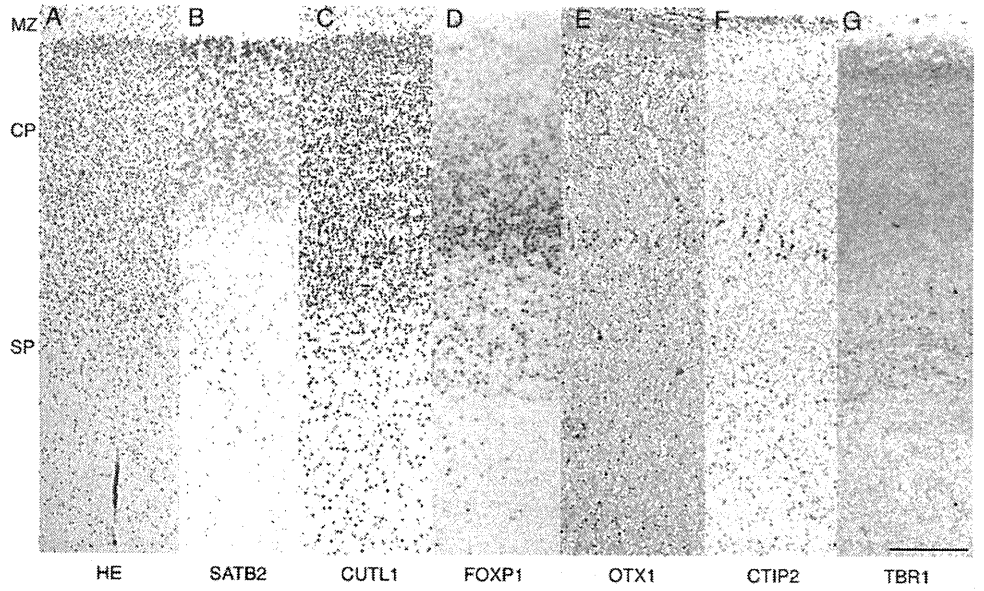

Figure 1. Layer-specific marker expression of the neocortex at 23 GWs. Around 20 GWs, the 3-layer pattern, that is, the marginal zone (MZ), CP, and subplate (SP), are seen (*A*). SATB2 expresses in the upper region of CP (*B*). CUTL1 diffusely expresses in the whole cortex and intermediate zone (*C*). 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.

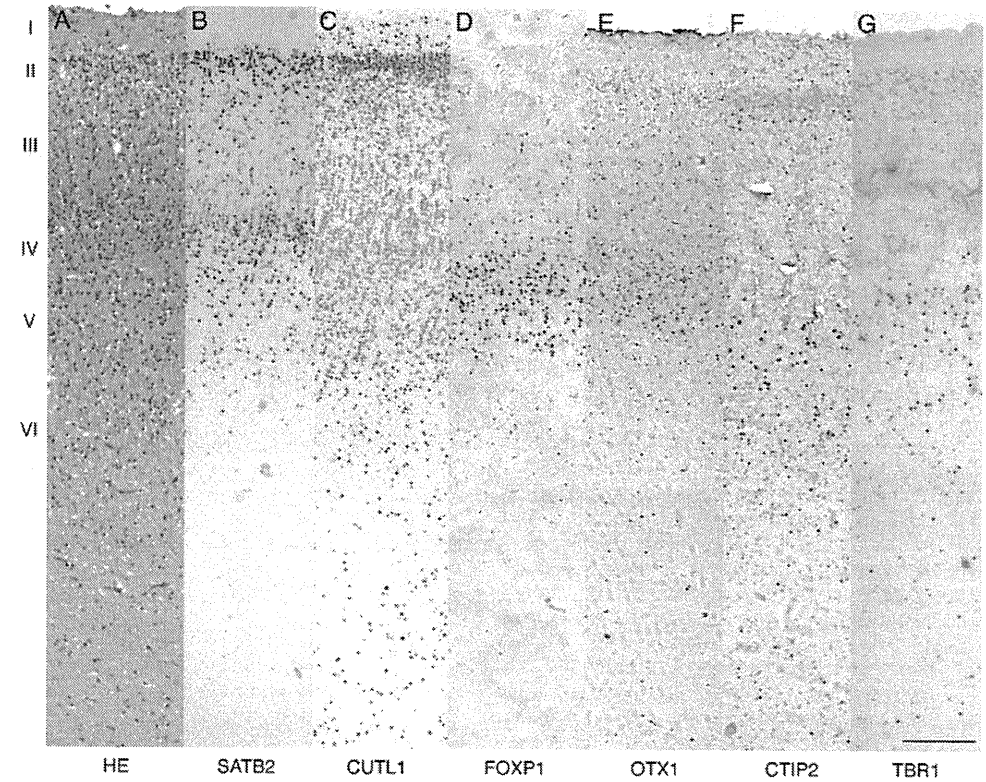

Figure 2. Layer-specific marker expression of the neocortex at 29 GWs. The 6-layer neocortex is shown (*A*). SATB2 expresses in layers II–V, especially layer II and upper region of layer IV (*B*). CUTL1 diffusely expresses in layers II–V and predominates in layer II (*C*). FOXP1 converges to layers VI 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.

