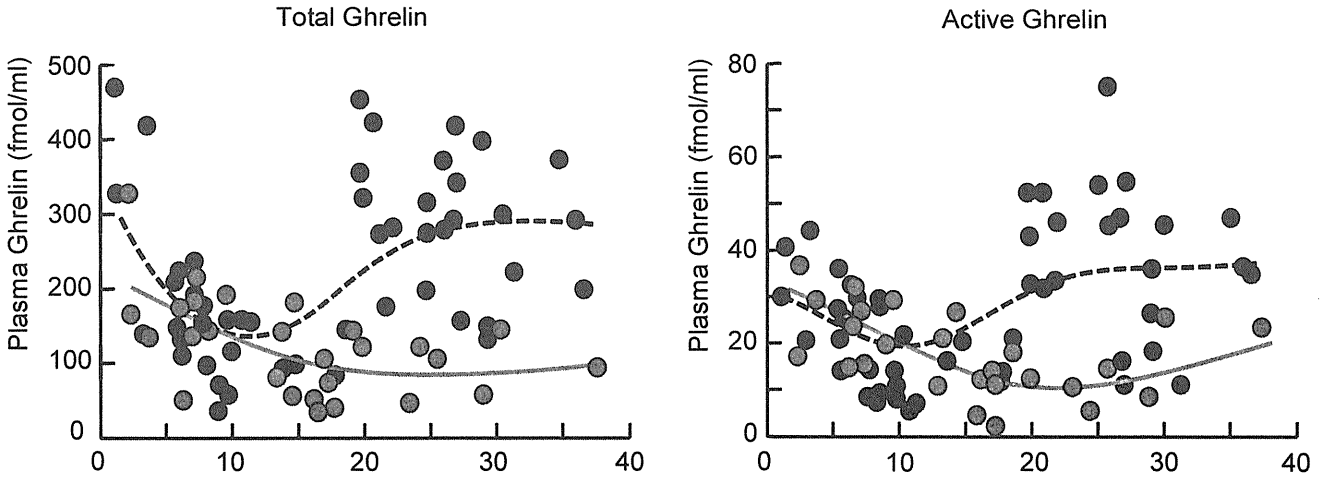
図１．年齢に伴う血漿中グレリン濃度の変化



赤丸：RTT女児，黒丸：コントロール女児　　(Int J Devl Neuroscience (2011))

II. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Nomura Y	Rett syndrome	Kompoliti K, Verhagen L.	Encyclopedia of Movement Disorders	Elsevier	New York	2010	38-41
野村芳子	Rett症候群の運動発達	久保田雅也、五十嵐隆	「小児科臨床ピクシス19」ここまでわかった小児の発達	中山書店	東京	2010	159-163

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kuki I, Kawawaki H, Okazaki S, Kimura S, Nakano T, Fukushima H, Inoue T, Tomiwa K, Itoh M.	Progressive Leukoencephalopathy with Intracranial Calcification, Congenital Deafness and Developmental Deterioration.	Am J Med Genet A	155	2832-2837	2011
Miyake K, Hirasawa T, Soutome M, Itoh M, Goto Y, Endoh K, Takahashi K, Kudo S, Nakagawa T, Yokoi S, Taira T, Inazawa J, Kubota T.	The protocadherins, PCDHB1 and PCDH7, are regulated by MeCP2 in neuronal cells and brain tissues: implication for pathogenesis of Rett syndrome	BMC Neuroscience	12	81	2011
Honda T, Fujino K, Okuzaki D, Ohtaki N, Matsumoto Y, Horie M, Daito T, Itoh M, Tomonaga K	Upregulation of insulin-like growth factor binding protein 3 in astrocytes of transgenic 4 mice expressing Borna disease virus phosphoprotein	J Virol	85	4567-4571	2011
Saito T, Hanai S, Takashima S, Nakagawa E, Okazaki S, Inoue T, Miyata R, Hoshino K, Akashi T, Sasaki M, Goto Y, Hayashi M, Itoh M	Neocortical layer-formation of the human developing brains and lissencephalies: consideration of layer-specific markers expression	Cereb Cortex	21	588-596	2011
Itoh M, Tahimic CG, Ide S, Otsuki A, Sasaoka T, Noguchi S, Oshimura M, Goto YI, Kurimasa A	Methyl CpG-binding Protein Isoform MeCP2_e2 Is Dispensable for Rett Syndrome Phenotypes but Essential for Embryo Viability and Placenta Development	J Biol Chem	Feb 28. [Epub ahead of print]		2012
Itoh M, Takizawa Y, Hanai S, Okazaki S, Miyata R, Shu H, Inoue T, Akashi T, Goto Y, Hayashi M	Partial loss of pancreas endocrine and exocrine cells of human ARX-null mutation: consideration of pancreas differentiation	Differentiation	80	118-122	2010
Hanai S, Saito T, Nakagawa E, Arai A, Otsuki T, Sasaki M, Goto Y, Itoh M	Abnormal development of neurons in focal cortical dysplasia: neuronal mis-maturation from an immunohistochemical consideration	Seizure	19	274-279	2010

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hara M, Nishi Y, Yamashita Y, Matsuishi T	Ghrelin levels are reduced in Rett syndrome patients with eating difficulties	Int J. Devl Neuroscie nce	29	899-902	2011
Matsuishi T, Yamashita Y, Takahashi T, Nagamitsu S	Rett syndrome: The state of clinical and basic research, and future perspectives	Brain Dev	33	627-631	2011
Okabe Y, Kusaga A, Takahashi T, Mitsumasu C, Murai Y, Tanaka E, Higashi H, Matsuishi T, Kosai K	Neural Development of Methyl-CpG-Binding Protein 2 Null Embryonic Stem Cells: A System for Studying Rett Syndrome	Brain Res	1360	17-27	2010
Tomimatsu N, Mukherjee B, Deland K, Kurimasa A, Bolderson E, Khanna KK, Burma S	Exo1 plays a major role in DNA end resection in humans and influences double-strand break repair and damage signaling decisions	DNA Repair (Amst)	Feb 10. [Epub ahead of print]		2012
Hisatomi T, Sueoka-Aragane N, Sato A, Tomimasu R, Ide M, Kurimasa A, Okamoto K, Kimura S, Sueoka E	NK314 potentiates anti-tumor activity with adult T-cell leukemia-lymphoma cells by inhibition of dual targets on topoisomerase II-alpha and DNA-dependent protein kinase	*Blood*	Jan 18. [Epub ahead of print]		2011
Yasui DH, Scoles HA, Horike S, Meguro-Horike M, Dunaway KW, Schroeder DI, Lasalle JM	15q11.2-13.3 chromatin analysis reveals epigenetic regulation of CHRNA7 with deficiencies in Rett and autism brain	Hum Mol Genet	20	4311-4323	2011
Meguro-Horike M, Yasui DH, Powell W, Schroeder DI, Oshimura M, Lasalle JM, Horike S	Neuron-specific impairment of inter-chromosomal pairing and transcription in a novel model of human 15q-duplication syndrome.	Hum Mol Genet	20	3798-3810	2011
Abe S, Tanaka H, Notsu T, Horike S, Fujisaki C, Qi DL, Ohhira T, Gilley D, Oshimura M, Kugoh H	Localization of an hTERT repressor region on human chromosome 3p21.3 using chromosome engineering	*Genome Integrity*	1	6	2010
Kuroki Y, Honda K, Kijima N, Wada T, Arai Y, Matsumoto N, Iwata K, Shirakawa T	In vivo morphometric analysis of inflammatory condylar changes in rat temporomandibular joint	Oral Dis	17	499-507	2011
Mikami Y, Ishii Y, Watanabe N, Shirakawa T, Suzuki S, Irie S, Isokawa K, Honda MJ	CD271/p75NTR inhibites the differentiation of mesenchymal stem cells into osteogenic, adipogenic, chondrogenic, and myogenic lineages.	Stem Cells Dev	20	901-913	2011
Kikuiri T, Yoshimura Y, Tabata F, Hasegawa T, Nishihira J, Shirakawa T	Stage-dependent suppression of the formation of dentin-resorbing multinuclear cells with migration inhibitory factor in vitro	Exp Ther Med	3	37-43	2012

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takei H, Fujita S, Shirakawa T, Koshikawa N, Kobayashi M	Insulin facilitates repetitive spike firing in rat insular cortex via phosphoinositide 3-kinase but not mitogen activated protein kinase cascade	*Neuroscience*	170	1199-1208	2010
和田崇, 滝口旗一, 武内倫子, 黒木洋祐, 関信幸, 高森一乗, 白川哲夫	*Mecp2*欠損Rett症候群モデルマウスにみられる呼吸の異常と病態変化	障歯誌	730-736	31	2010
白川哲夫	スペシャルニードの子どもたちへの歯科医療・病診連携	小児歯誌	48	40	2010
野村芳子	レット症候群	精神科治療学	25	264-265	2010

III. 研究成果の刊行物・別刷

Cerebral Cortex March 2011;21:588-596
doi:10.1093/cercor/bhq125
Advance Access publication July 12, 2010

Neocortical Layer Formation of Human Developing Brains and Lissencephalies: Consideration of Layer-Specific Marker Expression

Takashi Saito[1,2], Sae Hanai[1,2], Sachio Takashima[3], Eiji Nakagawa[2], Shin Okazaki[4], Takeshi Inoue[5], Rie Miyata[6], Kyoko Hoshino[7], Takumi Akashi[8], Masayuki Sasaki[2], Yu-ichi Goto[1], Masaharu Hayashi[6] and Masayuki Itoh[1]

[1]Department of Mental Retardation and Birth Defect Research, National Center of Neurology and Psychiatry, Kodaira, 187-8502, Japan, [2]Department of Child Neurology, The Hospital of National Center of Neurology and Psychiatry, Kodaira, 187-8551, Japan, [3]Yanagawa Institute of Handicapped Children, International University of Health and Welfare, Fukuoka, 832-0058, Japan, [4]Department of Pediatrics, Osaka City General Hospital, Osaka, 534-0021, Japan, [5]Department of Pathology and Laboratory Medicine, Osaka City General Hospital, Osaka, 534-0021, Japan, [6]Department of Clinical Neuropathology, Tokyo Metropolitan Institute of Neuroscience, Fuchu, 183-8526, Japan, [7]Department of Pediatrics, Saitama Medical Center, Kawagoe, 350-8550, Japan and [8]Department of Pathology and Laboratory Medicine, Tokyo Medical and Dental University, Tokyo, 113-8510, Japan

Takashi Saito and Masayuki Itoh have contributed equally to this work.

Address correspondence to M Itoh, Department of Mental Retardation and Birth Defect Research, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan. Email: itoh@ncnp.go.jp.

To investigate layer-specific molecule expression in human developing neocortices, we performed immunohistochemistry of the layer-specific markers (TBR1, FOXP1, SATB2, OTX1, CUTL1, and CTIP2), using frontal neocortices of the dorsolateral precentral gyri of 16 normal controls, aged 19 gestational weeks to 1 year old, lissencephalies of 3 Miller–Dieker syndrome (MDS) cases, 2 X-linked lissencephaly with abnormal genitalia (XLAG) cases, and 4 Fukuyama-type congenital muscular dystrophy (FCMD) cases. In the fetal period, we observed SATB2+ cells in layers II–IV, CUTL1+ cells in layers II–V, FOXP1+ cells in layer V, OTX1+ cells in layers II or V, and CTIP2+ and TBR1+ cells in layers V and VI. SATB2+ and CUTL1+ cells appeared until 3 months of age, but the other markers disappeared after birth. Neocortices of MDS and XLAG infants revealed SATB2+, CUTL1+, FOXP1+, and TBR1+ cells diffusely located in the upper layers. In fetal FCMD neocortex, neurons labeled with the layer-specific markers located over the glia limitans. The present study provided new knowledge indicating that the expression pattern of these markers in the developing human neocortex was similar to those in mice. Various lissencephalies revealed abnormal layer formation by random migration.

