13 autistic subjects had seizures (46%). In five cases, the age of onset of seizures was from 14 months to 5 years of age. A 23-year-old autistic male had only one seizure, which was reported as the cause of his death. In one child, an abnormal EEG was detected, but without seizures.

Several forms of challenging behaviors and behavioral disorders were noted, including self-injurious behavior (six cases, 46%), aggression (four cases, 31%), hyperactivity

(three cases, 23%), obsessive compulsive disorder (two cases, 12%) and depression and mania (a single case of each).

For three of the 13 autistic subjects, the list of high-confidence copy number variations identified both by quantiSNAP and Partek HMM computational algorithm was posted on the ATP portal by Drs. Steve Scherer and Richard Wintle from The Center for Applied Genomics, Toronto. The copy number variations detected in the three autistic subjects do not differ from those commonly observed [75], except for the loss of 25,505 kb within Neuropeptide S Receptor 1 (NPSR1) gene at 7p15–p14 detected in a 22-year-old autistic male (B-6337). NPSR1 has not been linked to autism in the genomic reports [103, 112]; however, an association of NPSR1 copy number variation with allergies has been reported [11] that might be linked to the patient's history of allergies.

Originally, 38 brains, including 20 brains of autistic and 18 brains of control subjects, were assigned to this project. However, application of the clinical and neuropathological

Table 2 Behavioral and neurological signs, and the type and topography of developmental abnormalities

Brain bank #	Psychiatric disorders and neurological symptoms	Mental retardation (MR)	Seizures age of onset	Type and topography of developmental abnormalities
IBR425-02	Hyperactivity. Tantrums. Self-injurious behavior	–	–	No changes
UMB-1627	Aggression	–	–	Focal neuronal heterotopia in white matter of the anterior cingulate gyrus
B-6403	–	–	14 months	Subependymal nodular dysplasia in the wall of the occipital horn of the lateral ventricle. Two periventricular nodular heterotopias (2 and 4 mm in diameter) near the frontal horn of the lateral ventricle. Tuber-like expansion of the tail of caudate nucleus into the lumen of the ventricle. Flocculonodular dysplasia
B-5666	–	–	Abnormal EEG; no seizures	Cortical dysplasia in the middle and inferior temporal gyri with focal dyslamination, clustering of dystrophic neurons and severe local neuronal deficits. Several focal dysplastic changes within CA. Flocculonodular dysplasia affecting almost entire lobe
B-5342	Pervasive developmental disorder. Hyperlexia	Mild MR	4.5 months	Focal cortical dysplasia. Dysplasia of the granule layer of the dentate gyrus. Subcortical heterotopia in the inferior frontal gyrus. Heterotopia in vermis and in cerebellar white matter
B-5535	Hyperactivity. Self-injurious behavior including head-banging	Moderate to severe MR	2 years	Thickening of the subependymal cell layer. Focal dysplasia within CA1 pyramidal layer with neuronal deficit, abnormal neuron morphology and spatial orientation. Multifocal dysplasia of the dentate gyrus with distortion of the shape of granule and molecular cell layers. Focal dysplasia within vermis
B-6115	Sensory integration disorder	–	–	Flocculonodular dysplasia affecting the majority of lobe volume. Cortical angioma
UMB-1638	ADHD	Moderate MR	5 years	Focal dysplasia within CA1 with diffuse neuronal deficit but without glial activation
B-6337	Obsessive compulsive disorder. Mania. Tourette syndrome. Self-injurious behavior	MR	–	Minor focal flocculonodular dysplasia
IBR93-01	Hyperactivity. Aggressive and self-injurious behavior	Severe MR	23 years	Focal dysplasia within islands in the entorhinal cortex. Pineal gland cysts
B-6212	Obsessive compulsive disorder. Depression, aggression, and anxiety	Severe MR	–	Several areas of focal cortical dysplasia within frontal cortex and insula with local loss of vertical and horizontal organization. Merger of ventral portion of the claustrum with insula. Flocculonodular dysplasia
B-6276	Aggression and self-injurious behavior, anxiety and agitation	Moderate MR	–	Focal dysplasia within CA1 sector with focal neuronal deficit. Heterotopia within stratum oriens. Flocculonodular dysplasia affecting approximately 70% of the lobe
B-7090	Disturbed movement coordination (walking like drunk)	MR	3 years	Three focal dysplasias in the frontal cortex. Dysplasia of layers 1–3 in the entorhinal cortex with missing numerous islands of the stellate neurons. Severe hypoplasia of cerebellar lobes 1–4. Reduced convolutions within dentate nucleus

Developmental abnormalities in brains of autistic subjects

exclusion criteria reduced the size of the cohort to 27 brains. Based on the results of the ADI-R, two cases were excluded, including one case diagnosed with atypical autism, and one that did not meet ADI-R criteria. Based on postmortem evaluation, five more autistic cases were excluded: one due to severe postmortem autolytic changes, three due to severe global hypoxic encephalopathy related to the mechanism of death, and one due to multiple microinfarcts. Moreover, four brains of control subjects were disqualified due to severe postmortem autolysis. In all these brains, neuronal loss, changes of neuronal size and shape, and gliosis were so severe that they masked and distorted the qualitative and quantitative characteristics of the developmental alterations associated with autism.

Brain tissue preservation

Brains of 13 autistic and 14 age-matched control subjects were examined by postmortem MRI and neuropathologically. The postmortem interval (PMI) varied, ranging from 6 to 27.8 h in the control group (16 h on average; SD 6 h) and from 8 to 30 h in the autistic group (20 h on average; SD 12 h). The median PMI was 15 h.