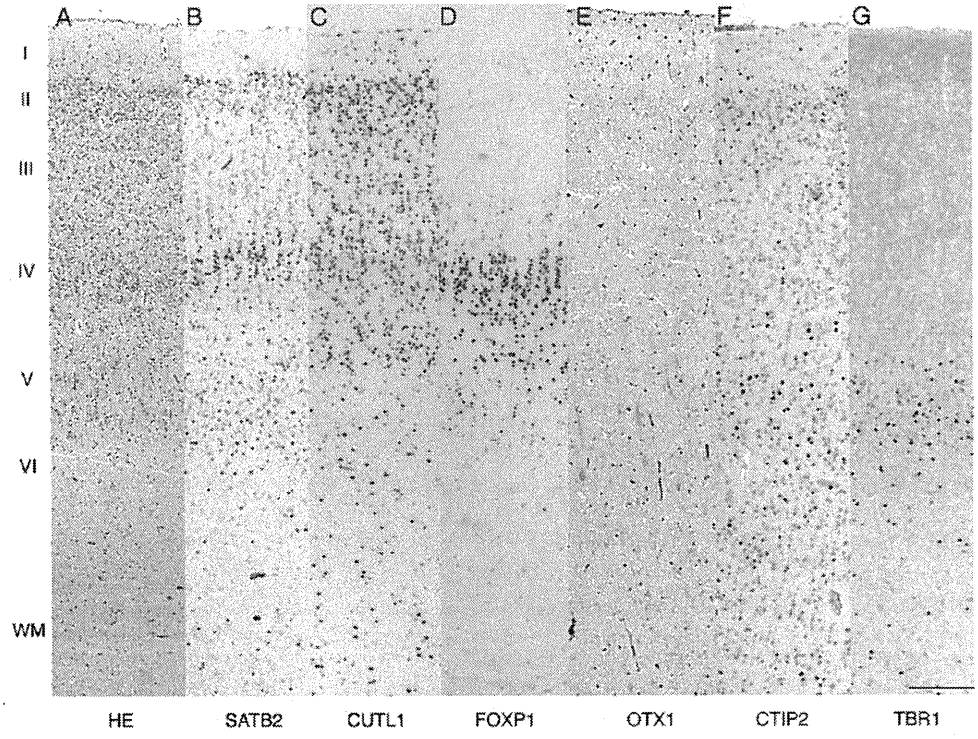

Figure 3. Layer-specific marker expression of the neocortex at 37 GWs. Expression of SATB2, CUTL1, FOXP1, OTX1, CTIP2, and TBR1 has a pattern similar to those at 29 GWs. OTX1 disappears in upper layer of neocortex (*E*). *A*, HE; *B–G*, SATB2, CUTL1, FOXP1, OTX1, CTIP2, and TBR1 immunohistochemistry,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

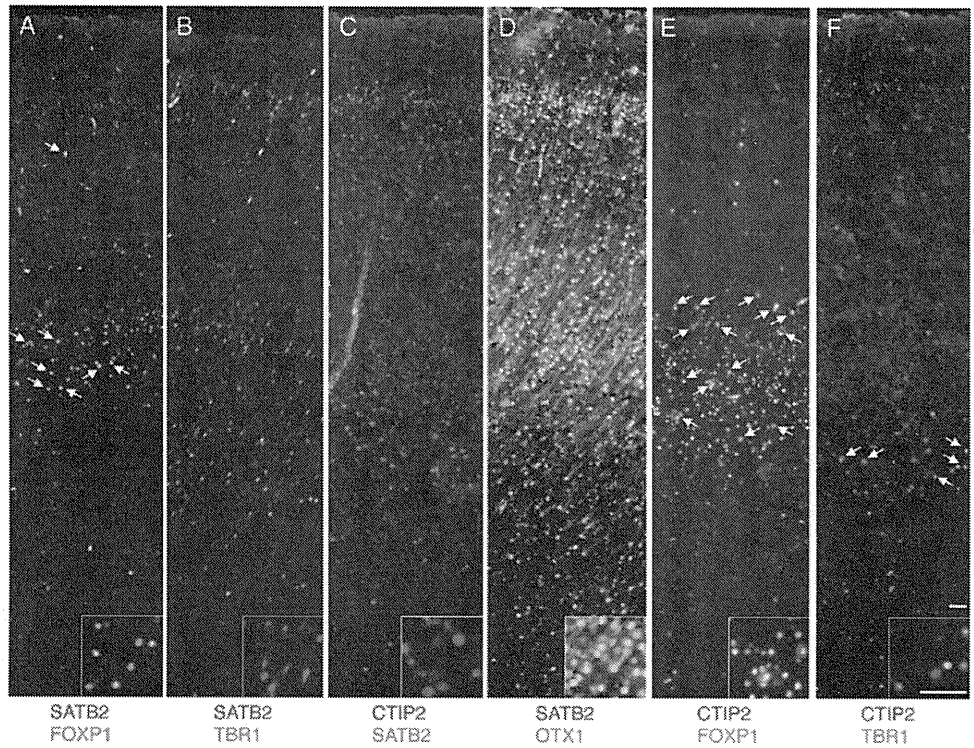

Figure 4. Immunofluorescence of layer-specific marker of neocortex at 29 GWs. FOXP1-immunopositive cells partially have SATB2 (merged color: arrows) in layers II–III and IV–V (*A*) and CTIP2 (merged color: arrows) in layers IV–VI (*E*). No double-positive cells for SATB2 and CTIP2 are scattered throughout 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.