Keywords: developing human neocortex, layer-specific marker, lissencephaly

Introduction

The experimental neurosciences have recently provided many new insights into the molecular mechanisms of mammalian cerebral formation. Past knowledge revealed that some molecules are regulated with a well-designed genetic algorithm during the developmental stages, with interrelated phenomena that include cell proliferation, fate determination and migration to the proper laminar, and final position in the cerebral cortex. Neocortical laminar formation is highly programmed by genetic control in the early embryonic period. At the decided time, projection neurons migrate into the cortical plate (CP) along the radial glial process from the subventricular germinal zone with an inside out pattern. At this neural migration stage, integration of reelin (RELN), Lis-1, doublecortin (DCX), and other molecules is required to form a complete neocortex (Guillemot et al. 2006; Mochida and Walsh 2004). Finally, mammalian brains commonly show a 6-layer neocortex, and each layer has a specific function with a synaptic connection. In each step, specific genes have important roles, and the molecular mechanism is well known in rodent brains (Arlotta et al. 2005; Alcamo et al. 2008). Satb2, a special AT-rich binding protein 2, generates callosal projection neurons in layers II–IV (Alcamo et al. 2008; Britanova et al. 2008). Ctip2, encoding a C2H2-type zinc finger protein, locates in layers V and VI and promotes corticospinal motor neuron projection (Arlotta et al. 2005; Britanova et al. 2008). Satb2 is a repressor of Ctip2 and makes not only the callosal projection but also the subcortical connections (Alcamo et al. 2008). Mouse Otx1, orthodenticle homeobox 1, is expressed in a number of cells in layers V and VI (Weimann et al. 1999). Tbr1, a member of the T-box homeobox gene family, expresses in preplate and layer VI in mouse fetal brain (Hevner et al. 2001) and layers I–III and layer VI in mouse adult brain (Bulfone et al. 1995). Tbr1 contributes to make corticocortical projection neurons (Hevner et al. 2001). Tbr1 expresses in the deep layer of the human fetus cortex (Sheen et al. 2006). A transcription factor Cutl1, drosophia homeobox CUT like 1, is expressed in pyramidal neurons of the upper layer (Nieto et al. 2004). Foxp1, a transcription factor of the winged-helix/forkhead family, expresses in layers III–V of mouse neocortex (Ferland et al. 2003) and layer V in human neocortex (Sheen et al. 2006). Foxp1 expresses in the deep layer of Miller–Dieker syndrome (MDS) neocortex (Sheen et al. 2006). However, many rodent studies show that the other layer-specific molecules also play very important roles in forming cortical lamination (Molyneaux et al. 2007) and that such gene disruption leads to profound cortical malformation (Mochida and Walsh 2004).

Lissencephaly, formed at the neuronal migration period, is classically recognized to be mainly of 2 types; smooth pacthygyria-agyria as type I lissencephaly and cobblestone lissencephaly as type II lissencephaly (Olson and Walsh 2002). Type I (classical) lissencephaly shows a thick 4-layer cortex and is typically known as MDS and double cortex syndrome. The causative genes of type I lissencephaly are known as RELN, Lis-1, DCX, and filamine. Interestingly, the gene products are associated with the microtubules and can alter the cytoskeleton size for cell movement (de Rouvroit and Goffinet 2001; Reiner and Sapir 2009) or its related molecules (Olson and Walsh 2002; Assadi et al. 2003). Typical type II (cobblestone) lissencephalies

of Muscle-eye-brain disease, Walker–Warburg syndrome (WWS), and Fukuyama-type congenital muscular dystrophy (FCMD), are caused by mutated genes encoding enzymes of alpha-dystroglycan glycosylation, such as POMGnT1, protein-O-mannosyltransferase (POMT) 1 and 2, and Fukutin, respectively (Mochida and Walsh 2004). The posttranslational glycosylated alpha-dystroglycan binds to extracellular matrix (Michele et al. 2002). Reduction of glycosylation leads to disruption of the glia limitans over which neurons migrate (Yamamoto et al. 2004).

Recently, it has been reported that X-linked lissencephaly with abnormal genitalia (XLAG), whose causative gene is *Aristaless*-related homeobox gene (*ARX*), is a new type of lissencephaly that shows a 3-layer neocortex (Dobyns et al. 1999; Kitamura et al. 2002; Bonneau et al. 2002; Okazaki et al. 2008). ARX has a homeodomain and decides the migration of interneurons in the ganglionic eminence. However, it is unknown why ARX dysfunction leads to abnormal radial neuronal migration in human XLAG, whereas *ARX*-null mice show reduced cortical proliferation but normal migration (Kitamura et al. 2002; Okazaki et al. 2008).

It is very important to reveal the molecular and morphological relationship between these malformed brains to understand human neocortical formation and pathophysiology, although little is known about the expression pattern of layer-specific markers in human developing brain (Hevner 2007). In the present study, we focus on layer formation and investigate the expression of layer-specific molecules in neocortices of human developing brains and lissencephalies.

Materials and Methods

Human Brain Tissues

All cerebral tissues used in the present study were approved for research usage by parents and Ethical Committees of the involved hospitals and institutes. For the developmental study, we used frontal cortices of the dorsolateral precentral gyri of 16 controls, showing no neuropathological findings (age 19 gestational weeks [GWs] to 1 year after birth) (Supplementary Material). In addition, we examined the same frontal cerebral hemispheres of lissencephaly, which were clinicopathologically diagnosed as MDS, XLAG, and FCMD (Supplementary Material). The postmortem interval (time from death to fixation) of all subjects was within 12 h (Supplementary Material). After removal, all brains were fixed in 10% buffered formalin or 4% paraformaldehyde for 2 weeks. Then, brains were dehydrated with 70–100% alcohol and embedded in paraffin. The serial sections were cut 6 μm thick for histological and immunohistological examination.

Histology and Immunohistochemistry

For investigation of brain architecture, the sections were stained with hematoxylin and eosin (HE) and Klüber-Barrera (KB) method. To investigate cortical layer formation, we performed immunohistochemistry using cortical layer-specific markers; polyclonal antibodies against TBR1 (dilution of 1:100; Abcam), FOXP1 (1:100; Abcam), and OTX1 (1:100; Abcam), as well as monoclonal antibodies against SATB2 (1:100; Bio Matrix Research Inc.), CUTL1 (1:100; Abnova), and CTIP2 (1:20; Abcam).

Our immunohistochemistry technique was previously described (Okazaki et al. 2008). Briefly, the serial sections were deparaffinized and rehydrated. For antigen retrieval, we performed an autoclave treatment (120 °C for 10 min in 10 mM citrate buffer, pH 6.0). Sections were incubated in primary antibodies at 4 °C for overnight, and then reacted with the secondary antibodies (Nichirei). We used amino ethyl carbazole (Nichirei) as a chromogen. For counterstaining, 0.2% methyl green was used. For double labeling, we used Alexafluor-488- and 568-conjugated secondary antibodies (Invitrogen Corporation) with 4′,6′-diamidino-2-phenylindole (DAPI). We observed the stained tissues with FLUOVIEW 500 fluorescent microscope (Olympus).

Results

Cortical Lamination of Normal Developing Brains

Generally, we confirmed cortical formation of all subjects with HE- and KB-staining. We observed the CP and intermediate zone around 20 GW (Fig. 1*A*). At this embryonic period, SATB2+ cells located in the upper region of CP (Fig. 1*B*). CUTL1+ cells were diffusely distributed in CP (Fig. 1*C*). FOXP1+ cells were restricted to the middle region of CP (Fig. 1*D*). OTX1+ cells and CTIP2+ cells are seen in the lower region of CP (Fig. 1*E,F*). The distribution of TBR1+ cells exhibited a 2-layer pattern of CP and SP (Fig. 1*G*).

At approximately 30 GW, the neocortex was divided into 6 layers (Fig. 2*A*). The distribution of SATB2+ cells was observed in layers II–V, predominantly in layers II and IV (Fig. 2*B*). CUTL1+ cells were diffusely seen in layers II–VI (Fig. 2*C*). FOXP1+ cells were in layer IV and the upper region of layer V (Fig. 2*D*). OTX1+ cells were concentrated in layers IV and V (Fig. 2*E*). CTIP2+ and TBR1+ cells were located in layers V and VI (Fig. 2*F,G*). The developmental expression pattern is shown in Supplementary Figure 1.

In the perinatal period, the expression pattern of the cortical layer-specific markers is very similar to that of around 30 GW (Fig. 3). In the late gestational period, SATB2 expressed in the superficial region of the neocortex and CUTL1, FOXP1, and CTIP2 gradually demonstrated in the deep region, while TBR1 was in the bottom. Interestingly, OTX1+ cells were only in layer V (Fig. 3*E*). After birth, SATB2+ and CUTL1+ cells appeared until 3 months of age, although the other markers had already disappeared (data not shown).

In order to investigate the relationships among these layer-specific markers, we performed double fluorescent staining of SATB2 and FOXP1, SATB2 and TBR1, CTIP2 and SATB2, SATB2 and OTX1, CTIP2 and FOXP1, and CTIP2 and TBR1 (Fig. 4, Supplementary Figure 1). FOXP1+ and SATB2+ merged (FOXP1+/SATB2+) cells were observed in the superficial CP of 23 GW but in the deep layer after 29 GW (Fig. 4*A*). Throughout the fetal period, FOXP1+/CTIP2+ cells might be in the deep layer (Fig. 4*E*), and many SATB2+/OTX1+ cells were in layers II and IV or V (Fig. 4*D*). However, SATB2+ cells did not express CTIP2+ (Fig. 4*C*). TBR1+ cells had no SATB2, but there were a few CTIP2 signals in layer VI (Fig. 4*B,F*). The double staining of layer-specific marker expression was shown in Supplementary Figure 1.