The brain hemispheres were removed using standard techniques, exercising extra care to avoid damaging the brain tissue. The brain was weighed in the fresh state. The fresh brain was sagittally cut through the corpus callosum and brainstem. Half of the brain was fixed in 10% buffered formalin. Following at least 3 weeks of fixation, the brain hemisphere was scanned using MRI. The aim of the MRI application was to determine the type of developmental changes detectable by MRI and to microscopically characterize MRI findings. All brains within this project were scanned (L.A.S.L.) using a standardized protocol (established and implemented for this and for other postmortem MRI studies by L.A.S.L. and M.L.). MRI scans were acquired on a 1.5 T GE Signa Imager (General Electric, Milwaukee, USA). The research scan consisted of a 124-slice T1-weighted fast gradient echo acquired in a coronal orientation perpendicular to the long axis of the hippocampus with a 1.5-mm slice thickness, which encompassed the entire brain hemisphere without gaps or wrap artifacts (FOV = 25 cm; NEX = 1; matrix = 256 × 192; TR = 35 ms; FA = 60°). All file names were assigned sequential code numbers, and demographic information was removed from image headers [9]. MRI scans were first screened in a diagnosis-blind manner, and the brains with abnormalities were re-evaluated by both radiologists and neuropathologists to determine the topography, type, and size of lesions detected with both methods.

The brain hemisphere was fixed with 10% buffered formalin. Formalin was washed out from the tissue during an overnight tap water rinsing. Brains were dehydrated

using a series of increasing ethyl alcohol concentrations (50% ethanol 3 days; 70% ethanol 4 days; 80% ethanol 3 days; 95% ethanol 4 days). The brain hemisphere was embedded in 8% celloidin [53]. During hardening, celloidin blocks were exposed to chloroform vapors for approximately 2.5 weeks, and celloidin blocks were then stored in 70% ethanol. For sectioning, the block was attached to the block holder with 10–15 ml of 8% celloidin. To fasten adhesion of the block to the holder, the block with the holder attached was immersed in 70% ethanol overnight. Serial 200- μ m-thick sections were separated with filter paper and stored in 70% ethanol. For the four control and four brains of autistic subjects, alternative series of 200- and 50- μ m-thick sections were preserved. To ensure the same probability of detection of changes in each case, every 200- μ m-thick section, with a distance 1.2 mm, was used in this project. Sections were washed in water for 2–3 h, stained with cresyl violet (CV) and mounted with Acrytol.

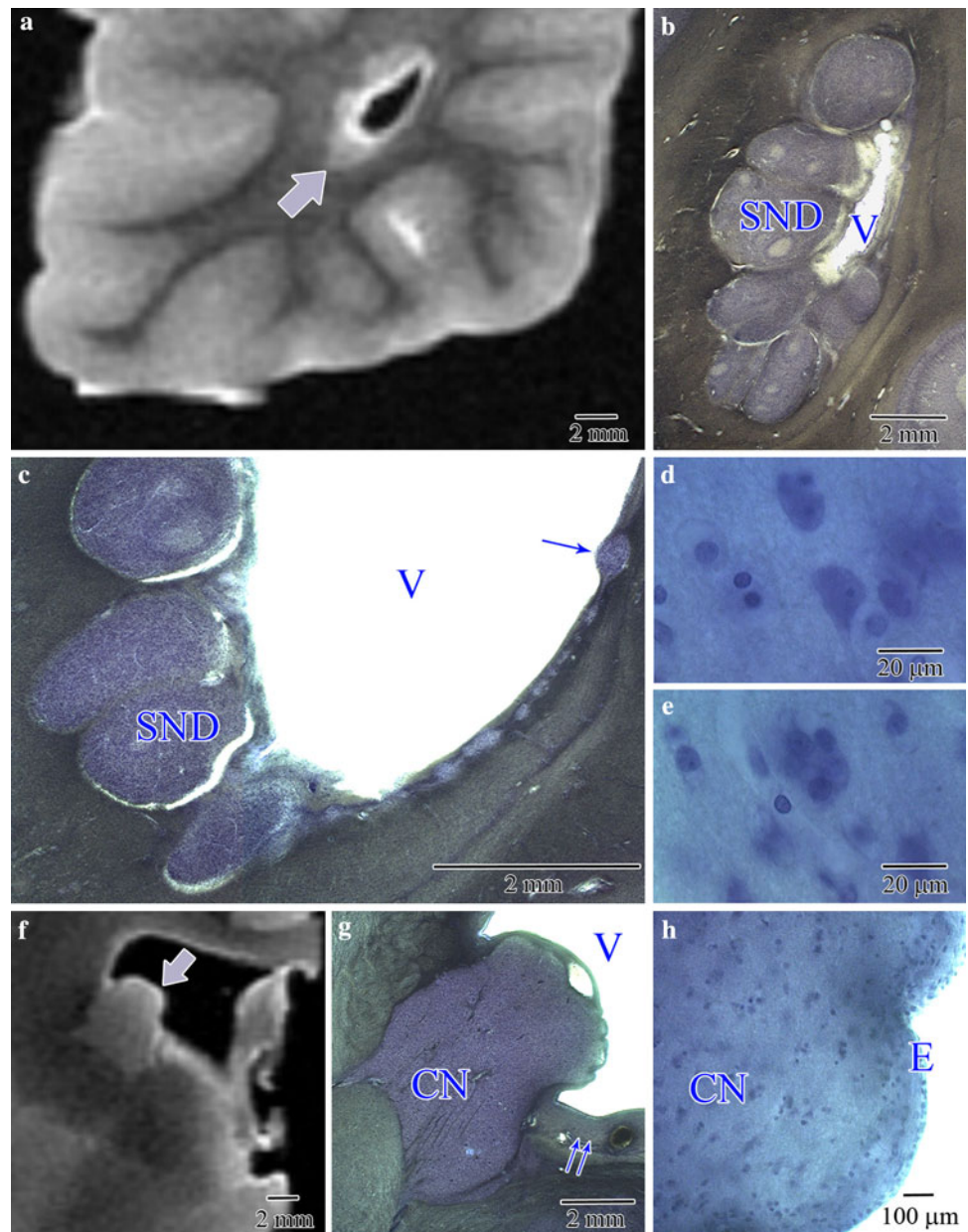
One neuropathologist (I.K.) examined, in a blind-to-diagnosis fashion, on average 120 hemispheric CV-stained sections per case with a 1.2-mm distance between sections. Two-step screening included examination at low magnification (28 \times) using Zeiss DL2 Documator and microscopic examination using objective lenses from 5 \times to 100 \times . Two other neuropathologists (T.W. and J.W.) examined all histological slides for which pathology was detected during the primary screening. The defects of neurogenesis, neuronal migration, and dysplastic changes that they detected were summarized in this report.

