Isolated Lissencephaly Sequence with Contiguous Gene Deletion Detected by FISH Analysis: A Case Report

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Abstract

Background : Lissencephaly is a clinically and genetically heterogeneous malformation of the brain, usually leading to a severe disabling condition and seizures. The recent discovery of molecular techniques and identification of lissencephaly genes (e.g. *LIS1* and *DCX*) has allowed etiologic diagnosis of this disorder feasible.

Objective : To describe a patient with lissencephaly in whom fluorescence *in situ* hybridization (FISH) determined etiologic diagnosis, providing precise genetic counseling and possible prenatal diagnosis for the family.

Clinical report and study results : The authors report a 4 month-old girl who presented with intractable, generalized myoclonic seizures at 1 month of age. The patient was born at 37 weeks' gestation, to a $G_4P_1A_2$ 36-year-old woman. Chromosome analysis from amniotic fluid performed for advanced maternal age revealed normal karyotype. Pregnancy was complicated by polyhydramnios. Computed tomographic scan of the brain at age one month showed a total absence of gyral formation. FISH of the metaphase chromosome from the patient, using Smith-Magenis and Miller-Dieker/ILS probe showed two signals of Smith-Magenis probe but only one signal of Miller-Dieker/ILS probe, indicating a microdeletion of 17p13.3 region including *LIS1* gene. Hybridization of the ILS probe on the metaphase chromosome of both parents was normal.

Conclusion : A confirmation of contiguous gene deletion in this patient lead to an etiologic diagnosis of lissencephaly. This information allowed precise genetic counseling, estimation of recurrent risk, and definite prenatal diagnosis available to the family. The authors suggest FISH 17p13.3 studies be performed in addition to a standard metaphase analysis in all patients with type I lissencephaly.

Key word : Microdeletion, Smooth Brain, *LIS1* Gene

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Lissencephaly is a rare congenital malformation of the brain designated by absent gyral formation due to failure of the normal pattern of neuronal migration[1]. This condition usually leads to a severe disabling condition and seizures. Neonates with this brain anomaly have poor responsiveness, poor feeding, and hypotonia. Early-onset seizures followed by decerebrate or decorticate postures are consistent findings. Confirmation is provided by ultrasound, computed tomography (CT) or magnetic resonance image (MRI) of the brain or at autopsy. Early death is common. Life expectancy, however, depends on the efficiency of respiratory supportive care and seizure control. Survivors are inevitably severely retarded and never achieve psychomotor development[1].

Dobyns classified three subtypes of lissencephaly, type I-III. Type I or classic lissencephaly is characterized by smooth neocortex, hypoplasia of corticospinal tract and normal or slightly reduced size of the cerebellum[2,3]. The overall cortical thickness is increased, with more gray matter and less white. Microscopically, the cortex consists of four, rather than normal six layers[1]. Commonly described syndromes with type I lissencephaly include Miller-Dieker syndrome (MDS), Norman-Roberts syndrome, and isolated lissencephaly sequence (ILS). Type II lissencephaly is mostly accompanied by additional malformation of the brain and eye in certain syndromes, such as Fukuyama muscular dystrophy, Walker-Warburg syndrome. Type III lissencephaly is characterized by agyria and by a granular-appearing cortex with microscopically six cortical layers with many immature neurons[1,4].

Miller-Dieker syndrome (MDS) has microcephaly, usually of postnatal onset, high forehead with wrinkles, bitemporal narrowing, prominent occiput, low and rotated ears, upturned nares, long and thin upper lip, micrognathia, and post-natal growth failure [1,5]. Malformations of other organs e.g. kidneys, heart, and gastrointestinal tract are not uncommon. In contrary, isolated lissencephaly has no facial dysmorphism and no associated malformation. High-voltage activity shown on electroencephalogram (EEG) has been reported as a typical finding in several patients with type I lissencephaly[6].

Herein, the authors describe a patient with lissencephaly in whom fluorescence *in situ* hybridization (FISH) allowed for an etiologic diagnosis, a precise genetic counseling and an accurate prenatal diagnosis for the family.