Layer-Specific Marker Expression of Various Lissencephalies

MDS brains were typical agyria and pachygyria with thick cortex and thin white matter. MDS showed a 4-layer neocortex as previously reported (Crome 1956): a molecular layer, an external cellular layer (layer I), a sparsely cellular layer (layer II), and an internal cellular layer (layer III) (Fig. 5*A*). In layers II, III, and IV, small neurons, which had immunoreactivities of SATB2, CUTL1, FOXP1, and TBR1, were observed diffusely but were few in number (Fig. 5*B–E*). Large pyramidal neurons in the upper layer II had TBR1 (Fig. 5*E*). The neocortex in XLAG exhibited a 3-layer pattern (Bonneau et al. 2002): a molecular layer (layer I), an intermediate layer with densely packed

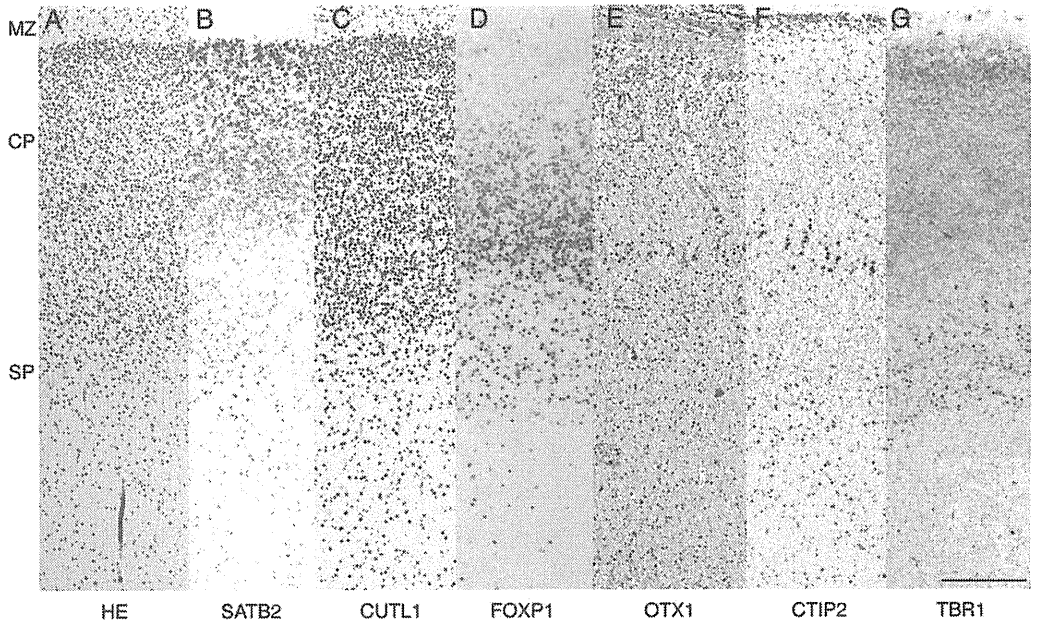


Figure 1. Layer-specific marker expression of the neocortex at 23 GWs. Around 20 GWs, the 3-layer pattern, that is, the marginal zone (MZ), CP, and subplate (SP), are seen (*A*). SATB2 expresses in the upper region of CP (*B*). CUTL1 diffusely expresses in the whole cortex and intermediate zone (*C*). FOXP1-positive cells locate in the middle region (*D*) and CTIP2-immunopositive cells (*F*) locate in the lower region of CP. OTX1 exhibits in CP and SP, predominantly lower region of CP (*E*). TBR1-immunopositive cells are in the lower region of CP and SP, as well as those fibers in CP (*G*). *A*, HE; *B–G*, SATB2, CUTL1, FOXP1, OTX1, CTIP2, and TBR1 immunohistochemistry, respectively. Scale bar: 100 μm.

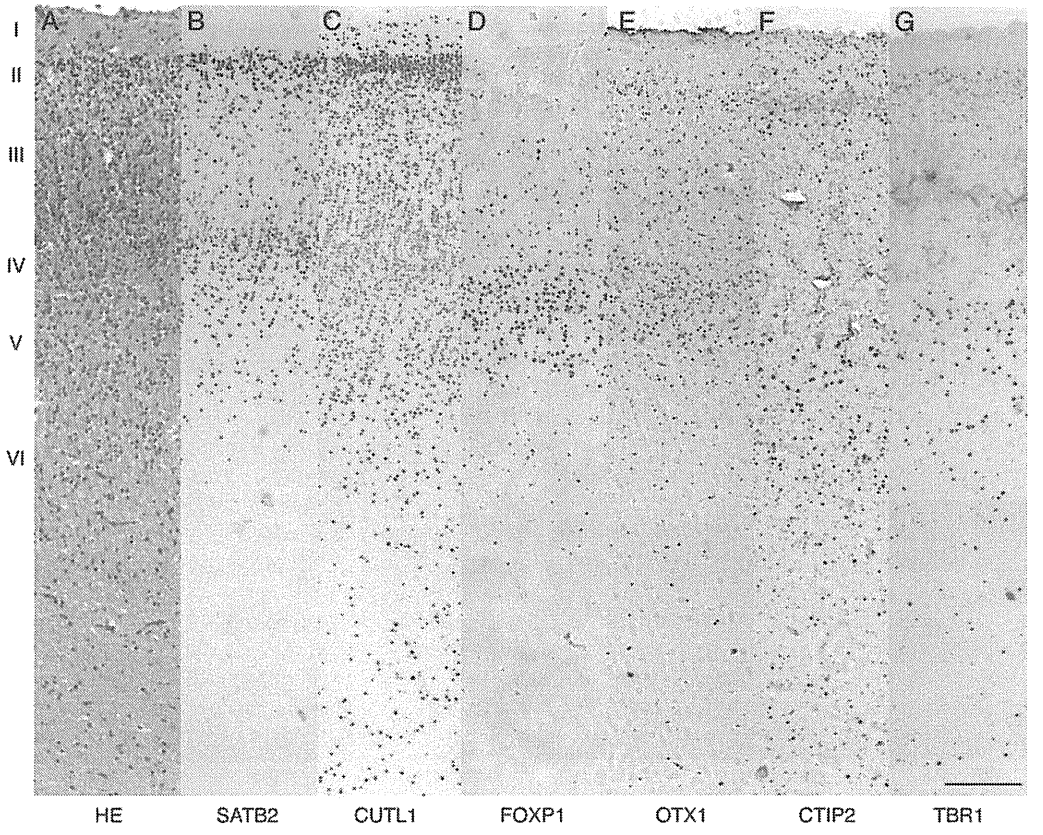


Figure 2. Layer-specific marker expression of the neocortex at 29 GWs. The 6-layer neocortex is shown (*A*). SATB2 expresses in layers II–V, especially layer II and upper region of layer IV (*B*). CUTL1 diffusely expresses in layers II–V and predominates in layer II (*C*). FOXP1 converges to layers VI and V (*D*). OTX expresses in upper layer and layers VI and V (*E*). CTIP2- and TBR1-immunopositive cells locate in layer V and layers V and VI (*F* and *G*). *A*, HE; *B-G*, SATB2, CUTL1, FOXP1, OTX1, CTIP2, and TBR1 immunohistochemistry, respectively. Scale bar: 100 μm.

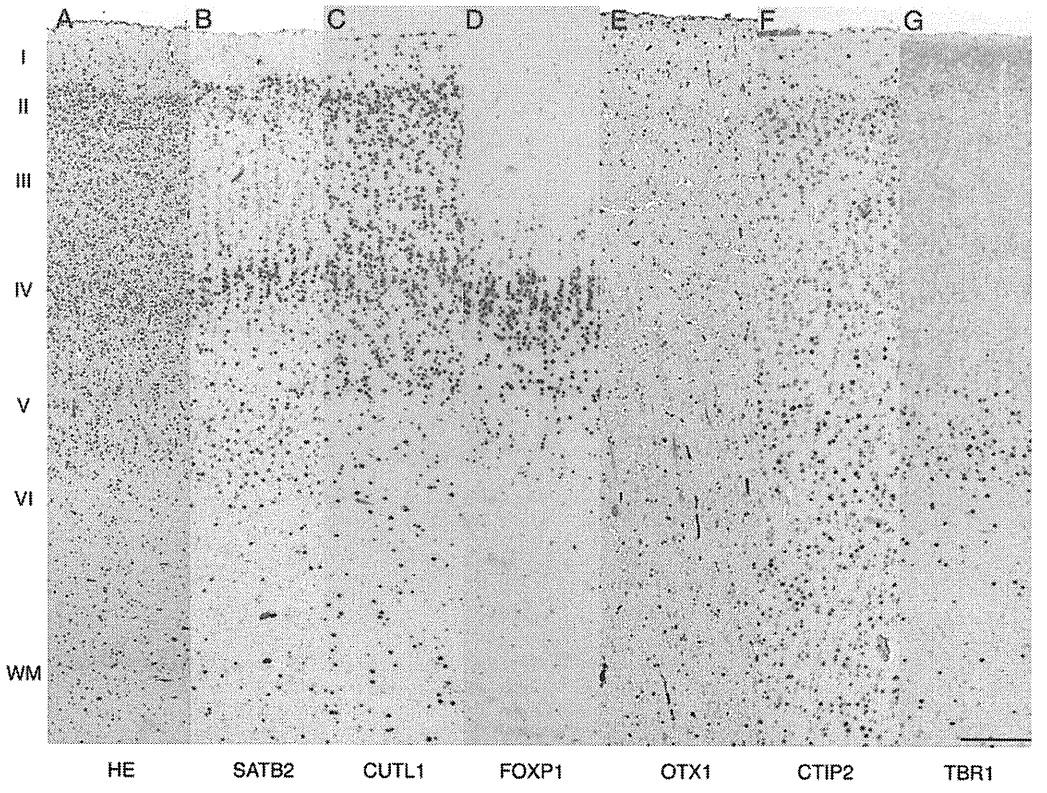


Figure 3. Layer-specific marker expression of the neocortex at 37 GWs. Expression of SATB2, CUTL1, FOXP1, OTX1, CTIP2, and TBR1 has a pattern similar to those at 29 GWs. OTX1 disappears in upper layer of neocortex (*E*). *A*, HE; *B–G*, SATB2, CUTL1, FOXP1, OTX1, CTIP2, and TBR1 immunohistochemistry, respectively. Scale bar: 100 μm.

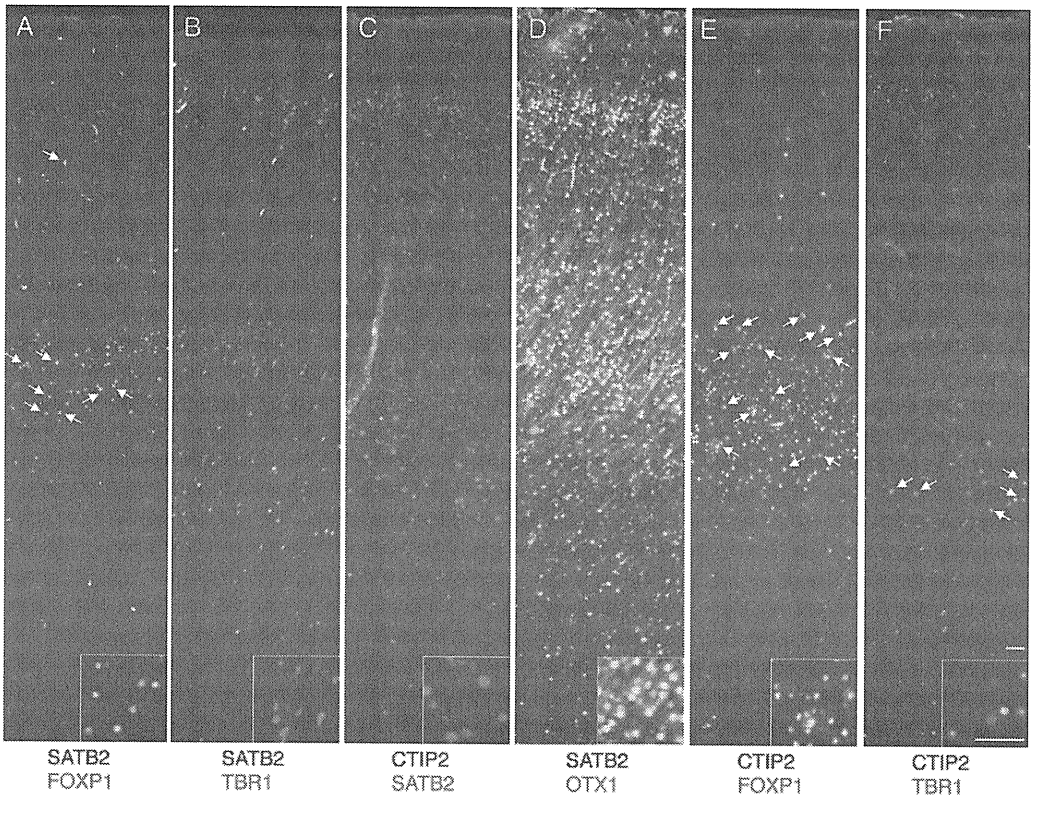


Figure 4. Immunofluorescence of layer-specific marker of neocortex at 29 GWs. FOXP1-immunopositive cells partially have SATB2 (merged color: arrows) in layers II–III and IV–V (*A*) and CTIP2 (merged color: arrows) in layers IV–VI (*E*). No double-positive cells for SATB2 and CTIP2 are scattered throughout all layers (*C*). No TBR1+ and SATB2+ cells are observed in layers V and VI (*B*), but a few TBR1+ and CTIP2+ cells are seen in layers V and VI (*F*). Many merged cells with SATB2 (red) and OTX1 (green) are diffusely demonstrated, predominantly in layers II and V (*D*). *A*, SATB2 (red) and FOXP1 (green) double fluorescence; *B*, SATB2 (red) and TBR1 (green); *C*, CTIP2 (red) and SATB2 (green); *D*, SATB2 (red) and OTX1 (green); *E*, CTIP2 (red) and FOXP1 (green); *F*, CTIP2 (red), and TBR1 (green). Scale bars: 20 μm.