Tissue acquisition for this program project is based on individual tissue transfer agreements between the program project's principal investigator and several tissue banks: (a) the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, (b) the Harvard Brain Tissue Resource Center and (c) the Brain Bank for Developmental Disabilities and Aging of the NYS Institute for Basic Research in Developmental Disabilities. Each brain hemisphere number given by the institution that received the donation was used as the only identifier of clinical records and tissue samples. Brain Bank identification of tissue samples is listed in Tables 1 and 2 to keep non-overlapping records of results of examination of brains in different projects and research groups. The Institutional Review Board of the New York State Institute for Basic Research in Developmental Disabilities approved the methods applied in this study.

Results

Neuropathological evaluation of serial coronal hemispheric sections from the cerebral and cerebellar hemispheres of

Fig. 1 Nodules in the wall of the lateral ventricle detected in postmortem MRI (a) in the brain of a 7-year-old male diagnosed with autism (B-6403) revealed features of subependymal nodular dysplasia (SND; b) in examination of CV-stained sections. c Numerous large and small nodules (arrow) dispersed within subependymal cell layer. They contained a few pyramidal-like neurons (d) and numerous poorly differentiated cells (e). Tuber-like expansion of the caudate nucleus (arrow) into the ventricle lumen is shown in MRI (f) and in CV-stained section (g). g A thick subependymal cell layer above and below (two arrows) the caudate nucleus (CN), and the absence of matrix in the tuber-like area. Under ependymal (E) cap of the caudate nucleus (CN) tuber-like expansion, small poorly differentiated neurons are present (h)



13 autistic and 14 control subjects revealed more details characterizing the topography and severity of changes than did standard sampling of brains for routine neuropathological evaluation. A broad range of changes was found. Developmental abnormalities included subependymal nodular dysplasia, heterotopia and very common dysplastic changes within the neo- and archicortex, hippocampus and cerebellum in 12 of 13 examined brains of the autistic subjects (92%) (Table 2). The general result of these developmental defects was a multifocal disorganization of gray and white matter. The developmental pathology observed in control brains was limited to one cerebellar dysplasia.

Alterations of the subependymal cell layer and subependymal nodular dysplasia

In two autistic subjects, there was a several-fold local increase in the thickness of the subependymal cell layer. Numerous subependymal nodules were found within a pathologically thickened subependymal cell layer, in the wall of the occipital horn of the lateral ventricle of a 7-year-old male, which reflects a subependymal nodular dysplasia (Fig. 1a–e). Nodules occupied 13.3 mm of the caudal portion of the occipital horn of the lateral ventricle. The diameter of round/oval nodules varied in size from 285 to 3,310 μm . While the smallest nodules were dispersed

within the subependymal cell layer, the large nodules expanded partially into the white matter, and partially into the lumen of the ventricles and were detectable on MRI (Fig. 1a) and CV-stained histological sections (Fig. 1b, c). The effect was narrowing of the ventricle and the tuberous appearance of the ventricular wall. There were large tubers that contained dysplastic neurons with a partially modified morphology of pyramidal, multipolar or bipolar large neurons (Fig. 1d) and irregularly shaped medium and small neurons. Neurons in the small nodules were small and poorly differentiated (Fig. 1e). In the large nodules, several hypocellular areas were observed. The nodules were free of oval or polygonal giant cells or ballooned glial cells, as well as signs of calcification.

In the brain with the subependymal nodular dysplasia, an abnormal tuberous expansion of the caudate nucleus was detected on MRI (Fig. 1f) and in histological sections (Fig. 1g, h). Only the ependyma separated the tuber-like expansion of the caudate from the ventricle lumen. The very thick subependymal cell layer that was present close to the caudate was substituted by loosely arranged and poorly differentiated neurons in the affected area.

Heterotopia

Heterotopias were found in the brains of four autistic subjects and no control subjects. The topography of the lesions was different in each case. Subcortical heterotopias were detected in the white matter of the anterior cingulate gyrus of a 5-year-old (Fig. 2a, b) and in the inferior frontal gyrus in an 11-year-old subject. Periventricular heterotopias were detected near the wall of lateral ventricle in 7-year old subject (Fig. 2c, d). A single heterotopia was noted in the stratum oriens of the hippocampus. In the cerebellum of the 11-year-old subject, heterotopias were detected in the vermis and the cerebellar white matter close to the dentate nucleus (Fig. 2f–h). These defects of migration were observed in two brains as a single aggregate of gray matter, in one brain as two aggregates and in one brain as three lesions measuring from 1 to 3 mm in diameter. Subcortical and periventricular heterotopias comprised poorly differentiated oval or multipolar neurons without spatial orientation (Fig. 2a) or had a distorted laminar organization (Fig. 2e). Cerebellar heterotopias had a distorted morphology of the granule and molecular layers with a few dispersed Purkinje cells (Fig. 2g, h).

Dysplasia within neocortex and archicortex, hippocampus and cerebellum

The multifocal neocortical dysplasia detected in four brains of autistic subjects (31%) was associated with a local loss

of vertical and horizontal organization of the neocortex, formation of abnormal layers, loss of orientation of neurons (Fig. 3a, b) and thickening of the affected portion of the cortical ribbon. A focal dysplasia in the entorhinal cortex, observed mainly in the second layer with a local absence of islands and/or reduced number of neurons, was found in the 23-year-old and the 60-year-old autistic subjects (15%) (Fig. 3c, d). A lack of giant multinuclear neurons and large, ballooned glial cells typical of focal cortical dysplasia indicated that the observed developmental changes in neocortex and archicortex reflect a more subtle cortical malformation, classified usually as focal cortical microdysgenesis.