CASE REPORT

CK was born to a $G_4P_1A_2$, a 36-year-old woman. CK's father was 40 years old. The couple were non-consanguineous, Chinese-Thai descendants, and healthy. The first and third pregnancy resulted in spontaneous miscarriages at 20 and 12 weeks, respectively. They had one 5-year-old boy who had been in good health. Amniocentesis and prenatal chromosomal analysis performed for advanced maternal age revealed 46,XX, normal karyotype. Pregnancy was complicated by polyhydramnios.

CK was vaginally delivered at 37 weeks. Apgar scores were 9 and 10 at 1 and 5 min, respectively. Birth weight was 2,010 g and length was 46 cm with head circumference of 31 cm (10^{th} centile). Soon after birth, CK was noted to have grunting and mild respiratory distress which slowly resolved in a few days. She was discharged home on the 7^{th} day-of-life. CK's seizures began at one month of age and had not been controlled by anticonvulsants.

On examination at 4 months, CK had a head circumference of 38 cm (3^{rd} centile), slightly high forehead with no frontal wrinkles, increased muscle tone, hyperreflexia, inverted nipples (Fig. 1). There was mild contraction of the proximal interphalangeal (PIP) joint of the left 4^{th} digit. The remainder of the physical examination was unremarkable. Ophthalmologic examination was within normal limit. Otoacoustic emission (OAE) hearing screening suggested normal hearing ability.

CT scan of the brain denoted smooth brain with figure of eight, mild ventricular dilatation, and unremarkable cerebellar hemispheres and brainstem (Fig. 2). EEG revealed independent and asynchronous epileptiform discharges, on bilateral hemispheres. Biochemical profiles revealed normal blood glucose, sodium, calcium, magnesium, and phosphorus levels.

Human metaphase chromosomes were prepared by short-term culture following standard procedure[7]. Both G- and Q-banding chromosomes were analyzed which revealed 46,XX, normal karyotype (Fig. 3). In addition, fluorescence *in situ* hybridization of the metaphase chromosome was performed using Smith-Magenis probe (17p13.1) as a control probe and Miller-Dieker/ILS probe (17p13.3) as a critical or disease probe. Prehybridization protocol was as described elsewhere[8]. Two signals of Smith-Magenis probe but only one signal of Miller-Dieker/ILS (isolated lissencephaly) probe were detected, indicating a submicroscopic deletion of 17p13.3 region including

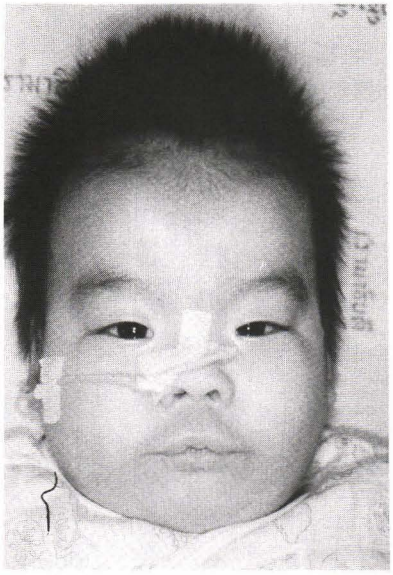

Fig. 1. Facial appearance of CK aged 4 months, noted slightly high forehead with no frontal wrinkles.