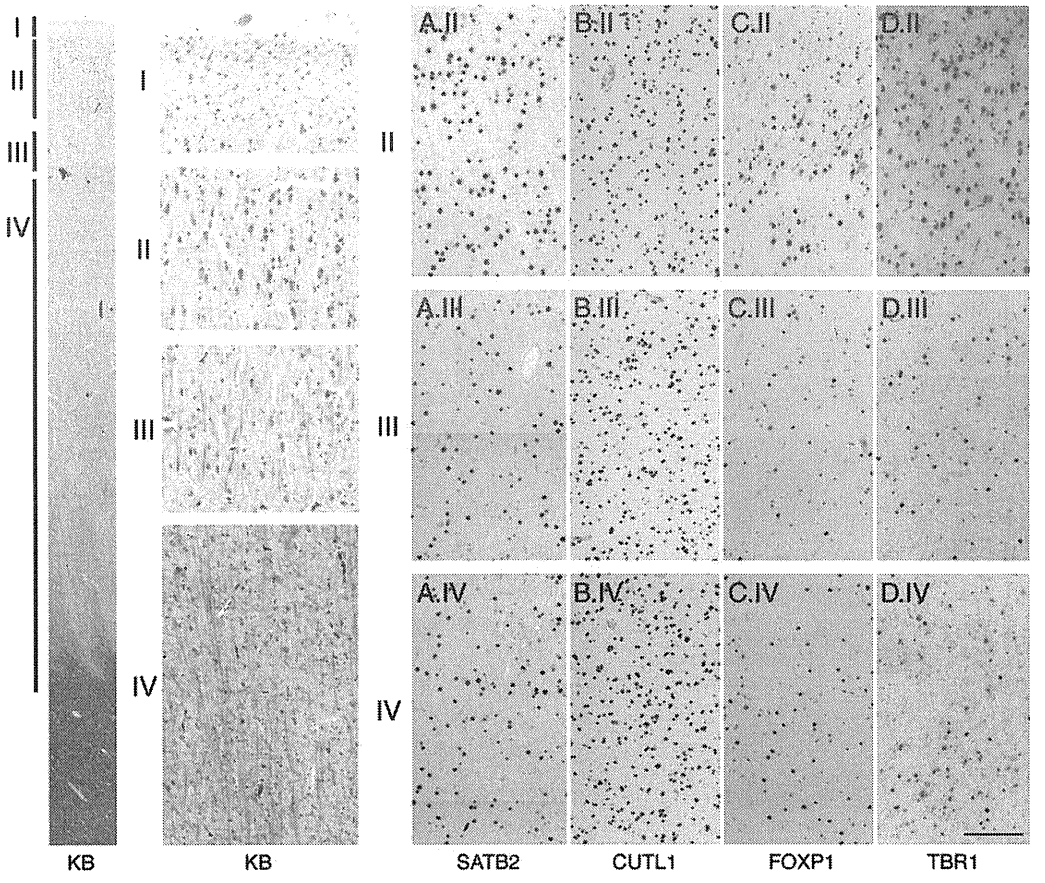


Figure 5. Layer-specific marker expression of the neocortex of 1-year-old patient with Miller–Dieker syndrome. Typical 4-layer pattern is shown. (*A*) SATB2, CUTL1, FOXP1, and TBR1 are diffusely expressed in layers II, III, and IV. Especially, TBR1-immunopositive cells locate in layer II (*E*). Enlargement of layer II shows *A.II*, *B.II*, *C.II*, and *D.II*. Enlargement of layer III shows *A.III*, *B.III*, *C.III*, and *D.III*. Enlargement of layer IV shows *A.IV*, *B.IV*, *C.IV*, and *D.IV*. Gross histology shows with KB staining. *A.II*, *A.III*, and *A.IV*, SATB2 in layers II, III, and IV; *B.II*, *B.III*, and *B.IV*, CUTL1; *C.II*, *C.III*, and *C.IV*, FOXP1; *D.II*, *D.III*, and *D.IV*, TBR1, respectively. Scale bar: 100 μm.

neurons (layer II), and a deep layer (layer III) (Fig. 6*A*). SATB2+ and CUTL1+ cells located in the intermediate layer and upper region of the deep layer (Fig. 6*B,C*). FOXP1+ cells and TBR1+ cells were also distributed in layer II and III (Fig. 6*D,E*). These labeled cells in the deep intermediate layer were large and dense but small and sparse in the upper region of the intermediate layer. Also, in the molecular layer, FOXP1+ and TBR1+ cells were few. No CTIP2+ and OTX1+ cells were observed in either malformed brain.

Usually, FCMD cerebral cortices show type II lissencephaly with cobblestone cortex. The cerebral cortices of FCMD fetus already revealed typical cobblestone lissencephaly (Fig. 7*A*). Neurons of the fetal neocortex migrated over the glia limitans. SATB2+, CULT1+, FOXP1+, CTIP2+, and TBR1+ cells were dense above the glia limitans and sparse below it (Fig. 7*B–D*), and TBR1+ cells were distributed predominantly below the glia limitans (Fig. 7*E*). However, no markers were detected in specimens from postnatal FCMD brains (data not shown).

The layer-specific marker expression pattern of 3 types of lissencephalies was summarized in Supplementary Figure 2.

Discussion

Very little is known about the molecular mechanism of human neocortex layer formation. Here, we presented new knowledge regarding the layer-specific marker expression in fetus development. Recent neuronal developmental studies have introduced some molecules as layer-specific markers. Among them, Satb2, Cutl1, Foxp1, Otx1, Ctip2, and Tbr1 are well-known transcriptional factors and highly conserved. The facts that SATB2 was relatively limited to layers II and IV of human fetus cortex and that Cutl1 was not known in human but was expressed in layers II–IV evidenced the same expression patterns of these molecules in rodent study (Nieto et al. 2004; Britanova et al. 2008). The migration pattern of callosal projection neurons may be the same as that in the mouse. FOXP1+ cells located in deep layers or layers IV–V before 30 GW and in layers IV–VI before birth. TBR1+ cells located in layers V–VI in the fetal period. FOXP1+ and TBR1+ cell localization in layers IV and V was similar to those in a previous human study (Sheen et al. 2006). However, TBR1+ cells were located beneath FOXP1+ cells but not colocalized. The restricted distribution of CTIP2+ cells in layer V may reflect the corticospinal projection formation, as indicated by mouse *ctip2* analysis (Arlotta et al. 2005). Interestingly, SATB2+ cells were located in the upper region of layer IV and FOXP1+ cells in the lower region of the same layer. This different localization indicates completely different neural functions between SATB2 and FOXP1, although the FOXP1 function in neocortex is unknown.

In mouse neocortex, Otx1+, Tbr1+, Ctip2+, Foxp1+, Cutl1+, and Satb2+ neurons are born around embryonic day 12.5, 10.0,

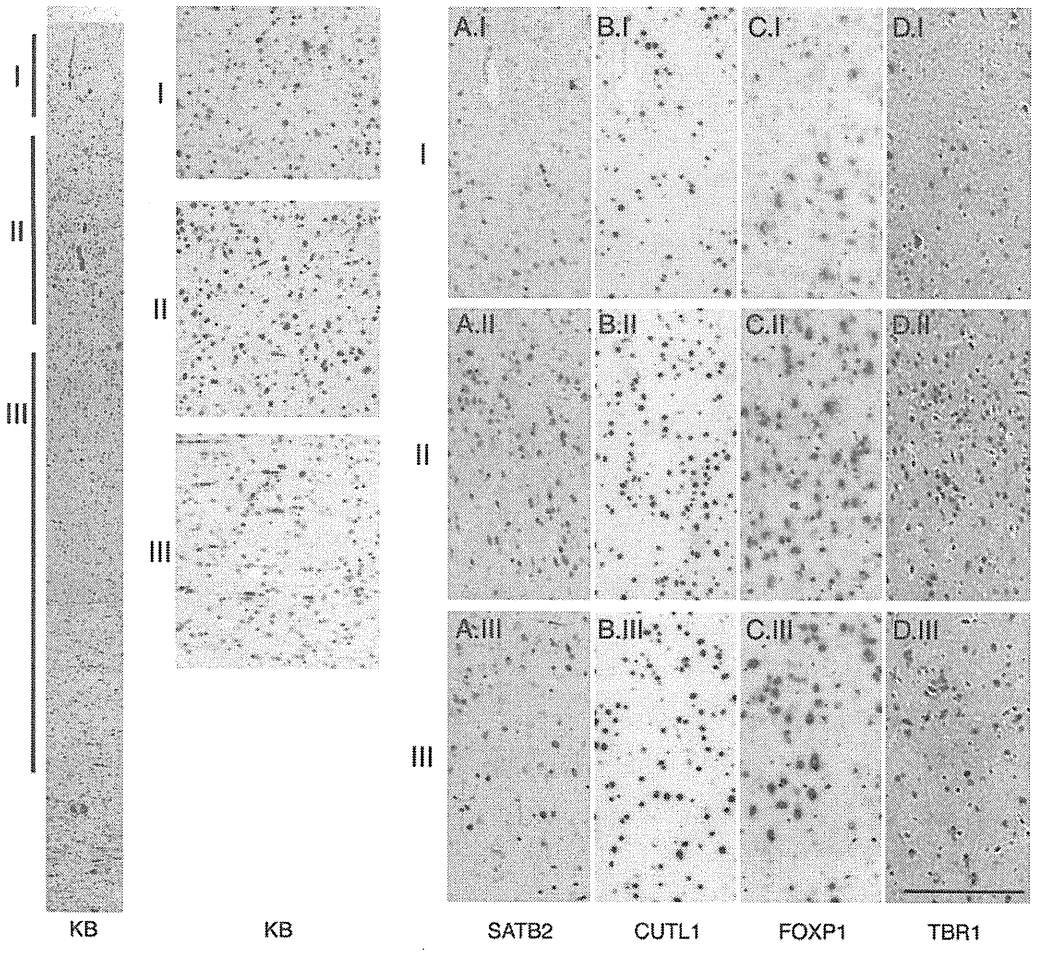


Figure 6. Layer-specific marker expression of the neocortex of 10-month-old boy with XLAG. Neocortex shows a thin 3-layer pattern. SATB2-, CUTL1-, FOXP1-, and TBR1-immunopositive cells locate diffusely (*A–D*). Gross histology shows with KB staining. *A.I*, *A.II*, and *A.III*, SATB2 in layers I, II, and III; *B.I*, *B.II*, and *B.III*, CUTL1; *C.I*, *C.II*, and *C.III*, FOXP1; *D.I*, *D.II*, and *D.III*, TBR1, respectively. Scale bar: 100 μm.