Two types of changes were observed in the dentate gyrus. An abnormal migration of granule neurons into the molecular layer resulted in the formation of an additional fragmentary granule cell layer (Fig. 3e). In other areas, granule cells formed irregular circles and loops (Fig. 3f).

In the CA1 sector of a 13-year-old male, several areas of dysplastic changes with a significant deficit of pyramidal neurons without gliosis were found (Fig. 3g). In affected areas, the size and shape of neurons varied over a wide range. Pyramidal neurons were very rare, whereas small irregular or oval-shaped, poorly differentiated neurons prevailed (Fig. 3h). In the dysplastic area in the CA1 sector of the 56-year-old autistic subject, an opposite trend was present, with thickening of the pyramidal layer and an increased packing of dysplastic neurons (not shown).

The most common developmental abnormality within the cerebellum was dysplasia, which was detected in seven autistic subjects (54%) and in the cerebellum of one control subject.

Flocculonodular dysplasia (Fig. 4a, b), usually affecting the entire nodule, was found in the cerebellum in six autistic subjects (46%). In the dysplastic areas, a thin granule layer formed the labyrinth, which was mixed with irregular islands of the molecular layer. Clusters of granule cells and a few Purkinje cells were dispersed within the distorted molecular layer. The only developmental abnormality detected in the control group was flocculonodular dysplasia in the cerebellum of the 51-year-old control subject (not shown). Local cortical dysplasia was also detected within the vermis of the 13-year-old autistic male. In the affected area, the cytoarchitecture of the molecular and granule layers and the Purkinje cells was completely disorganized (Fig. 4c, d).

In the cerebellum of the 60-year-old autistic male, severe hypoplasia affected lobes 1–4 (Fig. 4e). The thickness of the molecular and granular layer was decreased by almost 50% in comparison to that of the non-affected areas (Fig. 4e, f). The number of Purkinje cells was significantly reduced in the hypoplastic area. Hypoplastic changes within the portion of cerebellar cortex were observed,

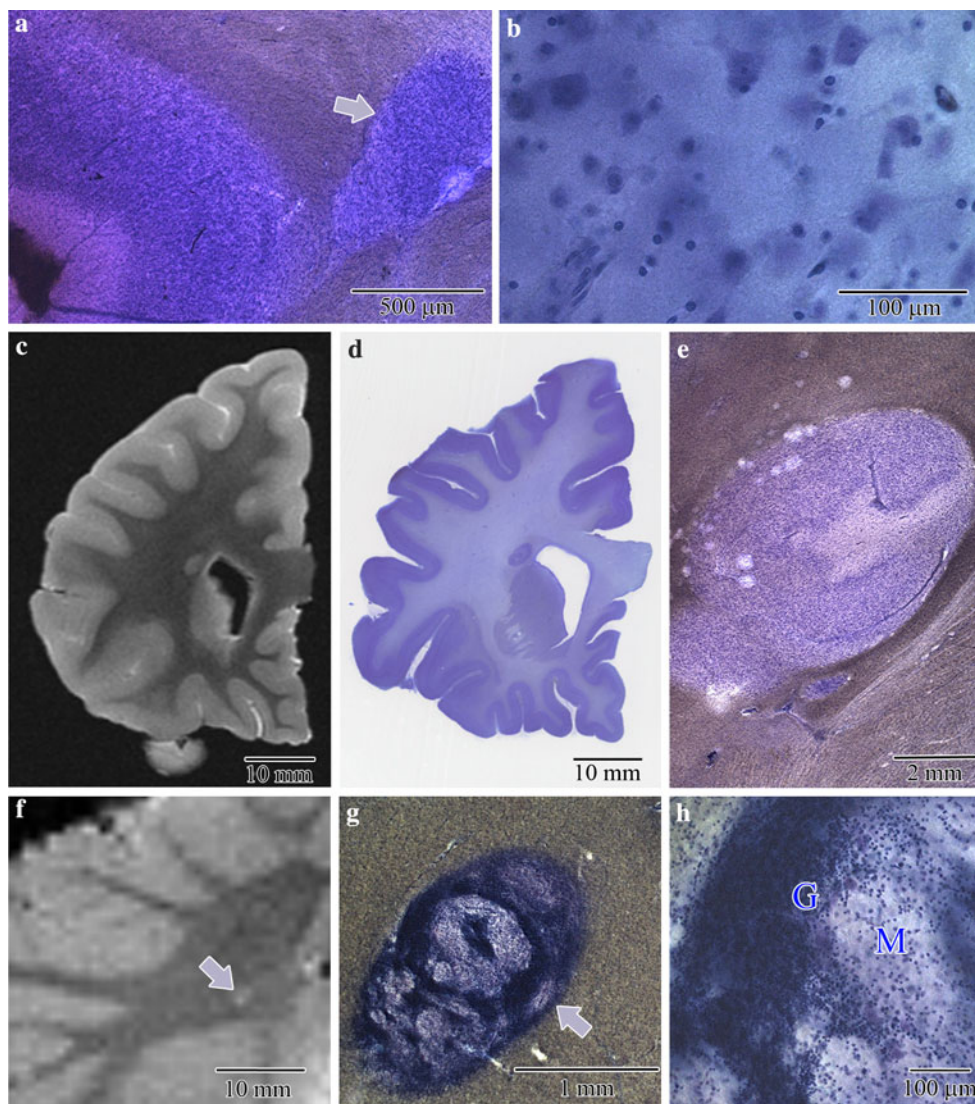


Fig. 2 Large subcortical heterotopia within anterior cingulate gyrus in a 5-year-old autistic child (UMB-1627) (**a**) contained dysplastic neurons without spatial orientation (**b**). Periventricular heterotopia near the frontal horn of the lateral ventricle (**c** MRI, **d**, **e** CV-stained section) shows a structure resembling molecular, granule and

pyramidal layers in a 7-year-old autistic subject (B-6403). MRI (**f**), low (**g**) and large (**h**) magnification of heterotopia (*arrow*) with dysplastic granule (*G*) and molecular layer (*M*) detected within cerebellar white matter in an 11-year-old autistic subject (B-5342)

together with a significantly reduced convolution of the dentate nucleus (Fig. 4g).