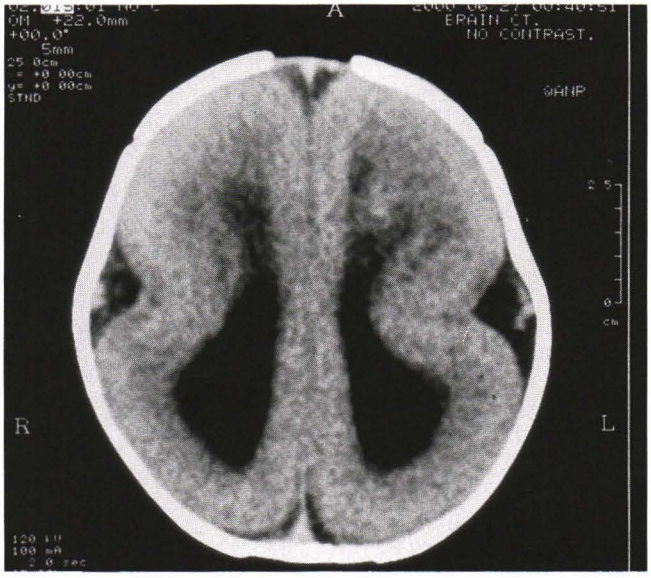

Fig. 2. CT scan of the brain showing absent gyri with figure of eight, and mild ventricular dilatation.

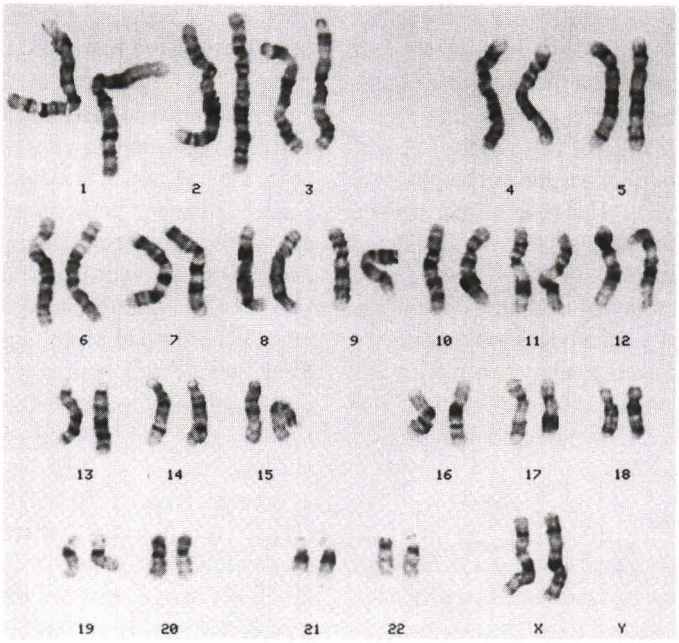

Fig. 3. G-banding karyotype from the patient's metaphase chromosome revealing 46,XX, normal female pattern.

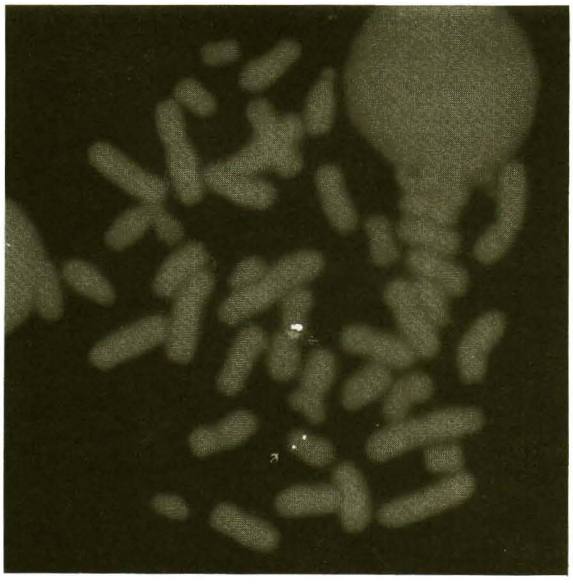

Fig. 4. Hybridization on the patient's chromosome showing two signals of Smith-Magenis (← control) probe but only one signal of Miller-Dieker/ILS (⬅ disease) probe.