12.0, 14.5, 13.0, and 13.5, respectively (Simeone et al. 1993; Bulfone et al. 1995; Hevner et al. 2001; Ferland et al. 2003; Leid et al. 2004; Nieto et al. 2004; Arlotta et al. 2005; Britanova et al. 2005). These labeling neurons originate from progenitor cells residing in the ventricular zone (VZ) and the subventricular zone (SVZ) of early developing brain. Early progenitor cells in VZ produce deep layer neurons expressing Ctip2. On the contrary, late progenitor cells in SVZ form upper layers, expressing Cutl1 (Nieto et al. 2004). The previous data that Satb2-null mice show loss of Cutl1+cells in the superficial layers (Alcamo et al. 2008) suggest the profound molecular relationship of Satb2 and Cutl1. Satb2+ cells directly contribute to the formation of a callosal projection of the bilateral neocortical connection (Alcamo et al. 2008), while Ctip2+ cells contribute to the formation of a corticospinal projection forming a long pathway between the neocortex and anterior horn of the spinal cord (Arlotta et al. 2005). Interestingly, the expression patterns of SATB2 and CTIP2 in human neocortex mimicked those of rodent, and SATB2+ cells were also found in part of layer V. Although SATB2+ cells and CTIP2+ cells were in layer V, these double-marked cells were not observable. This may indicate these cells have different functions. From rodent study, 2 major projection neurons, callosal and subcortical, are formed by Satb2 and Ctip2 interaction (Leone et al. 2008), which may be at work in the human fetal neocortex. The finding of no double-labeled cells with CTIP2 and SATB2 in human neocortex is compatible with the rodent data (Leone et al. 2008). Otx1 in mouse brain also expresses in layer V and contributes to the formation of the corticospinal projection (Frantz et al. 1994; Weimann et al. 1999). CTIP2+/OTX1+ cells may be closely related to the forming of the corticospinal projection. Interestingly, we found many SATB2+/OTX1+ cells in layer V. OTX1 may play an essential role in the specification of both callosal and corticospinal projection neurons, although the detailed interaction between OTX1 and CTIP2 remains unknown. Moreover, FOXP1+ cells expressed SATB2 and CTIP2 in layer V. It is unknown whether a relationship exists between Foxp1 and Satb2 or Foxp1 and Ctip2, although Ctip2 is known to colocalize with Foxp1 in mouse striatum (Arlotta et al. 2008). FOXP1 may also contribute callosal and corticospinal projection neurons. FOXP1 disappeared earlier than OTX1 (Figs 2 and 3 and Supplementary Figure 1). FOXP1 could strongly control forming corticospinal projection. Tbr1+ cells derived from the earliest progenitor cells locate in layer VI (Hevner et al. 2003) and contribute to the development of corticothalamic projection neurons (Hevner et al. 2001, 2002; Guillemot et al. 2006; Leone et al. 2008). In our data, the TBR1+ cells that expressed CTIP2 in layer VI may form corticothalamic projections, as in rodent studies.

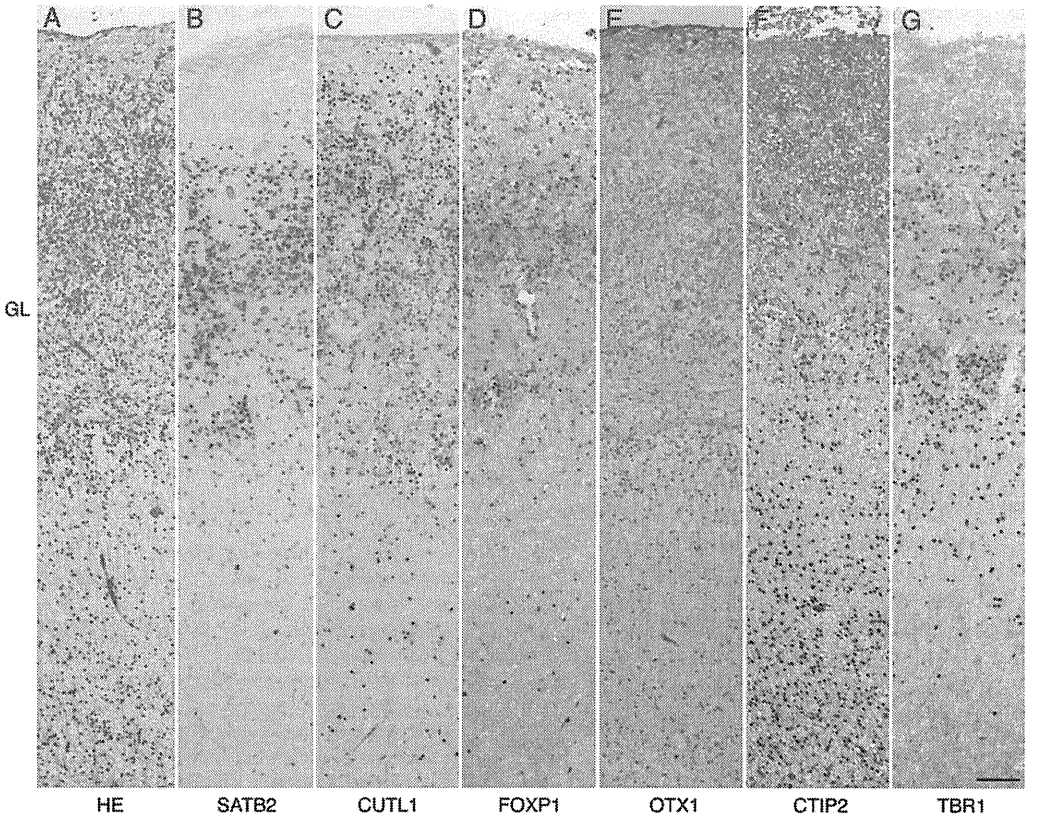


Figure 7. Layer-specific marker expression of the neocortex of 19-GW fetus with FCMD. Neocortex shows typical cobblestone lissencephaly feature. Many SATB2-, CUTL1-, FOXP1-, OTX1, CTIP2-, and TBR1-immunopositive cells migrate over the glia limitans (*B–G*), while some labeled cells locate under it. GL, glia limitans; *A*, HE; *B–G*, SATB2, CUTL1, FOXP1, OTX1, CTIP2, and TBR1 immunohistochemistry, respectively. Scale bar: 100 μm.

On the other hand, malformed neocortices revealed unique distributions of the layer-specific markers. In MDS, due to deletion of 17p13.3 with LIS1 gene, it has been thought that neurons of the superficial layer are neuronal components of the fundamental deep layers, and neurons of the deep layers consist of neuronal components of layers II–IV in the normal neocortex (Ferrer et al. 1987). Also, MDS neocortical lamination was found to have an inverted organization (Viot et al. 2004). However, recently the neocortex of 33 GW MDS has reportedly demonstrated FOXP1+ cell in the deep layers or TBR1+ cells in the first 3 layers (Sheen et al. 2006). MDS neocortical lamination was concluded to be preserved and noninverted. Our MDS findings supported noninverted lamination because of the diffuse expression pattern of all layer-specific markers. XLAG, caused by loss of function mutations of ARX gene concerned with differentiation and migration of γ-aminobutyric acidergic interneurons, shows a 3-layer lissencephalic neocortex (Kitamura et al. 2002; Bonneau et al. 2002; Cobos et al. 2005; Forman et al. 2005). Although ARX-null mice exhibit nearly normal layer formation of the cerebral cortex (Kitamura et al. 2002), the human XLAG neocortex was reported to consist of 3 layers with uniform pyramidal neurons (Bonneau et al. 2002; Okazaki et al. 2008). From our observation of layer-specific markers in layers II and III, XLAG might also be a random migration pattern. In human brain, ARX involves migration of not only interneurons but also projection neurons (Okazaki et al. 2008). XLAG neocortex may have an abnormal interneuron migration pattern, although in the present study this could not be demonstrated. Interestingly, our postnatal patients with MDS and XLAG revealed persistent expression of these layer-specific markers, which was not found in the normal neocortex. This suggests that MDS or XLAG neurons arrest in the premature or undifferentiated stage.

Further investigation is needed to determine why these layer-specific markers are expressed in postnatal brains, and the nature of their molecular function. Moreover, we investigated neocortices of typical type II lissencephaly, FCMD. Various-sized and/or disoriented neurons were widely scattered in the neocortex. In FCMD fetal brain, the layer-specific markers diffusely expressed over and under glia limitans (Fig. 7). Obviously, the FCMD fetal neocortex had completely lost its layer formation. The layer-formation pattern of WWS fetus presents the same result as ours (Hevner 2007). This type II lissencephaly, cobblestone lissencephaly, may commonly have this pathological construction. Postnatal FCMD demonstrated no expression of the layer-specific markers and was different from MDS and XLAG. Neuronal maturation of FCMD neocortex may be more advanced than other types of lissencephalies. This leads us to conclude that FCMD patients have a relatively low incidence of epilepsy and some cases are mild (Guerrini and Filippi 2005; Spalice et al. 2009).

Our study suggests that the laminar formation pattern of human and rodent neocortices is fundamentally the same. One of the characteristics of the human neocortex is its gyration, which is 1000-fold in the neocortical surface area between human and rodent (Bystron et al. 2006; Rakic 2009). It is thought that not only the number of neuronal progenitors but also the number of radial glial cells in human brain is much

larger than in the rodent. As a result, the human neocortex must fold and form gyrations. However, in case of abnormal expression of migration- or proliferation-related genes or environments such as trauma and infection, the number of neuronal progenitor cells, and radial glial cells may serve to reduce and influence the migration pattern.

We may conclude that the neocortex of lissencephalies is formed by a unique type of neuronal migration. The late-birth cells in MDS may migrate randomly but not the early-birth cells. In XLAG, SATB2+, and TBR1+ cells distribute in the relatively deep layers, but CUTL1+ and FOXP1+ cells may follow a random migration pattern. FCMD shows the most random pattern. We must seek to understand the mechanism behind these differences. The molecular mechanism of neuronal movement is well known. Lis-1 or Dcx is a modulator of radial migration and contributes to layer formation (Hirotsune et al. 1998; Meyer et al. 2002; Bai et al. 2003). In human layer formation, various projection neurons originate from VZ or SVZ and migrate radially depending on the time of cell birth. In interneuron development, Cutl1 and Cutl2 contribute to Reln expression and control the number of the interneuron subpopulation (Cubelos et al. 2008). However, little is known about interaction between the layer-specific markers (transcription factors) and neuron kinetic factors including Lis-1, Dcx and Reln. Further study is warranted to obtain more information in this regard.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Funding

Ministry of Health, Labor and Welfare of Japan (Intramural Research Grant [21B-5] for Neurological and Psychiatric Disorders of NCNP, and Research on Intractable Diseases 21-110 and 22-133 to M.I.).