Discussion

This neuropathological study revealed a broad spectrum of focal developmental abnormalities and pre- and perinatally acquired lesions in 92% of the brains of autistic subjects and striking inter-individual differences in the type and topography of changes. Evidence that different features of autism are caused by different genes associated with different brain regions [48] suggests a link between regional

developmental alterations in the brain and different components of the autistic phenotype.

Altered neurogenesis in autism

Increased brain mass in autistic children and some autistic adults [89], increase in the numerical density of neurons [13, 14], reduced size of neurons [7] and brain structure-specific delay of neuronal growth [111] indicate alterations in neuronal and brain growth in autistic individuals. The subventricular zone of the lateral ventricles [26] and the dentate gyrus [33] are active sites of neurogenesis in adult humans. Several of our findings support the hypothesis of

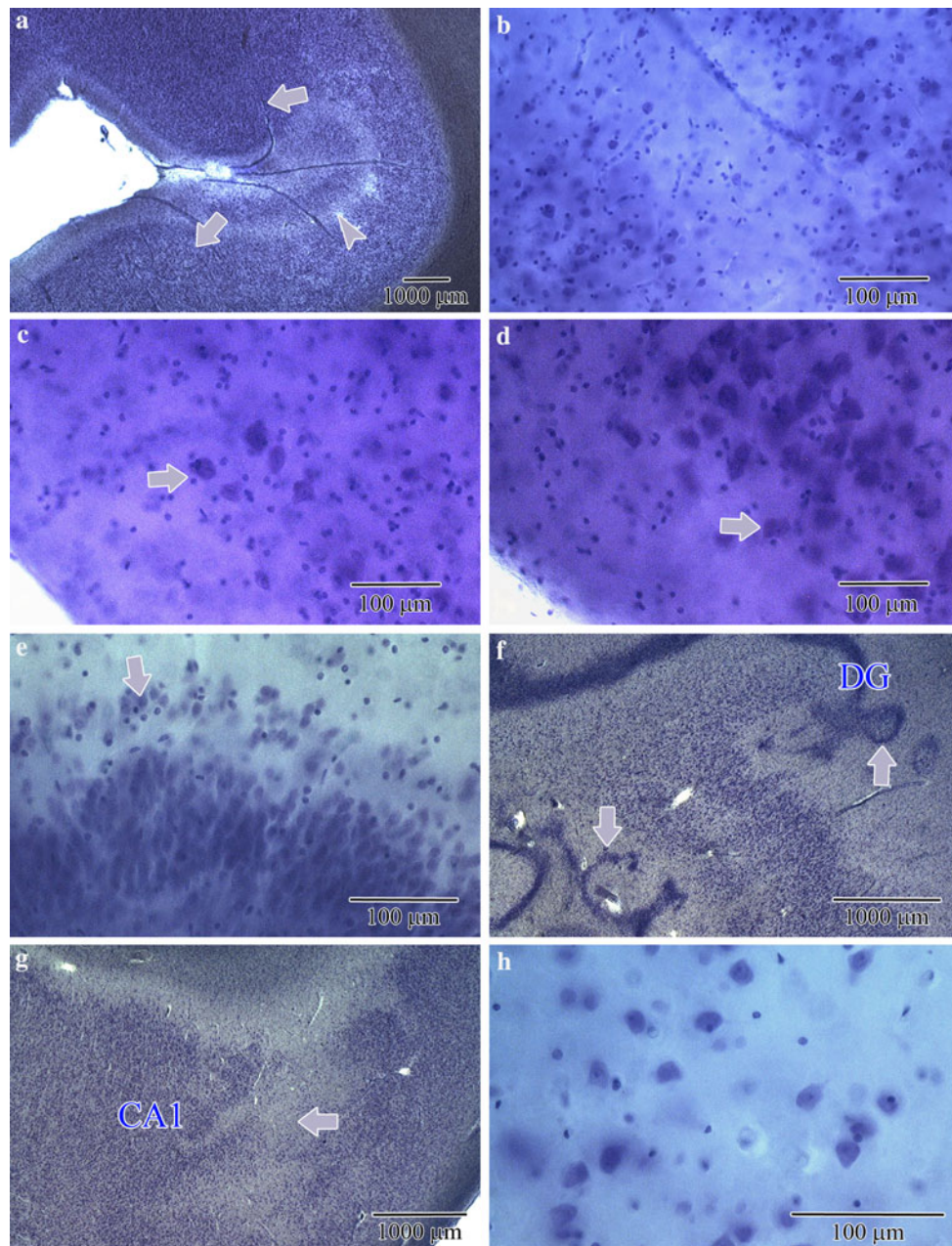


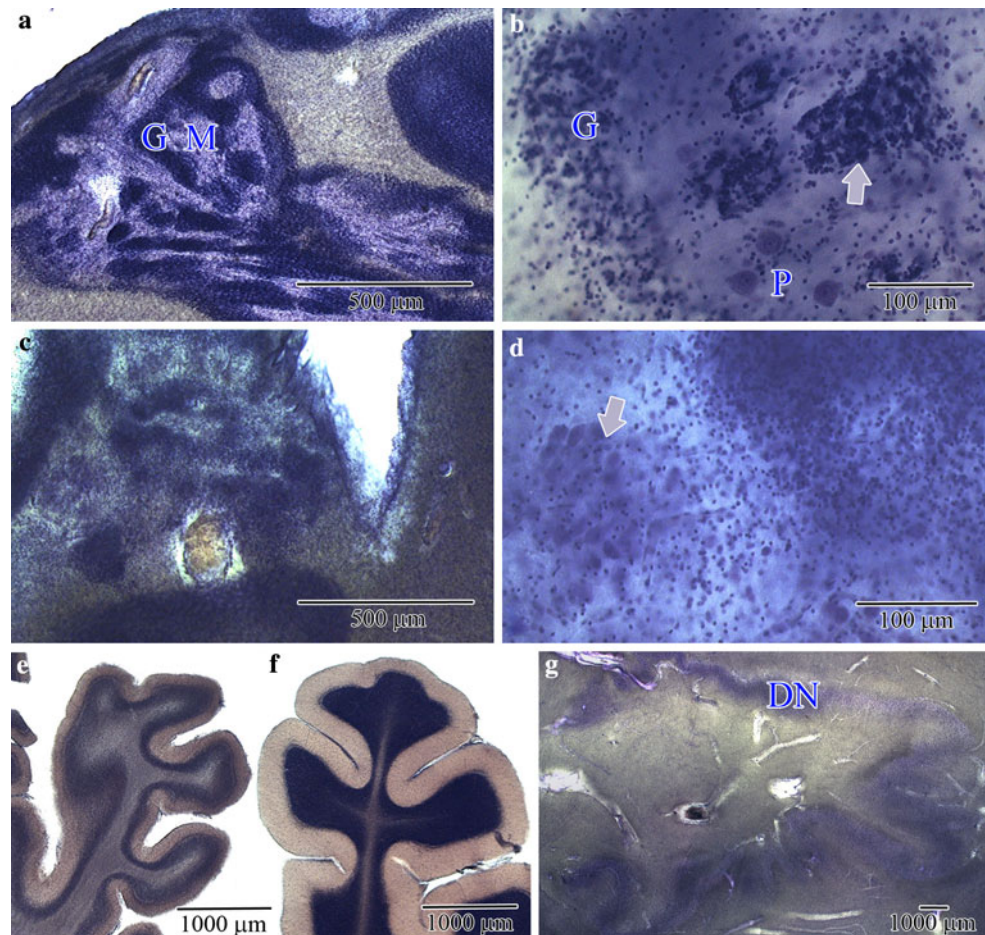
Fig. 3 Dysplastic changes within neocortex (**a, b**), entorhinal cortex (**c, d**), dentate gyrus (**e, f**) and the cornu Ammonis (**g, h**). Focal dysplasia in frontal cortex with loss of vertical and horizontal cytoarchitecture (*two arrows*) and abnormal (*arrowhead*) laminar organization (**a**). Dysplastic neurons within affected area (B-6212) (**b**). Microdysgenesis within the entorhinal cortex with deficit of stellate neurons in the islands (**c**) and normal morphology of islands in adjacent cortex (**d**) in 60-year-old autistic subject (B-7090).

altered neurogenesis in autistic subjects. The increased thickness of the subependymal cell layer, subependymal nodular dysplasia, abnormal growth of the dentate nucleus and dysplasia of the granule layer in the dentate gyrus, detected in this study, appear to be signs of abnormal neurogenesis in the brains of three autistic subjects.

Microdysgenesis of the dentate gyrus with dispersion of granule cells within the molecular layer (**e, arrow**) and distortion of the granule cell layer shape (**f, arrows**) in 13-year-old autistic male (B-5535). CA1 sector microdysgenesis with local deficit of pyramidal neurons (**g, arrow**) without markers of gliosis but with signs of poor differentiation of dysplastic abnormally arranged neurons (**h**) in 13-year-old autistic subject (B-5535)

Subependymal nodules were reported in approximately 80% of patients with tuberous sclerosis, a disorder that is highly associated with epilepsy, autism and mental retardation [73]. Tuberous sclerosis nodules were detected in one fetus [12], suggesting that fetal development of subependymal nodules can lead to the early onset of epilepsy

