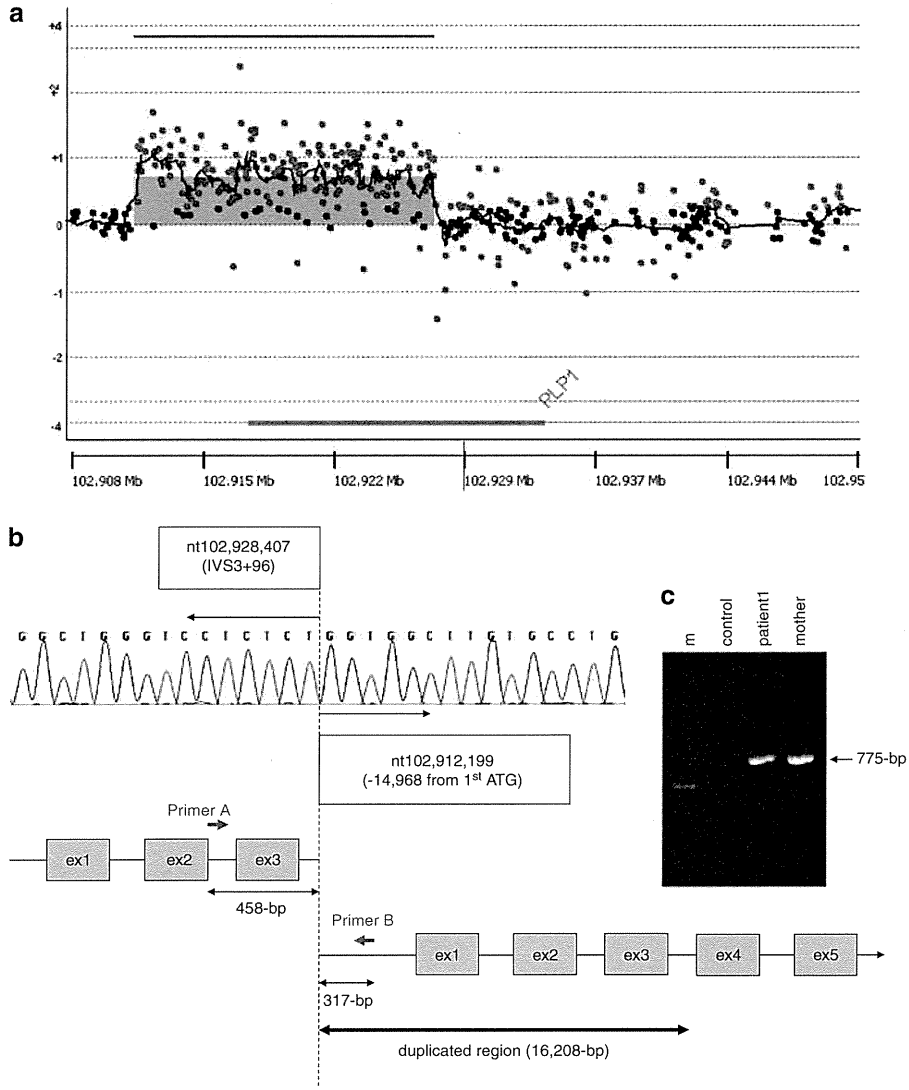

Figure 3 Molecular analyses of the *PLP1* duplication identified in Patient 1. (**a**) The result of a custom-designed aCGH shows partial duplication of *PLP1*. (**b**) A schematic representation of the breakpoint analyzed by PCR and subsequent sequencing. (**c**) A 775 bp PCR product including a breakpoint is amplified by Primer A and B and is shown in electrophoresis. m; molecular marker OneSTEP Ladder 50 (Nippon Gene).

the previous study.[12] Finally, total RNA samples extracted from the 12 iPS cell clones generated in this study were analyzed. Although we could detect the *PLP1* band in iPS cells from normal individual and Patients 2 and 3, we could not detect the *PLP1* band in the iPS cells generated from Patient 1, indicating null expression of *PLP1* caused by the partial duplication of *PLP1* (Figure 5). In Patients 2 and 3, *PLP1* signals appeared to be somewhat stronger than in controls, but because of the large variation in signal intensity among different cell lines, it was inconclusive in our limited experiments.

DISCUSSION

In this study, we identified different *PLP1* abnormalities in three patients with PMD. Patient 3 showed a novel missense mutation, c.636G>C (Tyr212Cys), which is in the extramembrane region of the PLP1 protein.[2] A missense substitution in the same codon, but resulting in a change into a different amino acid, c.634T>C (Tyr212Arg), has been reported to be a pathogenetic mutation by others.[13] Frequently, a cysteine residue changes the three-dimensional protein conformation drastically owing to disulfide bond formation with other cysteines.[14] Thus, the amino-acid substitution to cysteine in our patient is likely a pathogenetic mutation, causing PMD. Previous genotype-phenotype correlation study showed that the phenotype of patients with *PLP1* missense mutations was more severe than those with *PLP1* duplications.[1–3] Indeed, Patient 3 showed severely delayed psychomotor development complicated by respiratory and feeding difficulties. His condition can be classified as form 0 according to the classification proposed by Cailloux *et al.*,[13] as form 0 is the most severe form of PMD. Dysmyelination in this patient was particularly severe.