Notes

We thank Drs M. Morikawa, Tokyo Metropolitan Kiyose Children's Hospital, B. Akikusa, Matsudo Municipal Hospital, and H. Horie, Chiba Children's Hospital, for advice on the pathology in this study, and Dr K. Kitamura, National Center of Neurology and Psychiatry, for helpful comments on the manuscript. We are also indebted to Mrs Y. Shono, Tokyo Metropolitan Hachioji Hospital, and Mr S. Kumagai, National Center of Neurology and Psychiatry, for technical assistance. *Conflict of Interest*: None declared.

References

Alcamo EA, Chirivella L, Dautzenberg M, Dobreva G, Fariñas I, Grosschedl R, McConnell SK. 2008. Satb2 regulates callosal projection neuron identity in the developing cerebral cortex. Neuron. 57:364-377.

Arlotta P, Molyneaux BJ, Chen J, Inoue J, Kominami R, Macklis JD. 2005. Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. Neuron. 45:207-221.

Arlotta P, Molyneaux BJ, Jabaudon D, Yoshida Y, Macklis JD. 2008. Ctip2 controls the differentiation of medium spiny neurons and establishment of the cellular architecture of the striatum. J Neurosci. 28:622-632.

Assadi AH, Zhang G, Beffert U, McNeil RS, Renfro AL, Niu S, Quattrocchi CC, Antalffy BA, Sheldon M, Armstrong DD, et al. 2003. Interaction of reelin signaling and Lis1 in brain development. Nat Genet. 35:270-276.

Bai J, Ramos RL, Ackman JB, Thomas AM, Lee RV, LoTurco JJ. 2003. RNAi reveals doublecortin is required for radial migration in rat neocortex. Nat Neurosci. 6:1277-1283.

Bonneau D, Toutain A, Laguerrière A, Marret S, Saugier-Veber P, Barthez MA, Radi S, Biran-Mucignat V, Rodriguez D, Gélot A. 2002. X-linked lissencephaly with absent corpus callosum and ambiguous genitalia (XLAG): clinical, magnetic resonance imaging, and neuropathological findings. Ann Neurol. 51:340-349.

Britanova O, Akopov S, Lukyanov S, Gruss P, Tarabykin V. 2005. Novel transcription factor Satb2 interacts with matrix attachment region DNA elements in a tissue-specific manner and demonstrates cell-type-dependent expression in the developing mouse CNS. Eur J Neurosci. 21:658-668.

Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, Vogel T, Akopov S, Mitkovski M, Agoston D, et al. 2008. Satb2 is a postmitotic determinant for upper-layer neuron specification in the neocortex. Neuron. 57:378-392.

Bulfone A, Smiga SM, Shimamura K, Peterson A, Puelles L, Rubenstein JL. 1995. T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. Neuron. 15:63-78.

Bystron I, Rakic P, Molnar Z, Blackmore C. 2006. The first neurons of the human cerebral cortex. Nat Neurosci. 9:880-885.

Cobos I, Broccoli V, Rubenstein JL. 2005. The vertebrate ortholog of Aristaless is regulated by Dlx genes in the developing forebrain. J Comp Neurol. 483:292-303.

Crome L. 1956. Pachygyria. J Pathol Bacteriol. 71:335-352.

Cubelos B, Sebastian-Serrano A, Kim S, Redondo JM, Walsh C, Nieto M. 2008. Cux-1 and cux-2 control the development of reelin expressing cortical interneurons. Dev Neurobiol. 68:917-925.

De Rouvroit CL, Goffinet AM. 2001. Neuronal migration. Mech Dev. 105:47-56.

Dobyns WB, Berry-Kravis E, Havernick NJ, Holden KR, Viskochil D. 1999. X-linked lissencephaly with absent corpus callosum and ambiguous genitalia. Am J Med Genet. 86:331-337.

Ferland RJ, Cherry TJ, Preware PO, Morrisey EE, Walsh CA. 2003. Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. J Comp Neurol. 460:266-279.

Ferrer I, Fâbregues I, Condom E. 1987. A Golgi study of the sixth layer of the cerebral cortex. III. Neuronal changes during normal and abnormal cortical folding. J Anat. 152:71-82.

Forman MS, Sguler W, Dobyns WB, Golden JA. 2005. Genotypically defined lissencephalies show distinct pathologies. J Neuropathol Exp Neurol. 64:847-857.

Frantz GD, Weimann JM, Levin ME, McConnell SK. 1994. Otx1 and Otx2 define layers and regions in developing cerebral cortex and cerebellum. J Neurosci. 14:5725-5740.

Guerrini R, Filippi T. 2005. Neuronal migration disorders, genetics, and epileptogenesis. J Child Neurol. 20:287-299.

Guillemot F, Molnár Z, Takabykin V, Stoykova A. 2006. Molecular mechanisms of cortical differentiation. Eur J Neurosci. 23:857-868.

Hevner RF. 2007. Layer-specific markers as probes for neuron type identity in human neocortex and malformations of cortical development. J Neuropathol Exp Neurol. 66:101-109.

Hevner RF, Miyashita-Lin E, Rubenstein JLR. 2002. Cortical and thalamic axon pathfinding defects in Tbr1, Gbx2, and Pax6 mutant mice: evidence that cortical and thalamic axons interact and guide each other. J Comp Neurol. 447:8-17.

Hevner RF, Neogi T, Englund C, Daza RAM, Fink A. 2003. Cajal-Retius cells in the mouse: transcription factors, neurotransmitters, and birthdays suggest a pallial origin. Brain Res Dev Brain Res. 141:39-53.

Hevner RF, Shi L, Justice N, Hsueh Y, Sheng M, Smiga S, Bulfone A, Goffinet AM, Campagnoni AT, Rubenstein JL. 2001. Tbr1 regulates differentiation of the preplate and layer 6. Neuron. 29:353-366.

Hirotsune S, Fleck MW, Gambello MJ, Bix GJ, Chen A, Clark GD, Ledbetter DH, McBain CJ, Wynshaw-Boris A. 1998. Graded reduction of Pafah1b1 (Lis1) activity results in neuronal migration defects and early embryonic lethality. Nat Genet. 19:333-339.

Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, et al.

2002. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. Nat Genet. 32:359–369.

Leid M, Ishmael JE, Avram D, Shepherd D, Fraulob V, Dollé P. 2004. CTIP1 and CTIP2 are differentially expressed during mouse embryogenesis. Gene Expr Patterns. 4:733–739.

Leone DP, Srinivasan K, Chen B, Alcamo E, McConell SK. 2008. The determination of projection neuron identity in the developing cerebral cortex. Curr Opin Neurobiol. 18:28–35.

Meyer G, Perez-Garcia CG, Gleeson JG. 2002. Selective expression of doublecortin and LIS1 in developing human cortex suggests unique modes of neuronal movement. Cereb Cortex. 12: 1225–1236.

Michele DE, Barresi R, Kanagawa M, Saito F, Cohn RD, Satz JS, Dollar J, Nishino I, Kelley RI, Somer H, et al. 2002. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. Nature. 418:417–422.

Mochida GH, Walsh CA. 2004. Genetic basis of developmental malformations of the cerebral cortex. Arch Neurol. 61:637–640.

Molyneaux BJ, Arlotta P, Menezes JRL, Macklis JD. 2007. Neuronal subtype specification in the cerebral cortex. Nat Rev Neurosci. 8:427–437.

Nieto M, Monuki ES, Tang H, Imitola J, Haubst N, Khoury SJ, Cunningham J, Gotz M, Walsh CA. 2004. Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II-IV of the cerebral cortex. J Comp Neurol. 479:168–180.

Okazaki S, Ohsawa M, Kuki I, Kawawaki H, Koriyama T, Ri S, Ichiba H, Hai E, Inoue T, Nakamura H, et al. 2008. Aristaless-related homeobox gene disruption leads to abnormal distribution of GABAergic interneurons in human neocortex: evidence based on a case of X-linked lissencephaly with abnormal genitalia (XLAG). Acta Neuropathol. 116:453–462.

Olson EC, Walsh CA. 2002. Smooth, rough and upside-down neocortical development. Curr Opin Genet Dev. 12:320–327.

Rakic P. 2009. Evolution of the neocortex: a perspective from developmental biology. Nat Rev Neurosci. 10:724–735.

Reiner O, Sapir T. 2009. Polarity regulation in migrating neurons in the cortex. Mol Neurobiol. 40:1–14.

Sheen VL, Ferland RJ, Neal J, Harney M, Hill RS, Banham A, Brown P, Chenn A, Corbo J, Hecht J, et al. 2006. Neocortical neuronal arrangement in Miller Dieker syndrome. Acta Neuropathol. 111:489–496.

Simeone A, Acampora D, Mallamaci A, Stornaiuolo A, D'Apice MR, Nigro V, Boncinelli E. 1993. A vertebrate gene related to *orthodenticle* contains a homeodomain of the *bicoid* class and demarcates anterior neuroectoderm in the gastrulating mouse embryo. EMBO J. 12:2735–2747.

Spalice A, Parisi P, Nicita F, Pazzardi G, Del Balzo F, Iannetti. 2009. Neuronal migration disorders: clinical, neuroradiologic and genetic aspects. Acta Paediatr. 98:421–433.

Viot G, Sonigo P, Simon I, Simon-Bouy B, Chadeyron F, Beldjord C, Tantau J, Martinovic J, Esculpavit C, Brunelle F, et al. 2004. Neocortical neuronal arrangement in LIS1 and DCX lissencephaly may be different. Am J Med Genet A. 126A:123–128.

Weimann JM, Zhang YA, Levin ME, Devine WP, Brulet P, McConnell SK. 1999. Cortical neurons require otx1 for the refinement of exuberant axonal projections to subcortical targets. Neuron. 24:819–831.

Yamamoto T, Kato Y, Kawaguchi M, Shibata N, Kobayashi M. 2004. Expression and localization of fukutin, POMGnT1, and POMT1 in the central nervous system: consideration for functions of fukutin. Med Electron Microsc. 37:200–207.

ELSEVIER

Brain & Development 33 (2011) 627–631

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Review article

Rett syndrome: The state of clinical and basic research, and future perspectives

Toyojiro Matsuishi [a,*], Yushiro Yamashita [a], Tomoyuki Takahashi [b], Shinichiro Nagamitsu [a]

[a] *Department of Pediatrics and Child Health, Kurume University School of Medicine, Kurume 830-0011, Japan*
[b] *Division of Gene Therapy and Regenerative Medicine, Cognitive and Molecular Research Institute of Brain Diseases, Kurume University, Japan*

Received 28 September 2010; accepted 14 December 2010

Abstract

To clarify the pathophysiology of brain and spinal cord impairment in Rett syndrome (RTT), we report on the current status of research on Rett syndrome and review the abnormalities reported in neurotransmitters, neuromodulators and other biological markers in patients with RTT. We have previously investigated the levels of various factors in the blood, plasma, and cerebrospinal fluid (CSF) of RTT patients, including biogenic amines, lactate, melatonin, pyruvate and other citric acid cycle intermediates, substance P, β-endorphin and other neuropeptides, and a neuromodulator of β-phenylethylamine. In addition, we have performed near-infrared spectroscopy of the cerebral cortices in patients with RTT and genetic studies of the methyl-CpG-binding protein 2 (MECP2) in these patients. Taken together, the multiple abnormalities we and other authors have revealed in the various neurotransmitters/neuromodulator systems explain the pervasive effects of Rett syndrome. We also discuss the possible role of plasma ghrelin and present the results of our mouse study of the MECP2-null mutation using ES cells. Finally, we consider the potential for future analyses using our recently developed iPS cell system and discuss the future perspectives for the treatment and management of this disease.