Fig. 4 Flocculonodular dysplasia in cerebellum of 56-year-old autistic subject (B-6276) (**a**) with thin irregular granule (*G*) and molecular (*M*) layer. **b** Dysplastic granule layer (*G*), ectopic granule cells (*arrow*) in the molecular layer, and loosely dispersed Purkinje cells (*P*) (B-6276). Cortical dysplasia within vermis of 13-year-old autistic male (**c**) with dysplastic granule neurons mixed with heterotopic (*arrow*) large cells (**d**) (B-5535). **e** Severe hypoplasia of cerebellar lobe 3 and unmodified lobe 6 (**f**), respectively, within the cerebellum of a 60-year-old autistic male (B-7090). In the affected region, the thickness of the hypoplastic molecular and granule cell layer was reduced by about 50%. Almost half of the dentate nucleus (*DN*) was less convoluted than the unaffected part (**g**)



that was diagnosed at the age of 14 months in a neuropathologically examined autistic male. The subependymal nodules detected in this autistic male's brain are partially similar to tubers seen in subjects diagnosed with tuberous sclerosis [24]. The cause of subependymal nodular dysplasia in the examined subject is unknown. In the reported subjects, bilateral periventricular nodules are linked to mutations of the filamin A (FLNA) gene located on chromosome Xp28. Filamin A is an actin-crosslinking protein that is essential for cell locomotion [16], and nodule formation might be related to a defect in cell migration. The presence of miniature nodules that were built of poorly differentiated small neurons within the subependymal cell layer and an increase in nodular size with signs of growth and differentiation of neurons suggests that neurogenesis, differentiation and maturation of neurons were in progress within the subependymal germinal matrix of the 7-year-old autistic child. This interpretation of subependymal nodule genesis is consistent with lineage studies demonstrating that cells in nodules express cellular markers that are typical for progenitors derived from the subventricular germinal zone [35, 67]. However, in contrast to the subependymal nodules seen in subjects with tuberous sclerosis,

in the examined autistic subject, the nodules seen were small (from 258 to 3,310 μm in diameter), and did not have the characteristic ovoid or polygonal giant cells, 80–150 μm in diameter, giant cells with multiple and peripherally displaced nuclei [25], or balloon cells, which are considered the sine qua non histopathological features of the cortical tubers and subependymal nodules observed in tuberous sclerosis [73].