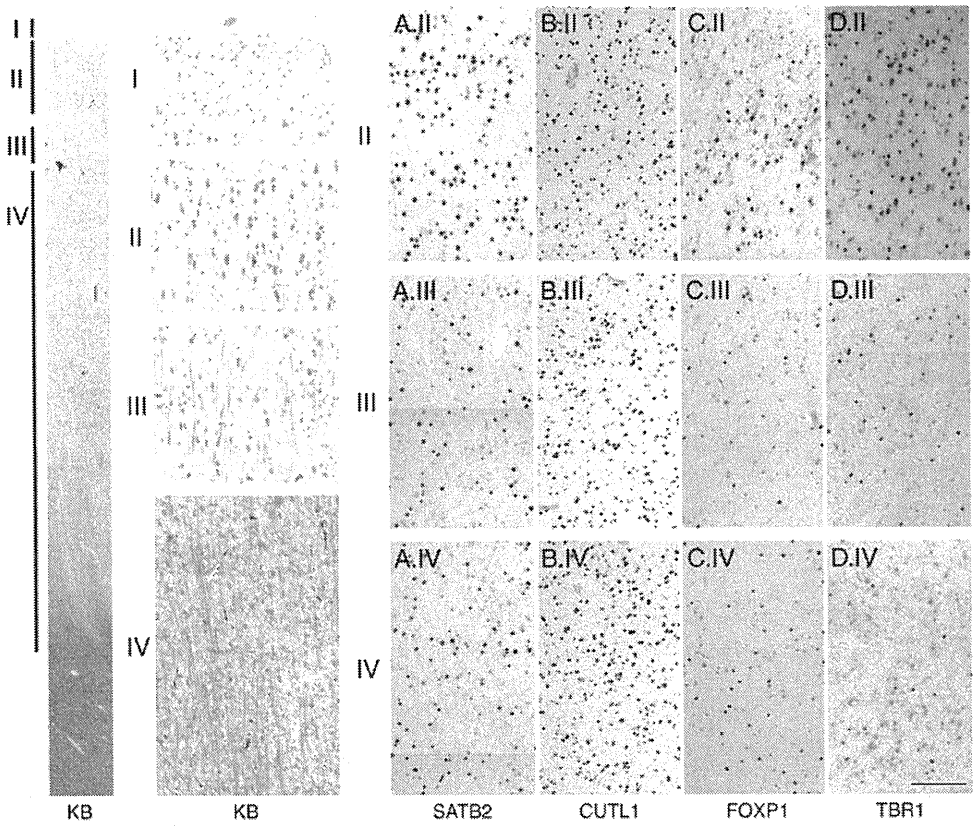

Figure 5. Layer-specific marker expression of the neocortex of 1-year-old patient with Miller–Dieker syndrome. Typical 4-layer pattern is shown. (*A*) SATB2, CUTL1, FOXP1, and TBR1 are diffusely expressed in layers II, III, and IV. Especially, TBR1-immunopositive cells locate in layer II (*E*). Enlargement 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,

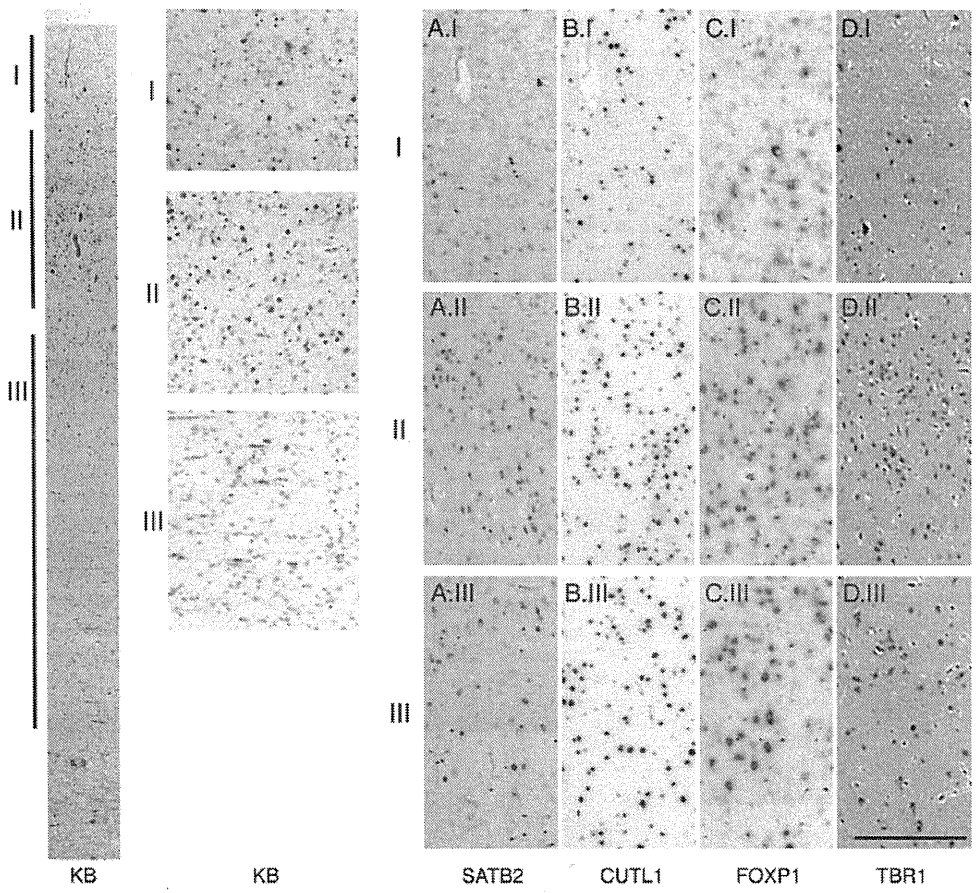

Figure 6. Layer-specific marker expression of the neocortex of 10-month-old boy with XLAG. Neocortex shows a thin 3-layer pattern. SATB2-, CUTL1-, FOXP1-, and TBR1-immunopositive cells locate diffusely (*A–D*). Gross histology shows with KB staining. *A.I*, *A.II*, and *A.III*, SATB2 in layers I, II,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