Keywords: Rett syndrome; Methyl-CpG-binding protein 2; Pathophysiology; Neurotransmitters; Neuromodulators; MECP2-null mutation mouse model

1. Introduction

Rett syndrome (RTT) is a neurodevelopmental disorder characterized by normal early psychomotor development followed by the loss of psychomotor and acquired purposeful hand skills and the onset of stereotyped movement of the hands and gait disturbance [1–4]. The gene was discovered in 1999 and the disease was found to be caused by a mutation of the methyl-CpG-binding protein 2 [5,6]. However, in many ways this clinically peculiar condition remains a mystery, with no clear correlations between the gene mutation and abnormal biological markers, neuropathology and/or unique clinical symptoms and signs [1–4,6].

RTT is unique among genetic, chromosomal and other developmental disorders because of its usually sporadic occurrence, extreme female gender bias, early normal development and subsequent developmental regression, autonomic dysfunction, stagnation in brain growth and distinctive neuropathology. MECP2 mutations lead to the RTT phenotype in females, and profound congenital encephalopathy in males [7]. Research needs to be directed toward clarifying the link between the MECP2 involvement and the alterations in biological, neurochem-

* Corresponding author. Address: Department of Pediatrics and Child Health, Kurume University School of Medicine, 67 Asahi-machi, Kurume City 830-0011, Japan. Tel.: +81 942 31 7565; fax: +81 942 38 1792.
E-mail address: tmatsu@med.kurume-u.ac.jp (T. Matsuishi).

0387-7604/$ - see front matter © 2010 The Japanese Society of Child Neurology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.braindev.2010.12.007

ical, and neurotransmitter/receptor systems, as well as toward developing new therapeutic modalities.

2. Neurotransmitters and biological markers

2.1. Biogenic amines

Nomura and Segawa have suggested that hypoactivity or underdevelopment in the biogenic amines might account for the range of abnormalities found in RTT. Specifically, they suggested that the disease might be associated with impairments in noradrenalin, serotonin, and dopamine based on a series of clinical, electrophysiological and polysomnographic studies. They have proposed that the following points are important in considering the pathophysiology of RTT. First, the characteristic symptoms and signs appear in sequence within a specific age from infancy. The earliest and pathognomonic manifestations of RTT are the autistic tendency and the decreased rate in head growth [8,9]. Their report has led to a proliferation of studies on biogenic amines in the cerebrospinal fluid (CSF) of RTT patients, as well as immunohistochemical studies, receptor studies, and neuroimaging studies. Together, these investigations have suggested that various neurotransmitters, neuromodulators, neurotrophic factors and neuronal markers may be involved in RTT. Zoghbi et al. have reported significant reductions in the levels of homovanillic acid (HVA) and in 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG) in the CSF of children with RTT [10]. However, Perry et al. reported no difference in these levels between RTT patients and controls [11]. Therefore, whether or not CSF biogenic amines are actually altered in RTT remains a matter of controversy. However, a recent report showed that HVA and 5-HIAA were decreased in RTT patients and the $MECP2^{null/y}$ mouse brain [12]. In another study, the biogenic amines dopamine, serotonin, and noradrenalin, and their respective metabolites HVA, 5-hydroxyindoleacetic acid, and MHPG, were measured in tissues from selected brain regions obtained at postmortem from four patients with RTT. A marked reduction in each of these substances was observed and these changes appeared to be age-related [13,14]. In addition, the endogenous levels of dopamine and its metabolites have been shown to be decreased throughout the neocortex and basal ganglia of patients with RTT [15]. Kitt et al. have reported a mild-to-moderate reduction in the number and cell size of the basal forebrain cholinergic neurons in RTT patients compared with controls, which might explain the impaired cognitive function and microcephalus [16].

2.2. β-Phenylethylamine

We have reported decreased β-phenylethylamine (PEA) levels in the CSF of patients with RTT [17]. PEA is an endogenous amine synthesized by decarboxylation of phenylalanine in the dopamine neurons of the nigrostriatal system, and plays an important role in both the dopaminergic and noradrenergic systems. We have also reported reduced levels of PEA in the CSF of patients with Parkinson's disease. The PEA level was also negatively correlated with the severity of the Parkinson's disease [18].

2.3. β-Endorphin, substance P, melatonin

Myer et al. [19] and Budden et al. [20] have reported elevated CSF β-endorphin in RTT. However, elevated β-endorphin was not found in the brain, suggesting that the alteration in β-endorphin may be a secondary change. We have reported that the level of substance P was markedly decreased in the CSF in patients with RTT, and this was considered to play a role in the features of autonomic dysfunction that occur in RTT, including constipation, small and cold feet, progressive limb muscle weakness and muscle atrophy [21]. Substance P is a neurotransmitter or neuromodulator in the peripheral as well as the central nervous system (CNS). Substance P activity is associated with dopaminergic neurons in the substantia nigra and the striatum, the central autonomic nuclei, the dorsal root ganglia, and the peripheral autonomic ganglia [22]. Hedner et al. reported that substance P acted on the respiratory control system by at least two different mechanisms: the bulbo-pontine time setting mechanism, and the inspiratory off-switch mechanism [23]. Deguchi et al. reported that the substance P immunoreactivity was significantly decreased in brain tissues, especially the solitary tract and nucleus, parvocellular and pontine reticular nuclei, and locus coeruleus, with less significant decreases in the substantia nigra, central gray matter of the midbrain, and other regions. Glial fibrillary acidic protein (GFAP)-positive astrocytes were increased in the areas in which SP immunoreactivity was decreased [24]. Neurotrophic effects of substance P on the hippocampal neurons have been reported [25]. Sleep disturbances such as screaming attacks, fragmented nighttime sleep, and excessive daytime sleeping are also common features in patients with RTT. These symptoms may be due to the decreased levels of melatonin, and in fact, such symptoms are ameliorated by exogenous melatonin treatment [26–28].

2.4. Neurotrophic factors and others

Nerve growth factor (NGF) is known to be a trophic factor, especially for the cholinergic neurons of the basal forebrain. NGF has been shown to be markedly decreased in the CSF of RTT patients, which may explain the decreased brain size [29]. Chen et al. [30] and Martinowich et al. [31] groups found that MECP2 binds selectively to brain-derived neurotrophic factor (BDNF) promoter and functions to regulate expression

of the BDNF gene. Overexpression of BDNF indeed extended the lifespan, restored locomotor activity levels, and relieved some symptoms of the MECP2 mutant phenotype [32,33]. Itoh et al. reported that MECP2 directly regulates expression of insulin-like growth factor binding protein 3 (IGFBP3) gene, which can be expected in turn to inhibit IGF-1 signaling [34,35].

Blue et al. reported significant changes in specific glutamate receptors, including NMDA, AMPA, and metabotropic type glutamate receptors in RTT [36]. Hamberger et al. have reported an elevation in the glutamate level in the CSF of children with RTT [37]. The elevations in NMDA receptors combined with the increased levels of CSF glutamate have suggested that excitatory neurotransmission is enhanced early in the course of the disease. Yamashita et al. measured benzodiazepine binding in stage IV RTT using single-photon computed tomography (SPECT) imaging techniques, and noted a significant reduction in the fronto-temporal cortex, suggesting a decrease in GABA receptors in adult RTT patients [38].

3. Energy metabolism: Rett syndrome is not a mitochondrial disease

Haas et al. have reported elevated CSF lactate and pyruvate in some patients with RTT [39]. Wakai et al. have reported morphological changes in the mitochondria in sural nerve biopsy specimens from patients with RTT [40]. We have also reported that the elevation in CSF lactate levels constituted a secondary biochemical change directly related to the abnormalities in respiration [41,42]. In a related study, we continuously monitored changes in cerebral oxygenation and hemodynamics in the frontal lobes of six patients with Rett syndrome during the awake state, which is associated with hyperventilation (HV) and breath-holding (BH), by near-infrared spectroscopy. We found that oxygenated hemoglobin (HbO_2) and total hemoglobin (HbT) decreased significantly during HV and BH in the awake state compared with the sleep state. The observed continuous decreases in HbO_2 and HbT may cause the focal ischemia and the increased lactate levels in the brain [43]. These finding suggested that RTT was not a primary mitochondrial disorder.

4. Neuropathological study

Armstrong reviewed the neuropathology of RTT and pointed out several important points as follows. The RTT brain is much smaller than a normal brain, and the volume is reduced in specific brain regions including the prefrontal, frontal, and anterior temporal regions. In addition, there are alterations of dendritic arborization in the above brain regions, and some Rett neurons have decreased expression of prealbumin and synaptophysin immunoreactivity and altered expression of neurotransmitters. Previous neuropathologic studies have also observed decreased melanin content of the zona compacta nigrae in the CNS of RTT patients [44]. Reduced expression of neuropeptides has been observed, including reduced immunoreactivity for tyrosine hydroxylase, a reduction of substance P in the parabrachial complex, and reductions of methionine enkephalin in the brainstem and the basal ganglia [45].

5. Methyl-CpG-binding protein 2 gene

Amir et al. reported on the clinical and laboratory features versus the genotype of MECP2. They also reported that the CSF HVA was significantly decreased in patients with truncating mutations compared with that in patients with missense mutations [46]. Methylation of DNA is essential for development in the mouse and plays an important role in the activation of the X-chromosome, genomic imprinting and gene silencing. The spectrum of MECP2 mutations reflects the importance of the methyl-CpG-binding domain and transcriptional repression domains [47]. Mutation analyses of the MECP2 gene have been performed in Japanese patients with RTT. The T158M mutation is a common mutation in the typical phenotype of RTT [48,49], while the R133C mutation was associated with the mildest cases with preserved speech [46,50]. We have already presented our preliminary clinical and basic research and reviewed the previous literature on RTT [51].

6. Future intervention and therapeutic strategies

6.1. Ghrelin

Ghrelin, a 28 amino acid peptide isolated from the rat stomach as an endogenous ligand for growth hormone secretagogue receptor (GHS-R) 1a and expressed in both the stomach and hypothalamus, exerts multiple physiological functions, including the stimulation of somatic growth, improvement of appetite, and enhancement of the motility of the gut [52]. Many of these functions are related to the clinical phenotypes of RTT, and thus this study investigated the plasma levels of ghrelin in 23 RTT patients in comparison to those in 39 healthy controls. The total ghrelin level in the patients with RTT was 127 ± 71 fmol/ml, and that in the controls was 228 ± 12 fmol/ml; the difference was statistically significant ($P < 0.01$). Thus ghrelin may play an important role in the pathophysiology of RTT.

6.2. MECP2-null mutation mouse model

In collaboration with Kosai et al. we developed an MECP2-null ES cell system using an adenoviral conditional targeting method [53]. We showed the roles of

MECP2 in neuronal development in terms of neuronal stem cells, neuronal and glial cell differentiation during all developmental stages, the function of differentiated dopaminergic neurons, and the maturation of neuronal cells. All these results should prove useful for understanding not only the biological roles of MECP2 but also the pathogenesis of RTT. Recently, we also developed an iPS cell system that may provide a novel strategy for developmental analysis at the molecular and cellular levels.

7. Conclusion

Finally, we should consider the potential for future mouse studies on MECP2-null mutation using ES cells and iPS cells and discuss the future perspectives for the treatment and management of this disease.

The reversal of early lethality and of some neurological abnormalities in MECP2-/Y mice through the postnatal supply of normal MECP2 has raised hopes for an effective treatment [54]. To this end, we should continue to explore new therapeutic modalities, including ghrelin, BDNF [32], and other factors [35].