Patient 2 had a 0.6-Mb duplication including *PLP1*. This size is typical for PMD patients with *PLP1* duplications.[8,15,16] This patient is now 43 years old and does not show any deterioration of neurological abilities. Despite being bedridden, he can verbally communicate with several words. His clinical condition can be classified as form 2, because his maximum motor ability was sitting. His dysmyelination is milder than that of Patient 3.

The most intriguing result in this study is the partial duplication of *PLP1* identified in Patient 1. Although there have been reports

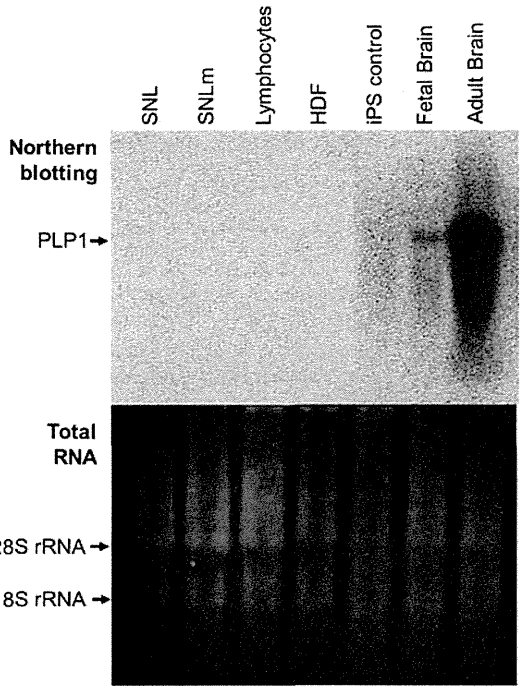

Figure 4 *PLP1* expression analysis by the northern blotting for various samples. (Upper) Predominant expression of *PLP1* is shown in the brain samples. Although control iPS cells show weak expression, there are no expressions of *PLP1* in the other samples. (Bottom) Agarose gel staining for the total RNAs before subsequent northern blotting indicates the same amounts of total RNAs loaded in each lane. SNLm, SNL-feader cells treated with mitomycin; HDF, human dermal fibroblasts

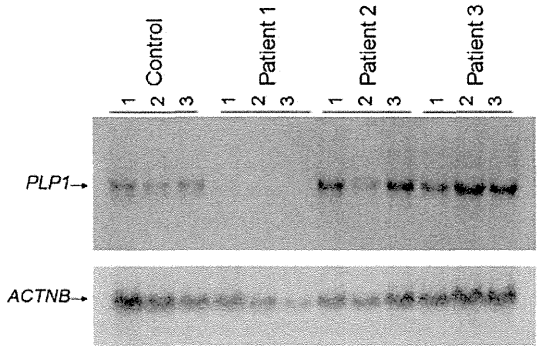

Figure 5 *PLP1* expression analyses using northern blotting. Three iPS cells of Patient 1 show no *PLP1* band, whereas the other iPS cells from normal control, Patient 2 and Patient 3 show expressions of *PLP1*. *ACTNB* (actin beta) is used for internal control.

of partial deletions in *PLP1* identified by multiplex ligation-dependent probe amplification analysis,[9] this is the first report of a partial duplication of *PLP1*. The duplicated segment included the promoter region and the first three exons. Therefore, we hypothesized that a very short mRNA or long fusion mRNA might be expressed by this duplication together with the normal mRNA. To confirm this hypothesis, we analyzed *PLP1* expression by using northern blot analysis, the only way to detect the length and the quantity of mRNAs. As expression levels of *PLP1* in skin fibroblasts were too low to be examined by northern blot analysis, we generated iPS cells from the patients. Contrary to expectation, northern blot analysis showed no *PLP1* bands in the iPS cell generated from the fibroblasts of Patient 1. Although there may be a limitation to detect short-unstable mRNAs in our method, this possibly indicated that the expression of *PLP1* mRNA was disturbed by the *PLP1* partial duplication identified in Patient 1. Regarding the clinical severities of the patients, Patient 1 showed milder phenotype than Patient 2, and his condition can be classified as form 3. Previous genotype-phenotype correlation study have shown that patients with *PLP1* missense mutations show severe manifestation associated with severe hypomyelination, which is recognized as the consequence of accumulated mutant protein in the endoplasmic reticulum as a gain-of-toxic function of the mutant protein.[2] Excessive PLP1 protein resulting from genomic duplications may accumulate in late endosomes/lysosomes, promoting its incorporation into other myelin components.[1] In contrast, patients with *PLP1* null mutations escape severe impairments because of the absence of any gain-of-toxic function.[2,13,17] Indeed, knockout mice with a functionally null *Plp1* gene do not develop classical signs of *Plp1*-related disease; their oligodendrocytes develop normally and synthesize compact myelin sheaths.[3,18] However, the mice show ultrastructural abnormalities, including swelling of the small-diameter axons and late-onset axonal degeneration.[3,19] Consequently, a loss-of-function mutation of *PLP1* does not induce oligodendrocyte cell death, possibly serving as a mechanism underlying the milder phenotypic consequences observed in patients with null *PLP1* mutations. Although length-dependent axonal degeneration has been described in *PLP1* null mutations,[20] there is no information about peripheral neuropathy in Patient 1. Thus, it was unclear whether the clinical condition of Patient 1 is compatible with that of *PLP1* null mutations. However, the lack of *PLP1* expression in iPS cells derived from Patient 1 clearly demonstrated that the underlying mechanism of PMD in Patient 1, with a partial *PLP1* duplication, is different from the other two patients in this study.