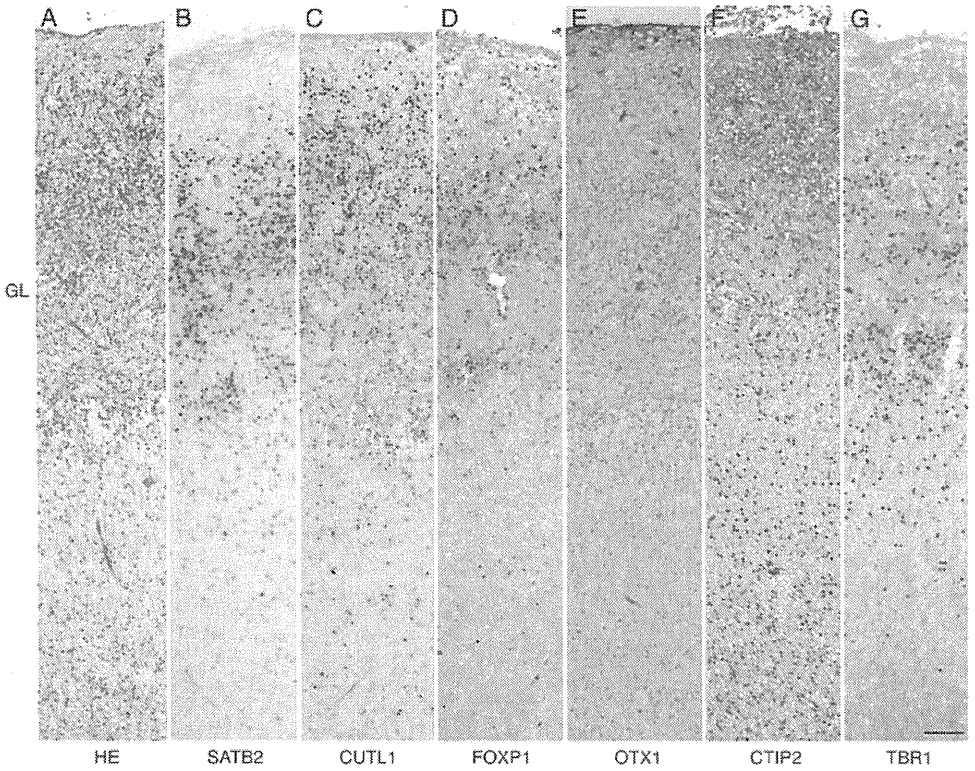

Figure 7. Layer-specific marker expression of the neocortex of 19-GW fetus with FCMD. Neocortex shows typical cobblestone lissencephaly feature. Many SATB2-, CUTL1-, FOXP1-, OTX1, CTIP2-, and TBR1-immunopositive cells migrate over the glia limitans (*B–G*), while some labeled cells locate under it. GL,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

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Notes

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

A New Microdeletion Syndrome of 5q31.3 Characterized by Severe Developmental Delays, Distinctive Facial Features, and Delayed Myelination

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Chromosomal deletion including 5q31 is rare and only a few patients have been reported to date. We report here on the first two patients with a submicroscopic deletion of 5q31.3 identified by microarray-based comparative genomic hybridization. The common clinical features of both patients were marked hypotonia, feeding difficulty in infancy, severe developmental delay, and epileptic/nonepileptic encephalopathy associated with delayed myelination. Both patients also shared characteristic facial features, including narrow forehead, low-set and abnormal auricles, bilateral ptosis, anteverted nares, long philtrum, tented upper vermilion, edematous cheeks, and high palate. The deleted region contains clustered PCDHs, including and *PCDHG*, which are highly expressed in the brain where they function to guide neurons during brain development, neuronal differentiation, and synaptogenesis. The common deletion also contains neuregulin 2 (*NRG2*), a major gene for neurodevelopment. We suggest that 5q31.3 deletion is responsible for severe brain developmental delay and distinctive facial features, and that the common findings in these two patients representing a new microdeletion syndrome. We need further investigations to determine which genes are responsible for the patients' characteristic features. © 2011 Wiley-Liss, Inc.

Key words: microdeletion; 5q31.3; array-based comparative genomic hybridization (aCGH); developmental delay; protocadherin (PCDH); neuregulin 2 (*NRG2*)

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INTRODUCTION

Interstitial deletions of the long arm of chromosome 5 are rare, except in the 5q35.2q35.2 region that includes the 2-Mb *NSD1* locus which is associated with Sotos syndrome [Visser and Matsumoto, 2003]. Although patients with proximal deletions that encompass the 5q15 to q22 region experience mild developmental delays, those with distal deletions that encompass the 5q22 to q31 region are more severely handicapped, fail to thrive, and present with significant craniofacial dysmorphism and joint dislocations or contractures [Garcia-Minaur et al., 2005]. Furthermore, there are only a few reports of patients with deletions encompassing the 5q31.3 region [Felding and Kristoffersson, 1980; Kramer et al., 1999; Arens et al., 2004].

Recently, we encountered 2 patients with severe developmental delay and distinctive facial features. Microarray-based comparative genomic hybridization (aCGH) analyses identified a common microdeletion of 5q31 in both patients. Radiological examination yielded characteristic finding with delayed myelination in both patients. The details of these cases are discussed in this report. Data on the patients were deposited in the DECIPHER database (Database of Chromosomal Imbalances and Phenotype in Humans using Ensembl Resources, https://decipher.sanger.ac.uk), and the corresponding DECIPHER number is given.

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CLINICAL REPORTS

Patient 1 (DECIPHER #TWM253734)

A Japanese boy was born at 40 weeks 5 days gestation by caesarean when labor had failed to begin. He is the first child of a 30-year-old father and a 26-year-old mother at the time of his birth. His birth weight was 2,925 g (−0.3 SD), length 50 cm (+0.5 SD), and head circumference 35 cm (+1.3 SD). Postaxial polydactyly of the right hand was noted. Patent ductus arteriosus (PDA) and a small ventricular septal defect (VSD) were revealed by echocardiography; PDA was surgically treated when he was 52 days old, and the small VSD was observed but not treated. He showed failure to thrive due to severe hypotonia and feeding difficulty, and aspiration was suspected because of recurrent pneumonia. Tube feeding was initiated at 6 months of age. Although he had no epileptic episodes, his electroencephalography showed spike waves on the right side of the posterior and occipital regions during natural sleep. Auditory brainstem response revealed obscure III waves in both sides, and the threshold was 40 dB. His median nerve conductive velocity (NCV) showed a delay with 32.5 m/s (−2.1 SD) on the left and 30.5 m/s (−2.5 SD) on the right. His posterior tibial NCV was also revealed to be delayed with 27.6 m/s (−3.2 SD) on both sides. These findings indicated peripheral neuropathy.