Acknowledgements

This study was partly supported by a Grant on Research on Psychiatric and Neurological Diseases and Mental Health (#19-8), a Grant from the Research Project for Overcoming Intractable Diseases (#21-Nan-chi-ippan-110) and a Grant on Research on Learning Disorders (#19-6) from the Ministry of Health, Labour and Welfare of Japan, and was also partly supported by a Grant-in-Aid for Scientific Research C (#18591172) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

We are grateful to Drs. Ken-ichiro Kosai, Yasunori Okabe, Yoshihiro Nishi, Munetsugu Hara, Jyunko Yoh, and Yasuyuki Kojima for their contribution. Finally, we would like to acknowledge the patients and the family members of the Rett Syndrome Association (Sakuranbo-kai). This manuscript was presented as a presidential report of the 52nd Annual Meeting of the Japanese Society of Child Neurology (in Japanese) which had been held on May 20–22, 2010, Fukuoka Japan.

References

[1] The Rett Syndrome Diagnostic Criteria Work Group. Diagnostic criteria for Rett syndrome. Ann Neurol 1988;23:425–8.
[2] Kerr AM, Nomura Y, Armstrong D, Anvret M, Belichenko PV, Budden S, et al. Guidelines for reporting clinical features in cases with MECP2 mutations. Brain Dev 2001;23:208–11.
[3] Hagberg B, Hanefeld F, Percy A, Skjeldal O. An update clinically appreciable diagnostic criteria in Rett syndrome. Comments to Rett syndrome clinical criteria consensus panel satellite to European Pediatric Neurology Society Meeting, 11 September 2001, Germany: Baden Baden, Eur J Pediatr Neurol 2002; 6: 1–5.
[4] Chahrour M, Zoghbi HY. The story of Rett syndrome: from clinic to neurobiology. Neuron 2007;56:422–37.
[5] Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 1999;23:185–8.
[6] Bienvenu T, Chelly J. Molecular genetics of Rett syndrome: when DNA methylation goes unrecognized. Nat Rev Genet 2006;7:415–26.
[7] Franke U. Rett syndrome and MECP2-status of knowledge 10 years after the genes. Invited speaker of Segawa program. No to Hattatsu 2010;42:S88.
[8] Nomura Y, Honda K, Segawa M. Pathophysiology of Rett syndrome. Brain Dev 1987;9:506–13.
[9] Segawa M, Nomura Y. Polysomnography in the Rett syndrome. Brain Dev 1992;14:S46–54.
[10] Zoghbi H, Milstien H, Butler IJ, Smith OB, Kaufman S, Glaze DG, et al. Cerebrospinal fluid biogenic amines and biopterin in Rett syndrome. Ann Neurol 1989;25:56–60.
[11] Perry TL, Dunn HG, Ho H-H, Crichton JU. Cerebrospinal fluid values for monoamine metabolites, gamma aminobutyric acid, and other amino compounds in Rett syndrome. J Pediatr 1988;112:234–8.
[12] Samaco RC, Mandel-Brehm C, Chao H-T, Ward CS, Fyffe-Maricich SL, Ren J, et al. Loss of MeCP2 in aminergic neurons causes cell-autonomous defects in neurotransmitter synthesis and specific behavioral abnormalities. PNAS 2009;106:21966–71.
[13] Lekman A, Witt-Engerstrom I, Gottfries J, Hagberg B, Percy AK, Svennerholm L. Rett syndrome: biogenic amines and metabolites in postmortem brain. Pediatr Neurol 1989;5:357–62.
[14] Ide S, Itoh M, Goto Y. Defect in normal developmental increase of the brain biogenic amine concentrations in the mecp2-null mouse. Neurosci Lett 2005;386:14–7.
[15] Wenk GL. Alteration in dopaminergic function in Rett syndrome. Neuropediatrics 1995;26:123–5.
[16] Kitt CA, Troncoso JC, Price DL, Naidu S, Moser H. Pathological changes in substantia nigra and basal forebrain neurons in Rett syndrome. Ann Neurol 1990;28:416–7.
[17] Satoi M, Matsuishi T, Yamada S, Yamashita Y, Ohtaki E, Mori K, et al. Decreased cerebrospinal fluid levels of β-phenylethylamine in patients with Rett syndrome. Ann Neurol 2000;47:801–3.
[18] Zhou G, Shoji H, Yamada S, Matsuishi T. Decreased cerebrospinal fluid β-phenylethylamine in Parkinson's disease. J Neurol Neurosurg Psychiatry 1997;63:754–8.
[19] Myer EC, Tripathi HL, Dewey WL. Hyperendorphinism in Rett syndrome: cause or result? Ann Neurol 1988;24:340–1.
[20] Budden SS, Myer EC, Buttler IJ. Cerebrospinal fluid studies in the Rett syndrome: biogenic amines and beta endorphins. Brain Dev 1990;12:81–4.
[21] Matsuishi T, Nagamitsu S, Yamashita Y, Murakami Y, Kimura A, Sakai T, et al. Decreased cerebrospinal fluid levels of substance P in patients with Rett syndrome. Ann Neurol 1997;42:978–81.
[22] Mai JK, Stephens PH, Hope A, Cuello AC. Substance P in the human brain. Neuroscience 1986;17:709–39.
[23] Hender J, Hender T, Wessberg P, Jonason J. Interaction of substance P with respiratory control system in the rat. J Pharmacol Exp Ther 1983;228:196–201.
[24] Deguchi K, Antalffy BA, Twohill LJ, Chakraborty S, Glaze DG, Armstrong DD. Substance P immunoreactivity in Rett syndrome. Pediatr Neurol 2000;22:259–66.
[25] Whitty CJ, Kapatos G, Bannon MJ. Neurotrophic effects of substance P on hippocampal neurons in vitro. Neurosci Lett 1993;164:141–4.

[26] McArthur AJ, Budden SS. Sleep dysfunction in Rett syndrome: a trial of exogenous melatonin treatment. Dev Med Child Neurol 1998;40:186–92.
[27] Miyamoto A, Oki J, Takahashi S, Okuno A. Serum melatonin kinetics and long-term melatonin treatment for sleep disturbance in Rett syndrome. Brain Dev 1999;21:59–62.
[28] Yamashita Y, Matsuishi T, Murakami Y, Kato H. Sleep disorder in Rett syndrome and melatonin treatment. Brain Dev 1999;21:570.
[29] Lappalainen R, Lindholm D, Riikonen R. Low levels of nerve growth factor in cerebrospinal fluid of children with Rett syndrome. J Child Neurol 1996;11:296–300.
[30] Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, et al. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 2003;302:885–9.
[31] Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, et al. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 2003;302:890–3.
[32] Chang Q, Khare G, Dani V, Nelson S, Jaenisch R. The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. Neuron 2006;49:341–8.
[33] Larimore JL, Chapleau CA, Kudo S, Theibert A, Percy AK, Pozzo-Miller L. Bdnf overexpression in hippocampal neurons prevents dendritic atrophy caused by Rett-associated MECP2 mutations. Neurobiol Dis 2009;34:199–211.
[34] Itoh M, Ide S, Takashima S, Kudo S, Nomura Y, Segawa M, et al. Methyl CpG-binding protein 2 (a mutation of which causes Rett syndrome) directly regulates insulin-like growth factor binding protein 3 in mouse and human brains. J Neuropathol Exp Neurol 2007;66:117–23.
[35] Tropea D, Giacometti E, Wilson NR, Beard C, McCurry C, Fu DD, et al. Partial reversal of Rett syndrome-like symptoms in MeCP2 mutant mice. Proc Natl Acad Sci USA 2009;106:2029–34.
[36] Blue ME, Naidu S, Johnston MV. Development of amino acid receptors in frontal cortex from girls with Rett syndrome. Ann Neurol 1999;45:541–5.
[37] Hamberger A, Gillberg C, Palm A, Hagberg B. Elevated CSF glutamate in Rett syndrome. Neuropediatrics 1992;23:212–3.
[38] Yamashita Y, Matsuishi T, Ishibashi M, Kimura A, Onishi Y, Yonekura Y, et al. Decrease in benzodiazepine receptor binding in the brain of adult Rett syndrome. J Neurol Sci 1998;154:146–50.
[39] Haas RH, Rice MA, Trauner DA, Meritt A. Ketogenic diet in Rett syndrome. Am J Med Genet 1986;24(Suppl 1):5225–46.
[40] Wakai S, Kameda K, Ishikawa YI, Miyamoto S, Nagaoka M, Okabe M, et al. Rett syndrome: findings suggesting axonopathy and mitochondrial abnormalities. Pediatr Neurol 1990;6:164–6.
[41] Matsuishi T, Urabe F, Komori H, Yamashita Y, Naito E, Kuroda Y, et al. The Rett syndrome and CSF lactic patterns. Brain Dev 1992;14:68–70.
[42] Matsuishi T, Urabe F, Percy AK, Komori H, Yamashita Y, Schultz RS, et al. Kato H Abnormal carbohydrate metabolism in cerebrospinal fluid in Rett syndrome. J Child Neurol 1994;9: 26–30.
[43] Murakami Y, Yamashita Y, Matsuish T, Iwanaga R, Kato H. Cerebral oxygenation and hemodynamics during hyperventilation and sleep in patients with Rett syndrome. Brain Dev; 20: 574–578.
[44] Armstrong DD. Rett syndrome neuropathology review 2000. Brain Dev 2001;23:S72–6.
[45] Saito Y, Ito M, Ozawa Y, Matsuishi T, Hamano K, Takashima S. Reduced expression of neuropeptides can be related to respiratory disturbances in Rett syndrome. Brain Dev 2001;23:S122–6.
[46] Amir RE, Van den Veyver IB, Schultz R, Malicki DM, Tran CQ, Dahle EJ, et al. Influence of mutation type and X chromosome inactivation on Rett syndrome phenotypes. Ann Neurol 2000;47:670–9.
[47] Nan X, Bird A. The biological function of the methyl-CpG binding protein MECP2 and its implication in Rett syndrome. Brain Dev 2001;23:S32–7.
[48] Obata K, Matsuishi T, Yamashita Y, Fukuda T, Kuwajima K, Horiuchi I, et al. Mutation analysis of the Methyl-CpG binding protein 2 gene (MECP2) in patients with Rett syndrome. J Med Genet 2000;37:608–10.
[49] Fukuda T, Yamashita Y, Nagamitsu S, Miyamoto K, Jin JJ, Ohmori I, et al. Methyl-CpG binding protein 2 gene (MECP2) variations in Japanese patients with Rett syndrome: pathological mutations and polymorphisms. Brain Dev 2005;27:211–7.
[50] Yamashita Y, Kondo I, Fukuda T, Morishima R, Kusaga A, Iwanaga R, et al. Mutation analysis of the methyl-CpG-binding protein 2 gene (MECP2) in Rett patients with preserved speech. Brain Dev 2001;23:S157–60.
[51] Matsuishi T, Yamashita Y, Kusaga A. Neurobiology and neurochemistry of Rett syndrome. Brain Dev 2001;23:S58–61.
[52] Yoshihara F, Kojima M, Hosoda H, Nakazato M, Kangawa K. Ghrelin: a novel peptide for growth hormone release and feeding regulation. Curr Opin Nutr Metab Care 2002;5:391–5.
[53] Okabe Y, Kusaga A, Takahashi T, Mitsumasu C, Murai Y, Tanaka E, et al. Neural development of methyl-CpG-Binding protein 2-null embryonic stem cells: a system for studying Rett syndrome. Brain Res 2010 in press.
[54] Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. Science 2007;315:1143–7.