Immortalized lymphocytes and skin fibroblasts derived from patients are often used for expression studies or biological analyses, as these cells are easy to be obtained and handled. However, many tissue-specific genes are not sufficiently expressed by these cells; *PLP1* being one of them. Although there are reports examining *PLP1* expression by RT–PCR, using mRNA extracted from skin fibroblasts,[4,5,21] the expression of *PLP1* mRNA in skin fibroblasts is too low to be examined by northern blotting as shown here. In this study, our microarray database search showed over 40 times higher *PLP1* expression in iPS cells than that in skin fibroblasts. Our initial northern blot analysis confirmed faint but detectable *PLP1* expression in iPS cells, whereas no expression was observed in skin fibroblasts, lymphocytes or SNL feeder cells. This study also confirmed a lack of *PLP1* expression in SNL feeder cells. Therefore, this study demonstrates that iPS cells express endogenous *PLP1*, and that the possibility of contamination from SNL feeder cells or original skin fibroblasts can be excluded. Although being detectable by northern blotting, *PLP1* expression in iPS cells appeared to be much lower than that in mature oligodendrocytes and may be simply cryptic rather than functional. If so, this allows us to evaluate the native transcriptional level of each mutant (and wild-type) *PLP1* allele, which is the primary focus of our study. Meanwhile, terminal differentiation of iPS cells into the oligodendrocyte lineage would result in an enhanced *PLP1* expression with functional consequence. However, this requires technological breakthrough in the induction of terminal differentiation into oligodendrocyte lineage, which is currently unavailable.

In conclusion, we identified the first PMD patient having a partial *PLP1* duplication. The absence of *PLP1* expression in iPS cells, generated from the patient's skin fibroblasts, proved the underlying effects of the partial *PLP1* duplication for the PMD development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the patients and their families for their cooperation. This work was mainly supported by JST PRESTO program (KS) and was partially supported by Grant-in-Aid for Scientific Research on Innovated Areas 'Foundation of Synapse and Neurocircuit Pathology' and Grant-in-Aid for scientific research from Health Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare, Japan (TY). This work was supported in part by Grant-in-Aid for Scientific Research (A) 21249062, Japan Society for the Promotion of Science (JSPS) and 'High-Tech Research Center' Project for Private Universities: matching fund subsidy from the Ministry of Education, Culture, Sports, Science and Technology, 2006–2010 'The Research Center for the Molecular Pathomechanisms of Epilepsy, Fukuoka University', Research Grants (21B-5) for Nervous and Mental Disorder from the Ministry of Health, Labor and Welfare and Health and Labor Science Research Grant 21210301, KB220001 from the Ministry of Health, Labor and Welfare, and Adaptable and Seamless Technology Transfer Program through Target-driven R&D (A-STEP) Exploratory Research, Japan Science and Technology Agency (JSP) (SH). KS thanks Hayashi Memorial Foundation for Female Natural Scientists for grant aid support.

WEB SITE: NCBI GEO; http://www.ncbi.nlm.nih.gov/guide/genes-expression/ OMIM; http://omim.org/