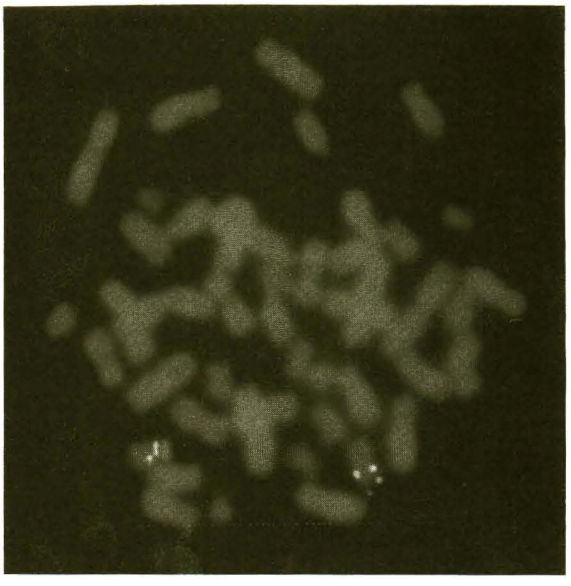

Fig. 5. Hybridization of the metaphase chromosome from CK's mother revealing no deletion detected (two ← and two ⬅ signals). FISH analysis on the father's metaphase chromosome was also normal (data not shown).

LIS1 gene (Fig. 4). Karyotypes and hybridization of the Miller-Dieker/ILS probe on the metaphase chromosome of both parents were normal (Fig. 5).

Of note, at the time of this report, CK was 22 months old, having normal weight and height, 11.2 kg and 87 cm, respectively. Her head circumference was small 43.3 cm, far below 3rd centile. Facial appearance had not changed. As one could guess, she had had several episodes of aspirated pneumonia necessitating gastrostomy tube operation, and recurrent urinary tract infections secondary to urinary stasis accompanying severe neurological devastation. Her kidney ultrasound and vesico-ureterography (VCUG) resulted in normal findings.

DISCUSSION

Based on clinical and radiological findings, CK is a de novo case of type I, isolated lissencephaly. The authors have demonstrated a submicroscopic deletion of 17p13.3 region including *LIS1* gene underlying her lissencephaly.

Lissencephaly is a genetically heterogeneous disorder. Absence of gyri can be associated with chromosomal abnormalities, contiguous gene deletion, single gene mutation, or unidentified cause[9-12]. In 1989, Ledbetter et al first described a molecular technique in detecting microdeletion involving 17p13.3 region in patients with type I lissencephaly [13]. Later, Dobyns et al have shown that contiguous gene deletion and point mutation involving *LIS1* gene are two common pathogenic mechanisms leading to Miller-Dieker syndrome and isolated lissencephaly [14,15]. Contiguous gene deletion has accounted for most Miller-Dieker syndrome cases, with only half of these being cytogenetically detected on high resolution (prometaphase chromosome) analysis. In isolated lissencephaly, the size of the deletion is smaller and non-visible on high resolution banding. Using a molecular tool, FISH of Miller-Dieker/ILS probe (17p13.3), it increases sensitivity in detecting deletion up to 90 per cent of Miller-Dieker syndrome and 40-60 per cent of isolated lissencephaly[14-16]. The greater severity seen in Miller-Dieker syndrome may be associated with the loss of another cortical development gene in the deleted chromosomal segment[17].

Point mutation of *LIS1* gene is an underlying mechanism in 40 per cent of isolated lissencephaly cases. Diagnostic test by this means is possible only

by the DNA analysis method, which is not commercially available at the present time.

LIS1 gene has 11 exons, encoding a beta-subunit of brain platelet-activating factor acetylhydrolase (PAFAH1B1)[18]. PAFAH1B1 is involved in a variety of biologic and pathologic processes, including the movement of neuronal nuclei with extending processes during differentiation and development[18-20]. Targeted mutagenesis in mouse models resulted in a viable and fertile heterozygotes, but early lethal homozygotes. Cortical neurons and glia cells were aberrant in the developing cortex, and the neurons migrated slowly[21].

Mutations of another distinct gene, double cortin (*DCX* or *XLIS*) on Xq22.3-q23 can lead to an X-linked formed lissencephaly[22]. Double cortin mutations in males result in type I lissencephaly, whilst the same mutations in females result in a less severe phenotype, seizure disorders with subcortical band heterotopia[22-24].