The enlarged caudate nucleus detected in the brain of the 7-year-old autistic subject is consistent with MRI reports documenting an increased volume of basal ganglia, including the caudate, in autism [54, 55, 66, 99]. A disproportionate increase of the caudate nucleus volume [66] suggests that in brains of some autistic individuals, extended neurogenesis within the subependymal cell layer may contribute to abnormal growth of the caudate nucleus. A similar process has been observed in the brains of people with Huntington disease, showing enhanced neurogenesis in the subependymal layer and suggesting renewal of the neuronal population in a degenerating caudate nucleus [26]. The caudate nucleus is a part of the fronto-striatal network involved in several functional domains that are impaired in autism, including lower order repetitive motor

behavior; intense circumscribed patterns of interests and higher order rituals and compulsions [41], and defects in cognitive functions [19, 109], planning and problem-solving skills [78, 98], short- and long-term memory [40] and learning [88].

Defective migration in autism

Heterotopia is a sign of altered migration leading to an abnormal distribution of gray matter nodular masses with disorganized or rudimentary lamination within the periventricular area (periventricular heterotopia) or subcortical white matter (subcortical heterotopia) [2]. In the examined cohorts, heterotopias were detected in the brains of four autistic subjects and in the brain of one control subject. Heterotopias are associated with mutations in the filamin 1 gene (FLNA1) [39, 46] and the chromosome X-linked DCX gene that codes for doublecortin, a protein expressed during brain development in migrating neurons, and in the cortical plate [29, 44, 45], which is involved in the formation of the microtubules necessary for neuronal migration [15]. Periventricular nodular heterotopia has been reported to be associated with pharmaco-resistant seizures in 80–90% of patients [31]. In the examined cohort, two periventricular heterotopias were detected in the brain of a child with subependymal nodular dysplasia and seizures diagnosed at 14 months of age (B-6403). Early onset epilepsy, diagnosed at the age of 4.5 months, might be related to the multiple heterotopias found within the frontal inferior gyrus, vermis and cerebellar white matter, coexisting with a focal cortical dysplasia and dentate gyrus dysplasia (B-5342).

Cortical, hippocampal and cerebellar dysplasia in autism

The most common form of developmental changes detected in the examined brains was focal dysplasia, which was observed in 11 (85%) of the autistic subjects. The morphology of focal dysplasias appears to reflect signs of abnormal migration, neuronal immaturity and altered cell arrangement, resulting in focal distortion of cytoarchitecture. In spite of similarities, the dysplastic changes in the neocortex and archicortex, dentate gyrus and cornu Ammonis and cerebellum also reveal a brain structure-specific pattern of dysplastic changes in autism.

Dysplasias encompass a spectrum of changes ranging from a mild form of cortical disruption, without cellular abnormalities, to the most severe form with cortical dyslamination, with abnormal morphology of neurons and astrocytes [93, 96, 107]. Focal cortical dysplasias with giant neurons and balloon cells [107, 113] are histopathologically similar to tubers containing giant cells in tuberous

sclerosis complex [25, 73], suggesting a common pathogenic basis [113]. However, activation of the mammalian target of rapamycin (mTOR) pathway observed in the tuberous sclerosis complex is not present in focal cortical dysplasia [8, 80]. The giant neurons and ballooned cells, which are histopathological features of tuberous sclerosis and focal cortical dysplasia, were absent both in the subependymal nodules and in the focal cortical dysplasia observed in the examined autistic cohort. These findings suggest that in spite of similarities, the pathomechanisms of developmental alterations are different in the examined autistic subjects than those in tuberous sclerosis heterotopias or focal cortical dysplasia. The development of the giant neuron- and balloon cell-free dysplasias observed in the autistic subjects might be related to differences in cause and/or mechanism. The detection of changes similar to focal cortical dysplasia in association with prenatal ischemia [65] or in shaken infant syndrome [74] may support these speculations.

Ectopias and dysplastic changes were reported in the brains of autistic subjects, by several groups [4, 62–64, 91]. Bailey et al. [4] detected olivary dysplasia in the brain of three of the five autistic subjects, and ectopic neurons related to the olivary complex in two cases. Moreover, in the brains of four autistic subjects, cortical dysgenesis was found. In the brains of the autistic subjects, a strikingly consistent finding was cingulate cortex disordered lamination [62–64, 100]. A recent study of the cingulate cortex of nine autistic subjects revealed a developmental malformation with irregular lamination in three cases, and an increased number of neurons within the subcortical white matter in two [100]. Simms et al. [100] suggest that the excessive number of neurons in the subcortical white matter reflects the lack of proper resolution of the transient zone in the developing brain of autistic subjects. Studies by Fatemi et al. [37, 38] link the migration and lamination defects to a striking reduction of reelin (by 40%) and Bcl-2 (by 34–51%) in the brains of autistic subjects. Our studies along with others' suggest that in the majority of autistic subjects, heterotopias and dysplastic changes are the local sign of general developmental defects of migration with a broad spectrum of topographic, morphological, and functional outcomes.

In the examined brains of autistic subjects, signs of neuronal immaturity were a common finding. Failure of maturation of neuronal precursors caused by altered expression of cytoskeletal proteins and loss of neuronal polarity results in defects in migration to the destined layer and in incorrect vertical and horizontal orientation [93]. The immaturity of dysplastic neurons is reflected in the expression of a variety of proteins and mRNA that are not present in mature neurons an altered expression of developmentally regulated cytoskeletal elements [3, 23, 61, 76],

which are known to be crucial for dendrite arborization, spine formation, axon outgrowth and maintenance of cell size and shape. Reduced cell size, dendritic arborization and spine expression are characteristic of dysplastic neurons [6, 93]. Cortical dysplasias are the most epileptogenic lesions of the brain [107] and are observed in up to 25% of all epileptic surgeries [102]. More subtle cortical malformations or dysgenesis encountered in adults with epilepsy may lack the histological criteria for focal cortical dysplasia. They have been described as mild cortical dysplasia or microdysgenesis [77].