1 Inoue, K. PLP1-related inherited dysmyelinating disorders: Pelizaeus-Merzbacher disease and spastic paraplegia type 2. *Neurogenetics* **6,** 1–16 (2005).
2 Garbern, J.Y. Pelizaeus-Merzbacher disease: Genetic and cellular pathogenesis. *Cell. Mol. Life Sci.* **64,** 50–65 (2007).
3 Woodward, K.J. The molecular and cellular defects underlying Pelizaeus-Merzbacher disease. *Expert. Rev. Mol. Med.* **10,** e14 (2008).
4 Hobson, G.M., Huang, Z., Sperle, K., Sistermans, E., Rogan, P.K., Garbern, J.Y. *et al.* Splice-site contribution in alternative splicing of PLP1 and DM20: molecular studies in oligodendrocytes. *Hum. Mutat.* **27,** 69–77 (2006).
5 Regis, S., Grossi, S., Corsolini, F., Biancheri, R. & Filocamo, M. PLP1 gene duplication causes overexpression and alteration of the PLP/DM20 splicing balance in fibroblasts from Pelizaeus-Merzbacher disease patients. *Biochim. Biophys. Acta.* **1792,** 548–554 (2009).
6 Inoue, H. Neurodegenerative disease-specific induced pluripotent stem cell research. *Exp. Cell. Res.* **316,** 2560–2564 (2010).
7 Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131,** 861–872 (2007).
8 Shimojima, K., Inoue, T., Hoshino, A., Kakiuchi, S., Watanabe, Y., Sasaki, M. *et al.* Comprehensive genetic analyses of PLP1 in patients with Pelizaeus-Merzbacher disease applied by array-CGH and fiber-FISH analyses identified new mutations and variable sizes of duplications. *Brain Dev.* **32,** 171–179 (2010).
9 Warshawsky, I., Chernova, O.B., Hubner, C.A., Stindl, R., Henneke, M., Gal, A. *et al.* Multiplex ligation-dependent probe amplification for rapid detection of proteolipid protein 1 gene duplications and deletions in affected males and carrier females with Pelizaeus-Merzbacher disease. *Clin. Chem.* **52,** 1267–1275 (2006).
10 Ohnuki, M., Takahashi, K. & Yamanaka, S. Generation and characterization of human induced pluripotent stem cells. *Curr. Protoc. Stem Cell Biol.* Chapter 4, Unit 4A2 (2009).
11 Yamamoto, T., Feng, J.H., Higaki, K., Taniguchi, M., Nanba, E., Ninomiya, H. *et al.* Increased NPC1 mRNA in skin fibroblasts from Niemann-Pick disease type C patients. *Brain Dev.* **26,** 245–250 (2004).
12 Iwaki, A., Muramoto, T., Iwaki, I., Furumi, H., Dario-deLeon, M.L., Tateishi, J. *et al.* A missense mutation in the proteolipid protein gene responsible for Pelizaeus-Merzbacher disease in a Japanese family. *Hum. Mol. Genet.* **2,** 19–22 (1993).
13 Cailloux, F., Gauthier-Barichard, F., Mimault, C., Isabelle, V., Courtois, V., Giraud, G. *et al.* Genotype-phenotype correlation in inherited brain myelination defects due to proteolipid protein gene mutations. Clinical European Network on Brain Dysmyelinating Disease. *Eur. J. Hum. Genet.* **8,** 837–845 (2000).
14 Dhaunchak, A.S. & Nave, K.A. A common mechanism of PLP/DM20 misfolding causes cysteine-mediated endoplasmic reticulum retention in oligodendrocytes and Pelizaeus-Merzbacher disease. *Proc. Natl Acad. Sci. USA* **104,** 17813–17818 (2007).
15 Lee, J.A., Inoue, K., Cheung, S.W., Shaw, C.A., Stankiewicz, P. & Lupski, J.R. Role of genomic architecture in PLP1 duplication causing Pelizaeus-Merzbacher disease. *Hum. Mol. Genet.* **15,** 2250–2265 (2006).
16 Woodward, K.J., Cundall, M., Sperle, K., Sistermans, E.A., Ross, M., Howell, G. *et al.* Heterogeneous duplications in patients with Pelizaeus-Merzbacher disease suggest a mechanism of coupled homologous and nonhomologous recombination. *Am. J. Hum. Genet.* **77,** 966–987 (2005).
17 Sistermans, E.A., de Wijs, I.J., de Coo, R.F., Smit, L.M., Menko, F.H. & van Oost, B.A. A (G-to-A) mutation in the initiation codon of the proteolipid protein gene causing a relatively mild form of Pelizaeus-Merzbacher disease in a Dutch family. *Hum. Genet.* **97,** 337–339 (1996).
18 Klugmann, M., Schwab, M.H., Puhlhofer, A., Schneider, A., Zimmermann, F., Griffiths, I.R. *et al.* Assembly of CNS myelin in the absence of proteolipid protein. *Neuron* **18,** 59–70 (1997).
19 Griffiths, I., Klugmann, M., Anderson, T., Yool, D., Thomson, C., Schwab, M.H. *et al.* Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* **280,** 1610–1613 (1998).
20 Garbern, J.Y., Yool, D.A., Moore, G.J., Wilds, I.B., Faulk, M.W., Klugmann, M. *et al.* Patients lacking the major CNS myelin protein, proteolipid protein 1, develop length-dependent axonal degeneration in the absence of demyelination and inflammation. *Brain* **125,** 551–561 (2002).
21 Mikesova, E., Barankova, L., Sakmaryova, I., Tatarkova, I. & Seeman, P. Quantitative multiplex real-time PCR for detection of PLP1 gene duplications in Pelizaeus-Merzbacher patients. *Genet. Test.* **10,** 215–220 (2006).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)