At 18 months of age, he showed delayed growth and microcephaly with height 76.4 cm (−1.6 SD), weight 8.7 kg (−1.7 SD), and head circumference 42.8 cm (−3.0 SD). He showed distinctive features including narrow forehead, low-set and abnormal auricles, bilateral ptosis, epicanthic folds, depressed nasal bridge, anteverted nares, long philtrum, tented upper vermilion, edematous cheeks, and high palate (Fig. 1A). His developmental milestones were markedly delayed with no contact eye movements, no smile response, and no head control. Brain magnetic resonance imaging (MRI) revealed reduced volume of the cerebrum and severely delayed myelination (brain appearance was that of an 8-month-old child) in T2-weighted imaging (Fig. 2A). Chromosomal G-banding showed a normal male karyotype.

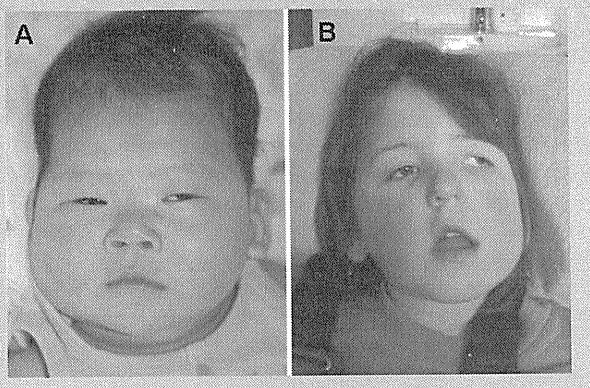

FIG. 1. Patient facial features. (A) Patient 1 at 18 months old. (B) Patient 2 at 6 years old.

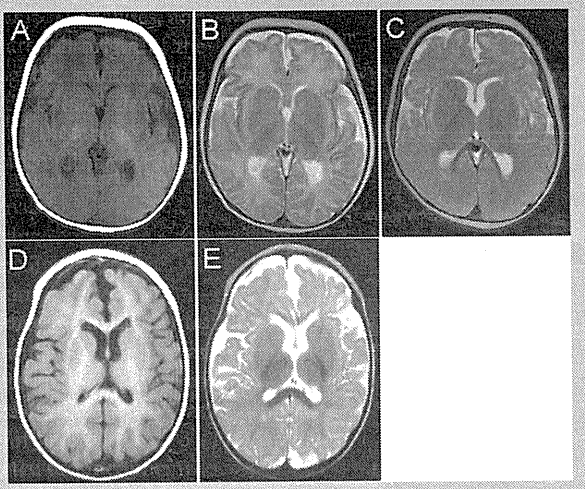

FIG. 2. Brain MRI findings with T1-weighted images (A, D) and T2-weighted images (B, C, E). Patient 1 at 4 months (A, B) and 18 months old (C). Patient 2 at her age of 4 years and 4 months old (D, E). Both patients showed reduced volume of the cerebrum associated with hypomyelination.

Patient 2 (DECIPHER #4681)

An 8-year-old French girl had no family history and no consanguinity in her parents. There was no complication during pregnancy. She was born with a birth weight of 3,700 g (+0.5 SD), a length of 52 cm (+1.0 SD), and a head circumference of 36 cm (+1.0 SD). Since early infancy, she showed feeding difficulties due to severe hypotonia. She had severe developmental delay with sitting at 11 months. Since the age of 12 months, she suffered epileptic seizures which were drug-resistant (hydrocortisone, clonazepam, topiramate, lamotrigine). Her epileptic status was diagnosed as Lennox–Gastaut syndrome.

She was of relatively small stature at a height of 121 cm (−1.5 SD), had a weight of 16.7 kg (−2.5 SD), and head circumference of 50 cm (−1.5 SD). She was not able to walk unassisted and was apraxic for speech. Her features were distinctive with narrow forehead, low-set ears, bilateral ptosis, downslanting palpebral fissures, anteverted nares, long philtrum, tented upper vermilion, edematous cheeks, and high palate (Fig. 1B). Strabismus was also noted. Brain MRI examination showed ventriculomegaly with reduced volume of the cerebrum, particularly in the frontoparietal regions, and marked hypomyelination (Fig. 2B). Conventional chromosome analysis showed a normal female karyotype.

MATERIALS AND METHODS

For further evaluation, microarray-based comparative genomic hybridization (aCGH) analyses, using Human Genome CGH Microarray 105A for Patient 1 and 44A for Patient 2 (Agilent Technologies, Santa Clara, CA), were performed according to the manufacturer's protocol, with genomic DNAs extracted from

peripheral blood samples. The identified aberrations were confirmed by fluorescence in situ hybridization (FISH) analyses, and both patients were also analyzed by FISH. Parental origin of the deletion in Patient 1 was determined using the microsatellite marker D5S1979 according to methods described elsewhere [Komoike et al., 2010]. Information regarding the primers used for the marker was obtained from the in-silico library (http://genome.ucsc.edu/).

RESULTS

Losses of genomic copies of 5q31.3 were identified in both patients. Patient 1 showed a 5.0-Mb deletion with molecular karyotyping as arr chr5q31.2q31.3(137,538,788–142,574,719)(hg18)x1 and Patient 2 showed a 2.6-Mb deletion with molecular karyotyping as arr chr5q31.3q31.3(139,117,448–141,682,547)(hg18)x1 (Fig. 3). FISH analyses with only one signal for the targeted probe confirmed the deletion (Fig. 4), and subsequent parental FISH analyses using the same probe showed no abnormality in their parents (data not shown), indicating de novo occurrence. Patient 1 shared the D5S1979 allele with his mother but not with his father (Fig. 4). This indicated that the deletion was paternally derived, and the final karyotype was ish del(5)(q31.2q31.3)(RP11-678N8x1) dn pat.