Microdysgenesis within the entorhinal cortex of the 23- and the 60-year-old autistic subjects in the examined cohort is unique because the selective deficit of neurons was limited almost exclusively to the stellate neurons in the second layer. It is possible that the observed dysgenesis is a result of defective migration of neurons to their intended destinations. The presence of a thicker molecular layer and the deeper location of islands in the entorhinal cortex of subjects with schizophrenia were previously interpreted as evidence that the stellate neurons do not reach their destinations during development, probably due to abnormal migration [36, 57]. Studies indicating the involvement of reelin and Bcl2 genes in the pathogenesis of schizophrenia [37, 47, 60] and the reduced expression of reelin and Bcl2 in people with autism suggest that these two genes play a role in abnormal brain development and contribute to the structural and functional anomalies seen in autism and schizophrenia [37].

The distortion of dentate gyrus development detected in two autistic subjects was reflected in granule cell migration into the molecular layer and formation of an additional granule cell layer. Distortion of the shape of the dentate granule cell layer with the formation of irregular circles and loops appears to be another piece of evidence suggesting abnormal neuronal migration and networking. Numerous factors up-regulate neurogenesis in the hippocampus [32], including seizures [70, 71], antidepressant drugs [59, 72] and lithium [18]. Several areas of dysplastic changes with significant deficits of pyramidal neurons were found in the CA1 sector in three autistic subjects, but thickening of the pyramidal layer and an increased packing of dysplastic neurons in the CA1 sector of the 56-year-old subject suggests a diversity of CA dysplasia patterns in autism. The lack of gliosis indicates that the observed pathology is a sign of microdysgenesis rather than an effect of hypoxic neuronal loss. A significant deficit of mature pyramidal neurons and the presence of small irregular or poorly differentiated oval neurons suggest the defect of neuronal maturation in autism.

We report a spectrum of focal developmental changes seen in the cerebellum of eight autistic subjects, including nodular (lobe X) [97] dysplasia in the cerebellum in five,

vermal dysplasia in one, severe focal hypoplasia in one, and heterotopias in one other subject. The presence of heterotopias only in one control subject is evidence of a strong tendency for focal developmental changes of cerebellar microarchitecture that were present in 61% of the autistic subjects. Flocculonodular dysplasia affecting almost the entire lobe indicates that mechanisms leading to focal dysplasia, which were present in five (38%) of the autistic subjects, show extremely strong topographic predilection. The observed focal dysplasia was associated with profound local disorganization of granule cells, Purkinje cells and molecular layers limited to a small cerebellar compartment receiving major projections from the vestibular complex involved in the oculomotor and postural system. Similar cerebellar dysplastic changes classified as heterotaxias (clusters of poorly organized mixed cells) were identified in 14% of normal infants but in 83% of infants with trisomy of different chromosomes [92]. The presence within the dysplastic nodule of both GABAergic Purkinje cells produced from the cerebellar ventricular zone, and the glutamatergic granule neurons produced from the rhombic lip, and the preservation of the cytoarchitecture in the adjacent cerebellar folia suggest that the final steps of migration and networking are disturbed mainly or exclusively in the nodule of the majority of autistic subjects. The characteristic feature distinguishing lobule X from the other lobules is the abundance of the transcription factor Tbr2 positive unipolar brush cells (UBCs) [30, 34], which amplify inputs from vestibular ganglia and nuclei, by spreading and prolonging excitation within the internal granular layer [84]. Abnormal networking of Purkinje cells, granule neurons, and UBCs may contribute to altered cerebellar coordination of locomotion and motor learning and planning, as well as of higher cognitive processing [58]. Flocculonodular dysplasia appears to be another sign of the mosaic of local developmental defects, most likely predetermined by the spatial patterning of germinal zones in developing rhombic lip [110], and coexisting with more general developmental defects resulting in the accelerated growth of the brain in early childhood [89], minicolumn pathology [13, 14], reduced neuron volume [7, 108, 111], and desynchronized neuronal growth in many brain regions [111] observed in autism.

Identification of sub-groups with signs of hyperplasia, hypoplasia and normal-sized cerebellum [95] reflects the heterogeneity of the autistic population. Piven et al. [87] reported that cerebellar volume correlates with an increased total brain volume. In the majority of autistic subjects, reduced size of the cerebellar hemisphere is observed [42, 82], but this trend is not detectable in cohorts of high-functioning autistic individuals [56]. Regional hypoplasia affects the vermis in autistic individuals relatively often [20, 22, 52] and may be associated with the

deficits in attention-orienting [49, 104], stereotypic behavior and reduced exploration observed in autism [86]. In the examined autistic cohort, selective and severe hypoplasia of lobes 1–4 associated with hypoconvolution of a large portion of the dentate nucleus appears to correspond to clinically detected defects of movement coordination. These findings suggest that differences in the type, topography and severity of cerebellar developmental defects may contribute to different clinical manifestations.

In the 4–7-year-old autistic children examined in this study, the volume of the Purkinje cells was 38% smaller than that of the age-matched control group [111]. Moreover, it has been reported that Purkinje cells of the autistic subjects revealed a 40% decrease in the expression of glutamic acid decarboxylase 67 (GAD67) mRNA [114]. In autism, the basket cells provide an increased GABAergic feed-forward inhibition to Purkinje cells. The result could be disruption in the timing of Purkinje cell firings and altered inhibition of the cerebellar nuclei, which could directly affect cerebello-cortical output and contribute to the changes in motor behavior and cognition observed in autism [115]. These findings and the reduced volume (by 26%) of the neurons of the dentate nucleus seen in the 4–7-year-old autistic children [111] suggest that in autism, interactions between the Purkinje cells and dentate nucleus are modified on the structural, molecular and functional levels.

The (a) detected changes within the subependymal cell layer with subependymal nodular dysplasia, (b) subcortical and periventricular heterotopias and (c) neocortex, archi-cortex, dentate gyrus, cornu Ammonis and cerebellar dysplasia reflect focal modification of neurogenesis, migration and alterations of the cytoarchitecture of brain cortex, subcortical structures and cerebellum in autism. Detection of dysplastic changes only in one control brain and of the broad spectrum of focal developmental alterations in the brains of 92% of the autistic subjects indicates that focal changes are a reflection of global developmental abnormalities and that regional changes may have their own contribution to the clinical heterogeneity of autism.

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