J. Spine Res. 3 : 1470-1474, 2012

原著 Original Article

肋骨異常を合併した先天性側弯症：成長期における自然経過の検討

Radiographic Analysis of Progression in Congenital Scoliosis (CS) with Rib Anomalies (RA) during Growth Period

川上紀明[*1] 辻 太一[*1] 柳田晴久[*2] 宇野耕吉[*3]
松本守雄[*4] 渡辺航太[*4] 山元拓哉[*5] 平野 徹[*6]
種市 洋[*7] 山崎 健[*8] 藤原憲太[*9]

Noriaki Kawakami[*1], Taichi Tsuji[*1], Haruhisa Yanagida[*2], Kouki Uno[*3], Morio Matsumoto[*4], Kota Watanabe[*4], Takuya Yamamoto[*5], Toru Hirano[*6], Hiroshi Taneichi[*7], Ken Yamazaki[*8], Kenta Hujiwara[*9]

要 旨

肋骨異常を合併した先天性側弯症の成長期における自然経過を明らかにする目的で70例の臨床データーを成長時期別に検討した．側弯は乳幼児期に最も悪化し，思春期がその次に続く結果であった．この悪化には肋骨変形が片側性，片側分節異常を伴う混合型の奇形椎，肋骨癒合/肋骨欠損などが存在する，広範囲な肋骨異常，などが大きく関与していた．この結果を加味して，手術時期を判断する目的で4段階の重症度分類を提唱した．

Abstract

Introduction : This study was aimed to evaluate the progression of various types of CS with RA during each of the growth periods, and to assess the severity of progression for strategic planning of expansion thoracoplasty (ET).

Material & Methods : 70 pts. (M-32 and F-38 with an average age of 2.6 years at the first visit.) from 13 institutions matched the inclusion criteria : CS with RA, no procedures that could influence the natural history, repeated plain X-ray check-ups at at least a 2-year interval during growth periods. The average F/U time was 5.4 years (2-14). X-ray images of 70 pts. were divided into 3 age groups, infantile (0-6), juvenile (5-11), and adolescent (11-18).

Results : 54 of 70 pts. had unilateral RA. The magnitude of scoliosis was 49.3° at the first visit and 65.7° at the final F/U. Scoliosis progressed most severely during infancy with the rate of 5.9°/y, followed by 3.9°/y during adolescence. Patients with rib defects or unilateral unsegmented bar showed higher progression rates during infancy. 4 grades in severity of progression (most severe.

[*1]国家公務員共済組合連合会名城病院整形外科脊椎脊髄センター〔〒460-0001 名古屋市中区三の丸1-3-1〕Department of Orthopedic Surgery, Meijo Hospital
[*2]福岡市立こども医療センター整形外科
[*3]独立医療法人神戸医療センター整形外科
[*4]慶應義塾大学医学部整形外科
[*5]鹿児島大学医学部整形外科
[*6]新潟大学医学部整形外科
[*7]獨協医科大学整形外科
[*8]岩手医科大学整形外科
[*9]大阪医科大学整形外科

severe, moderate, mild) were set up based on the relationship between SAL and scoliosis with cut-off values of 70%, 85% of SAL and 45°, 85° of scoliosis. Those grades were significantly related with types and location of RA and types of vertebral anomalies. [Conclusion] Scoliosis in CS with RA progressed most severely during infancy and was significantly related to the types and location of RA as well as the type of VA.

Key words：congenital scoliosis, congenital rib anomalies natural history

はじめに

幼少児期に高度に悪化する脊柱変形[1)2)]の中でも先天性に肋骨と脊柱の両方に生じる異常は胸郭不全症候群の代表的疾患であり，成長障害も加わり胸郭の三次元的変形と低形成，脊柱側弯や後弯・前弯などにより外見のみならず拘束性換気障害を来たし致死的となるものも認められる．しかし，Ramirez ら[3)]，Tsiricos ら[4)]の報告はあるものの，未だ本疾患群に対する自然経過の実態把握は不十分であり，どのような病態が胸郭不全症候群と言えるのかなど，悪化因子や治療成績に関係する様々な病態が十分解明されていない．また，成長に大きく影響されるため成長終了以前の早期に診断する診断基準が存在せず，治療方法に対するガイドラインも存在していない．平成21年以後，われわれは全国実態調査により自然経過や治療状況，悪化因子などを検討し，過去の治療の問題点と限界を検討してきた．その結果を2011年に本誌において肋骨異常を伴う先天性側弯症：その悪化因子として報告した[5)]．しかし，対象とした症例の年齢が様々であり，成長時期の影響が全く考慮されていないという問題点があった．