DISCUSSION

Both the patients in the present study showed an overlapping deletion of the region that included 5q31.3. The clinical features that were common for both patients were marked hypotonia, feeding difficulties in infancy, severe developmental delay, and epileptic/nonepileptic encephalopathy. Both patients also showed similar characteristic facial features, including a narrow forehead, low-set and abnormal auricles, bilateral ptosis, anteverted nares, long philtrum, tented vermilion of the upper lip, edematous cheeks, and high palate. Another characteristic finding was delayed myelination of the white matter, as identified by MRI examination. Thus, these findings are consistent, recognizable, and clinical features of 5q31.3 deletion.

To the best of our knowledge, five reports on patients with chromosome 5q31 deletions are available in the literature (Fig. 5). The first patient reported by Felding and Kristoffersson had manifestations similar to those of our patients [Felding and Kristoffersson, 1980]. Kramer et al. [1999] reported on a patient with 5q31q33 deletion whose condition was severely impaired; this patient showed congenital anomalies and died in the neonatal period. Arens et al. [2004] reported a patient with 5q22.1q31.3 deletion whose clinical findings included growth retardation, moderate psychomotor retardation, and mild facial dysmorphisms were similar to those of our patients. However, the severity of the developmental delay was milder than that of our patients, because she could walk without support and could speak a few words. These three patients were suspected to carry deletions of 5q31.3, but the deletion regions were ambiguous in conventional G-banding examination, and no neuroimaging test was available. We were thus unable to compare these patients with ours.

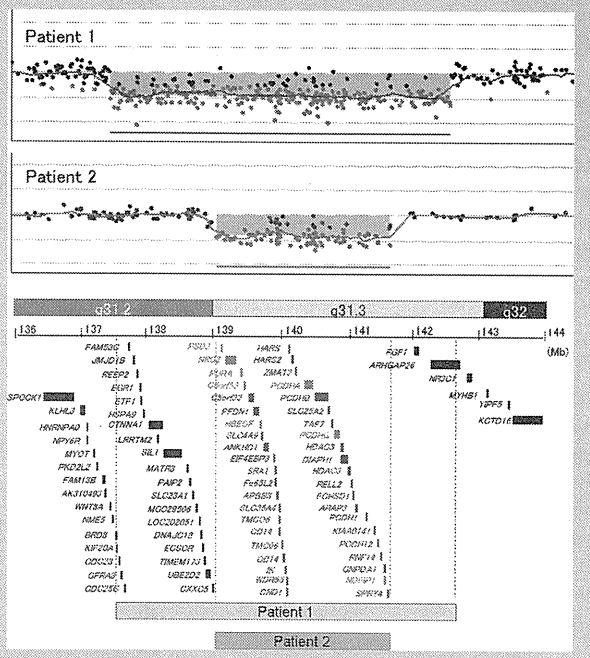

FIG. 3. The results of aCGH for chromosome 5. The aberrant areas of chromosome 5q31.2q31.3 are expanded in "Gene View" using CGH Analytics version 4.0 (Agilent) with the ADM-2 algorithm at a threshold of 6.0. The vertical axis indicates gain or loss of genome copy number and the horizontal axis indicates the physical position on chromosome 5. The aberrant areas are indicated by blue and red rectangles. Dots indicate the positions of the probes and green dots indicate $\log_2$ ratio under −0.5. Physical map corresponding to the highlighted area is depicted below the aCGH data. Rectangles indicate the positions of the UCSC genes (hg18). Colored rectangles indicate the genes included in the overlapped deletion region, and the genes showing high expression in the brain are indicated in red. Italic symbols indicate gene names.

Tzschach et al. [2006] reported on a patient with failure to thrive, psychomotor retardation, and mild facial dysmorphic features who carried a de novo deletion of 5q23.3q31.2, which did not overlap with those of our patients [Tzschach et al., 2006]. Mosca et al. [2007] reported a girl presenting with an abnormal cry, upslanting palpebral fissures, hypertelorism, anteverted nostrils, microretrognathia, growth retardation, and an adenoid cyst at the base of the tongue [Mosca et al., 2007]; the chromosomal deletion in this girl partially overlapped with that in Patient 1 of the present study, but the deletion did not involve the 5q31.3 band (Fig. 5).

In the present study, the common 2.6-Mb deletion region within the chromosomal band 5q31.3 is gene rich, containing 40 genes (UCSC Human genome browser, March 2006; http://genome.ucsc.edu/). The most intriguing finding is that the deleted region contained 5 genes classified as the protocadherin (PCDH) family which can be further divided into two main categories including clustered and non-clustered [Morishita and Yagi, 2007]. The clustered PCDHs including *PCDHA*, *PCDHB*, and *PCDHG*, which

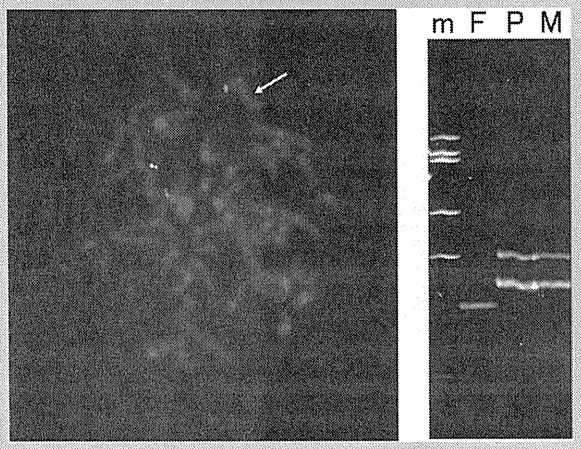

FIG. 4. Molecular cytogenetic validations for Patient 1. Two-color FISH analysis for patient 1 (left) using a combination of the BAC clones RP11-94J21 (red) and RP11-678N8 (green) as probes according to methods described elsewhere. A single green signal indicates the deletion of this region on the chromosome 5 (arrow). Microsatellite marker analysis of D5S1979 (right) separated on a polyacrylamide gel and visualized by staining with ethidium bromide. The bands for this patient are common only with those of the mother. m, molecular size marker; P, patient; F, father; M, mother. BAC clones RP11-94J21 and RP11-678N8 were selected from UCSC Human genome browser build 2006 (http://genome.ucsc.edu/cgi-bin/hgGateway).