The genotype-phenotype correlation has been demonstrated. The lissencephalic malformation was more severe posteriorly in individuals with *LIS1* mutations and more severe anteriorly in individuals with *XLIS* mutations. Hypoplasia of cerebellar vermis was more common with *XLIS* mutation[25]. Mutation analysis of *LIS1* and double cortin is essential in determining the etiology of the disease in patients, and helpful in determining the recurrent risk in the families[23].

Moreover, other distinct disorders, X-linked lissencephaly with ambiguous genitalia (XLA-G), and possibly autosomal recessive lissencephaly with cleft palate should also be warranted[26,27].

A confirmation of contiguous gene deletion in CK leads to an etiologic diagnosis of lissencephaly. Given both parents had no deletion of *LIS1* gene, the recurrent risk of this couple having another affected child is close to zero. However, gonadal mosaicism can not be completely excluded. This information is valuable in providing precise genetic counseling, estimation of recurrent risk, and definite prenatal diagnosis for the family.

Although this is a one case study, the authors hope that it will raise the awareness of general pediatricians and specialists that a diagnostic test is available and valuable to the affected family. In conclusion, given an extremely low sensitivity to detect microdeletion on standard metaphase chromosome analysis, the authors suggest FISH studies using probes specific to *LIS1* as the initial diagnostic assay for evaluation of patients with type I lissencephaly.

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REFERENCES

1. Hunter AGW. Brain. In: Stevenson RE, Hall JG, Goodman RM, eds. Human malformations and related anomalies. New York: Oxford University Press, 1993: 1-108.
2. Dobyns WB, Kirkpatrick JB, Hittner HM, Roberts RM, Kretzer FL. Syndromes with lissencephaly II: Walker-Warburg and cerebro-oculo-muscular syndromes and a new syndrome with type II lissencephaly. Am J Med Genet 1985; 22: 157-95.
3. Dobyns WB, Gilbert EF, Opitz JM. Further comments on the lissencephaly syndromes. Am J Med Genet 1985; 22: 197-211.
4. Plauchu H, Encha-Razavi F, Hermier M, Attia-Sobol J, Vitrey D, Verloes A. Lissencephalyc type III, stippled epiphyses and loose, thick skin: A new recessively inherited syndrome. Am J Med Genet 2001; 99: 14-20.
5. Allanson JE, Ledbetter DH, Dobyns WB. Classical lissencephaly syndromes: Does the face reflect the brain? J Med Genet 1998; 35: 920-3.
6. Hodgkins PR, Kriss A, Boyd S, et al. A study of EEG, electroretinogram, visual evoked potential, and eye movements in classical lissencephaly. Dev Med Child Neurol 2000; 42: 48-52.
7. Francke U. Quinacrine mustard fluorescence of human chromosome : characterization of unusual