本研究の目的は先天性側弯症の中で肋骨異常のある疾患の自然経過，病態をさらに詳細に調査することにより本疾患のそれぞれの成長時期における悪化状態とそれらに影響を与える因子を解明し，重症度を明確にすることである．

対象と方法

臨床データーを retrospective に調査検討した．2010年に行った本疾患におけるアンケート調査に対して回答のあった施設から，複数回の診察により経過観察が行われていた症例を有する13施設から患者情報，X 線写真の提供を受け検討した．対象症例は，未成熟な患者，肋骨異常を伴う先天性側弯症，手術治療なしで最低２年間自然経過を観察できたものとし，医原性のものは除外した．

提供を受けた症例を以下のごとく３群に分けて検討した．

１）生下時から５～６歳まで経過を観察(乳幼児期)

２）５～６歳から10～11歳まで経過を観察(学童期)

３）11歳以後18歳未満で経過を観察(思春期)

それぞれの症例において初診時，経過観察時の X 線写真(全脊柱正面，側面，あれば3DCT)，呼吸状態，全身状態を評価し，X 線画像に対しては，肋骨・脊椎の変形と側弯(変形)の進行との関係を検討した．

結　果

13医療機関から提供された症例中から上記症例選択基準にマッチした70患者(男性32名，女性38名)を対象とした．これらの患者の初診時年齢は平均2.6±3.5歳(０～13.6歳)，経過観察期間は5.4±3.4年(２～14年)であった．初診時の側弯は46.9±26.0°であったが，最終観察時65.7±30.7°まで進行していた($p<0.001$)．

１．成長時期と側弯の悪化の検討

乳幼児期の症例では立位歩行が不可能な時期と立位可能になってからの X 線画像には重力の影響が大きく影響しているため，臥位での乳幼児期と立位可能な幼児期のサブグループに分けて検討した．その結果，年齢，症例数，側弯 Cobb 角はそ

表1　各年代別の悪化速度

	乳幼児期	幼児期	学童期	思春期
年齢	1.1±1.0 (0～3)	4.3±1.2 (2～7)	8.9±2.0 (6～13)	14.1±2.0 (11～19)
症例数	55	61	39	24
側弯 Cobb角	47.3±23.0	58.4±29.7	56.2±30.7	72.0±30.5
進行度	5.0±5.6	2.3±2.1	3.8±2.2	

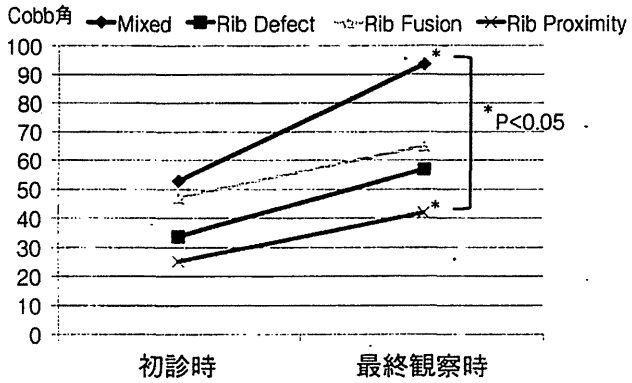

図2　側弯の大きさと肋骨奇形のタイプ
側弯は肋骨の異常が混合型，肋骨癒合，肋骨欠損，肋骨近接，の順で大きかった．特に混合型と近接には統計的有意差があった．

れぞれ乳幼児期で1.1±1.0歳，55例，47.3±23.0°，幼児期で4.3±1.2歳，61例，58.4±29.7°，学童期で8.9±2.0歳，39例，56.2±30.7°，思春期で14.1±2.0歳，24例，72.0±30.5°となっていた(表1)．進行度は乳幼児期から幼児期で5.0±5.6°/年，幼児期から学童期で2.3±2.1°/年，学童期から思春期で3.8±2.2°/年となっており，乳幼児期で最も悪化していた．特に臥位から立位への移行期における重力の影響を考慮した22例の検討では，初診時年齢0.2±0.3歳，側弯Cobb角52.8±24.6°が立位歩行開始後最初のX線撮影時それぞれ2.1±0.9歳，67.5±29.4°となっており，7.8±7.0°/年の最も大きい進行度を示していた．

2．肋骨奇形の部位・範囲と側弯角度

70例中54例は片側のみに肋骨異常があり，16例は両側性であった．片側性と両側性の初診時Cobb角と最終時Cobb角には有意差があったが，悪化度には有意差はなかった(図1)．肋骨異常のタイプは，52例が肋骨癒合で，単純X線写真では