are sequentially organized on the 5q31.3 region (Fig. 3) [Yagi, 2008]. The other non-clustered PCDHs, *PCDH1* and *PCDH12*, were also included in this region. PCDHs are highly expressed in the brain where they play roles in directing neurons during brain development, neuronal differentiation, and synaptogenesis [Akins and Biederer, 2006]. Although clustered PCDHs are suspected to have more important roles than non-clustered PCDHs in neuronal development, human diseases that are associated with clustered PCDHs have not yet been reported. The genomic organization of *PCDHA* and *PCDHG* includes multiple variable exons and a set of constant exons, similar to the gene encoding immunoglobulins and T-cell receptors [Morishita and Yagi, 2007; Takeichi, 2007]. These exons are combined by cis-splicing of the mRNA, leading to the production of a large number of isoforms and generating more than 50 transcripts from each gene, with various extracellular domain sequences [Morishita and Yagi, 2007; Takeichi, 2007]. Because of these characteristics, *PCDHA* and *PCDHG* are classified as clustered PCDHs. The expression mechanism of clustered PCDHs is also unique; different mouse neurons were found to express different sets of *Pcdha* and *Pcdhg*, indicating monoallelic gene expression that is unique to the clustered PCDHs [Esumi et al., 2005; Hirayama and Yagi, 2006].

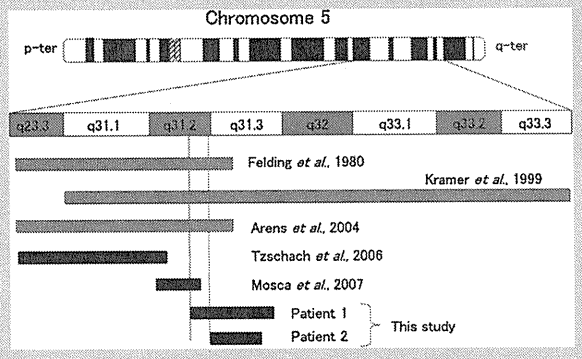

FIG. 5. Physical maps of the 5q31.3 region depict the regions deleted in the previously reported patients. Black rectangles indicate the range of the deletion, and the rectangles with diagonal lines indicate the suspected deletion region based on G-banded karyotyping. The reference author names are also indicated.

Although mutations of human *PCDHA*, *PCDHB*, and *PCDHG* have not been reported, hypomorphic *Pcdha* mutant mice exhibit enhanced contextual fear conditioning and abnormal spatial learning [Fukuda et al., 2008]. Morpholino-based reduction in levels of full-length Pcdh1a protein results in a dramatic increase in the extent of neuronal programmed cell death [Emond and Jontes, 2008]. These observations are similar to those in *Pcdhg−/−* mice that exhibit a loss of spinal interneurons [Wang et al., 2002]. Heterozygous mice of both *Pcdha* and *Pcdhg* have not been reported to show any neurological pathologies [Wang et al., 2002; Fukuda et al., 2008]; however, functional relevance of both *PCDHA* and *PCDHG* to human disorders cannot be denied, because mice heterozygous for the knockout alleles such as *Nsd1* and *Foxc1* show no manifestations [Rayasam et al., 2003; Aldinger et al., 2009]. Hemi-allelic deletions of the human homogues, *NSD1* and *FOXC1*, are associated with human disorders, i.e., Sotos syndrome and Dandy–Walker malformation, respectively. These findings suggest the biological difference between mice and human.

Another study showed that myelination functions as a trigger for the decline in Pcdha expression [Morishita et al., 2004]. Delayed myelination was another characteristic of our patients and may be associated with the deletions of *PCDHA*. Furthermore, *PCDHA* and *PCDHG* exhibit monoallelic expression [Esumi et al., 2005]. Thus, partial monosomy of 5q31.3 may affect the function of *PCDHA* and/or *PCDHG*.

By use of the UCSC genome browser, 6 genes other than *PCDHA* and *PCDHG* were found to be highly expressed in the brain among the 40 genes included in the common deletion region (Supplemental Table SI online). Neuregulin 2 gene (*NRG2*) was one of the 6 genes. *NRG2* is a member of the neuregulin family of signaling proteins that mediate cell–cell interactions in the nervous system and other organs [Rimer, 2007]. Recent genetic, transgenic, and postmortem brain studies support a potential contribution of NRG1-erbB4 signaling in schizophrenia [Banerjee et al., 2010]. Furthermore, *NRG2* is predominantly expressed by neurons in the central nervous system and exerts its effects on the perisynaptic Schwann cells at the neuromuscular junction [Longart et al., 2004; Rimer, 2007], suggesting a possible association of *NRG2* with neurological diseases. The findings of histological examinations of

the brain of *Nrg2* transgenic mice did not differ from those of the wild-type or heterozygous mice; however, homozygous knockout mice showed severe growth retardation, increased morbidity, and reduced reproductive capacity [Britto et al., 2004]. Thus, the peripheral neuropathy in Patient 1 may be attributable to *NRG2* deletion.

In this study, we reported the first two patients with deletions of the 5q31.3 region. We suggest that the deletion of 5q31.3, including clustered PCDHs and *NRG2*, lead to severe developmental delays, distinctive facial features, and delayed myelination. These characteristic manifestations comprise a new recognizable microdeletion syndrome. Although many genes in this region are highly expressed in the brain, the genes that specifically contributed to the unique characteristics of our patients could not be determined, because the crucial functions of the genes involved in the deletion region remain to be elucidated. Further studies need to be conducted to identify the genes that were associated with the characteristic features of our patients. Microcephaly was observed in Patient 1, but the head circumference of Patient 2 was within normal limit. Therefore, the gene associated with microcephaly in Patient 1 might be excluded from the deletion region that was common to both patients.