translocation. Am J Hum Genet 1972; 24: 189-213.
8. Tocharoentanaphol C, Cremer M, Schrock E, et al. Multicolor fluorescence in situ hybridization on metaphase chromosomes and interphase Halo-preparations using cosmid and YAC clones for the simultaneous high resolution mapping of deletions in the dystrophin gene. Hum Genet 1994; 93: 229-35.
9. Alvarado M, Bass HN, Caldwell S, Jamehdor M, Miller AA, Jacob P. Miller-Dieker syndrome. Detection of a cryptic chromosome translocation using in situ hybridization in a family with multiple affected offspring. Am J Dis Child 1993; 147: 1291-4.
10. Dobyns WB, Stratton RF, Parke JT, Greenberg F, Nussbaum RL, Ledbetter DH. Miller-Dieker syndrome: Lissencephaly and monosomy 17p. J Pediatr 1983; 102: 552-8.
11. Kingston HM, Ledbetter DH, Tomlin PI, Gaunt KL. Miller-Dieker syndrome resulting from rearrangement of a familial chromosome 17 inversion detected by fluorescence in situ hybridisation. J Med Genet 1996; 33: 69-72.
12. Masuno M, Imaizumi K, Nakamura M, Matsui K, Goto A, Kuroki Y. Miller-Dieker syndrome due to maternal cryptic translocation t (10;17) (q26.3; p13.3). Am J Med Genet 1995; 59: 441-3.
13. Ledbetter DH, Ledbetter SA, vanTuinen P, et al. Molecular dissection of a contiguous gene syndrome: Frequent submicroscopic deletions, evolutionarily conserved sequences, and a hypomethylated "island" in the Miller-Dieker chromosome region. Proc Natl Acad Sci USA 1989; 86: 5136-40.
14. Dobyns WB, Reiner O, Carrozzo R, Ledbetter DH. Lissencephaly. A human malformation associated with deletion of the *LIS1* gene located at chromosome 17p13. JAMA 1993; 270: 2838-42.
15. Dobyns WB, Curry CJ, Hoyme HE, Turlington L, Ledbetter DH. Clinical and molecular diagnosis of Miller-Dieker syndrome. Am J Hum Genet 1991; 48: 584-94.
16. Pilz DT, Macha ME, Precht KS, Smith AC, Dobyns WB, Ledbetter DH. Fluorescence in situ hybridization analysis with *LIS1* specific probes reveals a high deletion mutation rate in isolated lissencephaly sequence. Genet Med 1998; 1: 29-33.
17. Cardoso C, Leventer RJ, Matsumoto N, et al. The location and type of mutation predict malformation severity in isolated lissencephaly caused by abnormalities within the *LIS1* gene. Hum Mol Genet 2000; 9: 3019-28.
18. Hattori M, Adachi H, Tsujimoto M, Arai H, Inoue K. Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor acetylhydrolase [corrected]. Nature 1994; 370: 216-8.
19. Vallee RB, Faulkner NE, Tai CY. The role of cytoplasmic dynein in the human brain developmental disease lissencephaly. Biochem Biophys Acta 2000; 1496: 89-98.
20. Vallee RB, Tai C, Faulkner NE. *LIS1*: Cellular function of a disease-causing gene. Trends Cell Biol 2001; 11: 155-60.
21. Cahana A, Escamez T, Nowakowski RS, et al. Targeted mutagenesis of *LIS1* disrupts cortical development and *LIS1* homodimerization. Proc Natl Acad Sci USA 2001; 98: 6429-34.
22. Gleeson JG, Allen KM, Fox JW, et al. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 1998; 92: 63-72.
23. Gleeson JG. Classical lissencephaly and double cortex (subcortical band heterotopia): *LIS1* and doublecortin. Curr Opin Neurol 2000; 13: 121-5.
24. Clark GD. Cerebral gyral dysplasias: Molecular genetics and cell biology. Curr Opin Neurol 2001; 14: 157-62.
25. Dobyns WB, Truwit CL, Ross ME, et al. Differences in the gyral pattern distinguish chromosome 17-linked and X- linked lissencephaly. Neurology 1999; 53: 270-7.
26. Dobyns WB, Berry-Kravis E, Havernick NJ, Holden KR, Viskochil D. X-linked lissencephaly with absent corpus callosum and ambiguous genitalia. Am J Med Genet 1999; 86: 331-7.
27. Kerner B, Graham JM Jr, Golden JA, Pepkowitz SH, Dobyns WB. Familial lissencephaly with cleft palate and severe cerebellar hypoplasia. Am J Med Genet 1999; 87: 440-5.

รายงานผู้ป่วย 1 ราย ที่มีภาวะสมองเรียบร่วมกับมีการขาดหายของชิ้นส่วนโครโมโซม ตรวจโดยวิธี FISH

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ภาวะสมองเรียบไม่มีรอยหยัก เป็นความผิดปกติของสมองซึ่งมีความหลากหลายในทางคลินิค และเกิดจากสาเหตุทางพันธุกรรมได้หลายแบบ ผู้ป่วยจะมีความพิการทางสมองสูงและมีอาการชัก ความก้าวหน้าทางอณูพันธุศาสตร์และการค้นพบยีนที่ทำให้เกิดภาวะสมองเรียบ (เช่น ยีน LIS1 และ DCX) ทำให้สามารถตรวจหาสาเหตุทางพันธุกรรมของภาวะสมองเรียบได้