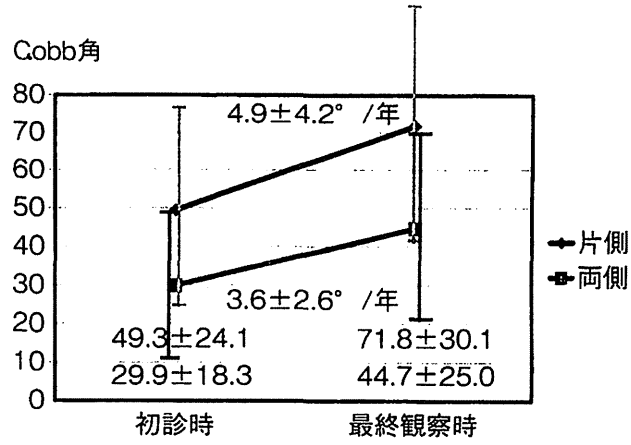

図1　側弯と肋骨異常のタイプ(片側性と両側性)
肋骨異常が片側性でも両側性でも経過観察期間内で側弯は悪化したが両者に統計的有意差はなかった．片側性の症例の方が側弯の角度は有意に高かった．

癒合が明確でなく近接していたもの8例，肋骨欠損4例であった．欠損と癒合の混合したものは6例認められた．側弯の大きさは混合型，癒合，欠損，近接の順で大きく，進行度では混合，欠損，癒合，近接の順であった(図2)．肋骨異常の部位は全体を3等分して上位，中位，下位に分けて検討した．全体の2/3以上にわたって異常を認めた症例は45例(全体-15，上位2/3-16，下位2/3-14)であり，肋骨異常が広範囲に存在する症例ほど側弯が大きかった．

3．片側のみに肋骨異常を伴う症例の検討(54例)

初診時年齢は平均2.6±3.6歳，側弯は49.3±24.1°であり，最終観察時では8.7±5.0歳．側弯は65.7±30.7°まで進行していた．側弯進行度は乳幼児期で5.9±7.6°/年，学童期で2.5±2.0°/年，思春期で3.9±2.3°/年となっており，乳幼児期での進行が大きかった(p=0.02)．肋骨異常のタイプと側弯の悪化には優位な関係は認めなかったが，肋骨欠損の有無での検討では有の症例では7.3±5.6°/年と明らかに無の症例の4.3±3.6°/年よりも進行性であった．特にその中でも乳幼児期の進行度には大きな差が認められた(有－10.7±1.8°/年，無－3.8±0.9°/年)．奇形椎のタイプと側弯進行度とは症例数にあまりに差があり有意な関係は認めなかったが，明らかに混合型の進行度が高かった．

表2　混合型における片側癒合の側弯悪化に対する影響

	側弯悪化率	初診時 Cobb 角	最終観察時 Cobb 角
UUB(+)	7.2±5.1	58.5±21.8	88.8±21.6
UUB(−)	3.2±2.6	46.4±23.1	61.0±31.6
	p=0.0032	p=0.0864	P=0.0017

表3　分節異常における片側癒合の側弯悪化に対する影響

	側弯悪化率	初診時 Cobb 角	最終観察時 Cobb 角
UUB(+)	3.6±2.8	44.1±24.9	64.8±27.1
UUB(−)	2.6±2.8	10.5±3.5	34.0±31.1
	p=0.6331	p=0.1008	P=0.1876

表4　肋骨異常の範囲と側弯の悪化

肋骨癒合の範囲	症例数	悪化率(°/年)
ほぼ全範囲	11	7.5±6.0*
2/3前後	20	5.1±6.4
1/3前後	23	3.5±3.2*

All pairs Tukey-Kramer 0.05
*p=0.0306

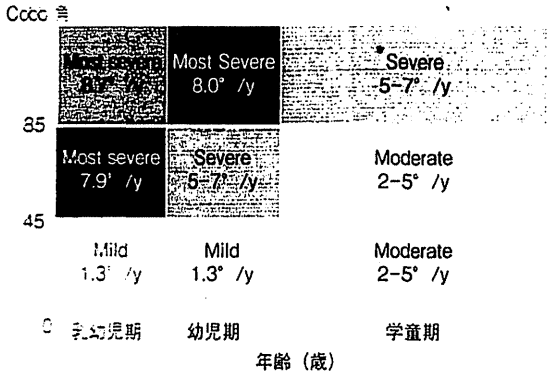

図4　成長期における側弯の悪化
成長時期における Cobb 角の大きさと悪化率の関係では，より幼少児期で側弯が高度であればあるほど側弯は高度に悪化していた．

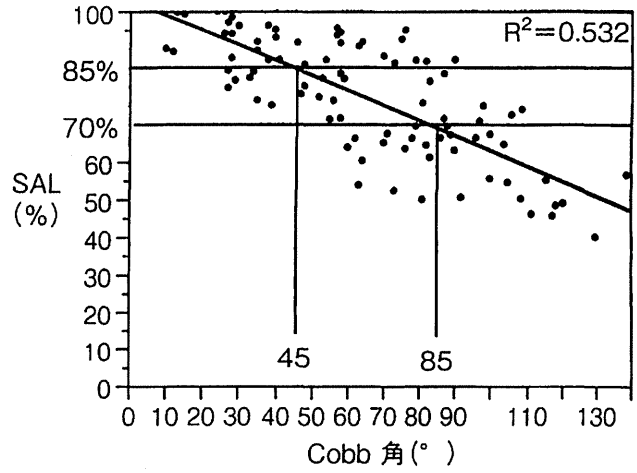

図3　側弯の角度と SAL
70例における SAL と側弯角度の関係は負の相関関係にあり(R^2=0.532)，SAL の85%，70%がそれぞれ側弯角度の45度，85度に一致していた．