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シンポジウム2：NICUと重症心身障害児(者)施設(病棟)との連携

NICUと重症心身障害児(者)施設(病棟)との連携

岩崎裕治[1)]　平元　東[2)]

2年前に東京都で脳内出血を起こした妊婦の受け入れができずに、その後死亡したという事件が新聞に取り上げられ、この頃からNICUの病床不足が社会問題として言われるようになった。このNICUの病床不足およびNICU長期入院児の問題は、以前から厚労省の研究班などでも検討されていたが、なかなか解決の糸口がみつからない状況であった。

楠田(厚労省科研費研究班「重症新生児のための療養・療育環境の拡充に関する総合研究」主任研究者田村正徳)によると、年間約210例のNICU長期入院児が発生し、年間約100例が退院できない、また長期入院となる基礎疾患として多いのは、早産児、新生児仮死、先天異常などだが、このうち新生児仮死が特に退院が難しいということである[1)]。このようにNICUに長期に入院となる子どもたちは、その障害のために療育を必要とすることもあり、重症心身障害児(者)(以下、重症児(者))施設(病棟)には、そのような長期入院児への対応や、在宅生活への支援が期待されている。今回のシンポジウムは、NICU長期入院児の問題の背景を明らかにし、NICU、重症児施設・重症児病棟、および厚労省からの報告をもとに、打開策を議論してもらいたいという本学術集会会長の佐々木先生の熱意で実現した。

療育施設の状況は、昨年度田村班で行ったアンケート結果によると、回答のあった111施設では、平成19、20年度に新規入所となった利用児(者)は646名で、そのうち40%程度が準･超重症児であり、医療的に重度の子たちが数多く入所してきている状況がある。またNICU長期入院児は75名入所しており、小児科長期入院児が133名でこの2つの群で特に準・超重症児の割合が多かった。残りの438名は在宅からの入所だった[2)]。このように、重症心身障害児(者)施設(病棟)には、NICUからの入所のみならず、PICUなどの救急病棟や小児科病棟への長期入院児も数多く受け入れており、また在宅生活を送っていても介護者の高齢化などで、入所を望まれる方も多い。そのため、重症心身障害児(者)施設(病棟)への入所待機児(者)は、3,000～5,000名ともいわれ、望んでもなかなか入所できないという状況がある[3)]。またNICU長期入院児への療育の提供などは療育施設の役割だが、NICUとの医療的なレベルや医療設備・環境、職員配置(特に看護師配置)などの違い、急変事の対応など受け入れには実際の課題も多い。在宅支援も療育施設の役割の一つであるが、在宅生活を送っている重症児(者)も、年々医療的な重症度が高くなっており、いわゆる準・超重症児は増加してきている[4)]。また在宅支援として短期入所も各施設で行っているが、人工呼吸管理を必要とする短期入所の希望が増加してきている[2)]。

このような状況を踏まえて、本シンポジウムでは各シンポジストの先生方には、それぞれの立場から、それぞれの施設の状況や、現在の対応、今後の検討課題などについて語っていただいた。

まず始めに、埼玉医科大学総合医療センター総合周産期母子医療センター長　田村正徳先生にお話をうかがった。田村先生からは、現在の新生児医療の現状や、NICU長期入院児への新生児側の取り組みの経緯、厚労省研究班での精力的な取り組み(NICU長期入院児の動態調査、在宅支援マニュアル作成、在宅支援ウェブサイト開設、コーディネーターの調査、中間施設の検討、療育施設状況調査など)につき紹介があった。次に、国立病院機構南京都病院の

1)東京都立東部療育センター
2)北海道療育園

連絡先　〒136-0075
東京都江東区新砂3-3-25
東京都立東部療育センター
(岩崎裕治)

宮野前健先生より、国立病院機構重症心身障害児病棟の現状、NICUとの連携、在宅支援の方向性などにつきお話があり、重症児施設は単なる後方支援施設ではなく、療育活動などをとおし、子どもたちの持つ可能性を引き出し、在宅への橋渡しを担う施設としての機能を持たなければならない、そのためのネットワーク作り、公的支援システムが必要とのことだった。続いて、やまびこ医療福祉センターの家室和宏先生に20年以上にわたるNICU長期入院児の受け入れをとおして浮かび上がってきた課題およびそれらへの対応としての、地域での連携・ネットワーク作りの実際・成果などにつきうかがった。最後に厚労省医政局指導課救急・周産期医療等対策室長の宮本哲也先生に、厚労省としての周産期医療についてのこれまでの施策や今後の方向性についてうかがった。

総合討論では、新生児医療が家族の絆を重視して障害などの現状の説明を控えることで、重症児施設・病棟への移行や、療育施設で対応する際にさまざまな問題が起きてくるなど、それぞれの立場の違いなどが理解でき、施設間でのコミュニケーションの大切さが浮き彫りになった。また厚労省の担当官のご出席もあったため、厚労省の方向性などへ質問も多く、今後の施策の考え方などについてもうかがうことができた。

今回のシンポジウムをとおして、NICU長期入院児の問題解決には、お互いの現状把握や情報交換を土台にした、各関連施設の連携の重要性を共有できた。その連携を作り出すためには、まず自ら踏み出すことが必要である。そしてこの連携をとおして、NICU長期入院児など重度の障害をもつ子どもたちや家族の生活がより豊かになることを期待する。

各地域でさまざまな試みが始まっている。各地域で、それぞれの施設が、お互いの状況の理解をベースに、関連施設とのさらに濃厚な、顔の見える連携を図っていくことがこの問題の解決の一歩であろう。

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