วัตถุประสงค์ : เพื่อรายงานผู้ป่วย 1 ราย ที่มีภาวะสมองเรียบ โดยใช้เทคนิคทางอณู–เซลล์พันธุศาสตร์ fluorescence *in situ* hybridization (FISH) เข้ามาช่วยในการวินิจฉัยหาสาเหตุของภาวะสมองเรียบ ทำให้สามารถให้คำปรึกษาแนะนำทางพันธุศาสตร์ที่ชัดเจนแก่ครอบครัวของผู้ป่วย และทำให้สามารถให้การตรวจวินิจฉัยก่อนคลอดได้ต่อไป

รายงานทางคลินิกและผลการศึกษา : ผู้ป่วยเด็กหญิงอายุ 4 เดือน มีอาการชักที่ไม่สามารถควบคุมได้ ตั้งแต่อายุ 1 เดือน ผู้ป่วยคลอดครบกำหนด เป็นบุตรคนที่ 2 แต่เป็นครรภ์ที่ 4 มารดาเคยมีการแท้งเอง 2 ครั้ง มารดาอายุ 36 ปีเมื่อตั้งครรภ์ผู้ป่วยและได้รับการเจาะน้ำคร่ำตรวจโครโมโซม ซึ่งผลพบว่าโครโมโซมของทารกในครรภ์ปกติ ภาวะแทรกซ้อนที่พบขณะตั้งครรภ์คือ น้ำคร่ำมากผิดปกติ ผู้ป่วยได้รับการทำ เอ็กซเรย์คอมพิวเตอร์ของสมองเมื่ออายุ 1 เดือน พบว่าสมองไม่มีรอยหยัก ผลตรวจโครโมโซมจากเลือดของผู้ป่วยพบว่าปกติ แต่ได้ทำการตรวจพิเศษเพิ่มเติมโดยใช้เทคนิค FISH โดยใช้ตัวนำจับควบคุม (control probe : Smith–Maginis) และตัวนำจับโรค (disease probe : Miller–Dieker/ILS) ผลพบว่าบนโครโมโซม 17 ของผู้ป่วยให้ปฏิกิริยาเรืองแสงกับตัวนำจับควบคุมบนทั้งสองโครโมโซม แต่ให้ผลเรืองแสงกับตัวนำจับโรคบนโครโมโซมเพียงข้างเดียว ซึ่งบ่งชี้ว่าผู้ป่วยมีการขาดหายเป็นบริเวณเล็ก ๆ (microdeletion) ของชิ้นส่วนโครโมโซมที่แขนข้างสั้นของโครโมโซม 17 (17p13.3) ซึ่งมียีน LIS1 ขาดหายไปด้วย ผลตรวจโครโมโซมและ FISH จากเซลล์ที่ได้จากเลือดของบิดาและมารดาของผู้ป่วยพบว่าปกติ

สรุป : การตรวจพบว่ามีการขาดหายเป็นบริเวณเล็ก ๆ ของชิ้นส่วนโครโมโซม 17p13.3 เป็นสาเหตุของภาวะสมอง–เรียบในผู้ป่วยรายนี้ ช่วยให้สามารถใช้ข้อมูลนี้ประกอบการให้คำปรึกษาแนะนำทางพันธุศาสตร์ การประเมินอัตราเสี่ยงของการมีบุตรเป็นโรคอีก และการให้การวินิจฉัยก่อนคลอดเป็นไปได้อย่างแม่นยำ เสนอว่าผู้ป่วยที่มีภาวะสมองเรียบแบบที่ 1 ทุกรายควรได้รับการตรวจ FISH 17p13.3 นอกเหนือไปจากการตรวจโครโมโซมมาตรฐาน

คำสำคัญ : Microdeletion, สมองเรียบ, ยีน LIS1

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