特に，片側癒合(unilateral unsegmented bar，以下 UUB)の有無でその進行度を評価すると，有の症例では6.2±0.7°/年と明らかに無の症例の3.2±0.8°/年よりも大きく進行していた．UUB は混合型と分節異常の両方に認められたが，混合型で UUB が合併した症例の方が分節異常における UUB よりも優位に進行度が大きかった(表2，3)．肋骨異常の範囲と側弯の進行の関係では，全範囲，2/3以上に肋骨異常を伴った症例では明らかにそれ以下よりも高度に悪化していた(表4)

4．両側肋骨癒合の症例における検討

初診時年齢は平均2.9±3.0歳，側弯は29.9±18.3°であり，最終観察時では6.8±3.8歳，側弯は44.7±21.5°まで進行していた．側弯進行度は乳幼児期で3.7±2.9°/年，学童期で2.2±2.1°/年，思春期で3.9±2.1°/年となっており，統計学的有意差は認められなかった．

5．側弯 Cobb 角と SAL，THR の関係

胸郭の左右対称性を表す SAL(Space Available for Lung)は経過観察期間中大きな変化はなく，胸椎から腰椎までの高さと胸椎の高さとの比 THR (Thoracic Height Ratio)にも有意差は存在しなかった．しかし，SAL と側弯の大きさとの相関関係は r^2=0.532と有意に負の相関関係を示し，側弯が45°で SAL は85%，側弯が85°で SAL は70%となり，胸郭変形と側弯の両方の観点から手術適応を検討する上で大変重要な境界値になると考えられた(図3)．側弯の大きさと各年代に分けて側弯の悪化率を整理すると図4のようになり，乳幼児期で側弯角度が85度以上となった症例で最も悪化速

度が大きかった.

6．併存疾患

Klippel-Feil 症候群が12例と最多で，以下，先天性脊髄異常11例，先天性心疾患８例，鎖肛４例，先天性腎奇形２例，等であった.

考　察

昨年度の結果では，全ての患者で側弯が進行しているわけではないこと，初診時の側弯角度や肋骨奇形の laterality，部位，脊椎奇形のタイプにより差が生じていることが概略で判明した[5]．しかし，この解析では成長時期との関わりから進行度を評価しておらず，いつどの時期に手術治療を行うべきか，どのような症例に早期手術を行うべきか，どのような症例は待期的にできるのか，などの判定には全く役立たなかった.

今回の結果では側弯の進行が成長時期により差があることがわかり，特に乳幼児期における進行度は最も大きく，それに肋骨異常のタイプや脊椎奇形のタイプが悪化因子として影響を与えていた．この結果は治療時期の決定に大変重要な情報となり，このような観点から本疾患にはやはり重症度を測る目安が必要であると考えられた．そこで，SAL と側弯の角度の相関解析から得られた回帰直線をもとに，側弯を45°と85°で区切りこれに年齢を加味して下記のように軽度，中等度，高度，超高度の４段階に分けた重症度分類を提唱した.

１．軽度(mild)：９～10歳以前の時点で45°未満までにしか悪化しない(２°/年以下)

２．中等度(moderate)：９～10歳以前の時点で45°以上，85°未満の間までの悪化にとどまる(２-５°/年)

３．高度(severe)：５～６歳までに85°以上に悪化する(５-７°/年)

４．超高度(most severe)：２～３歳までに85°以上に悪化する(８°/年以上)

この重症度分類に，さらに側弯の悪化を引き起こす悪化因子として昨年度と今年度の解析から得られた種々の因子(脊椎奇形のタイプ，片側癒合の有無，肋骨異常のタイプ，国庫椎弓欠損の有無，肋骨異常の範囲や部位など)を加味することで，いつ頃手術を行うべきかなどの治療計画を立てやすくなることが期待される．しかし，この重症度分類の臨床的有用性については今後の検討課題である．また，本研究の解析には胸郭不全症候群の呼吸機能のデーターが加えられておらず，診断基準策定においては呼吸機能や ADL からみた研究も行う必要がある.

結　語

肋骨異常を合併した先天性側弯症に伴う胸郭不全症候群の病態，実態を把握するため協力施設から得られた70例の臨床データを成長時期別に検討した．側弯は乳幼児期に最も悪化し，思春期がその次に続く結果であった．この悪化には片側性の肋骨変形，混合型の奇形椎，特に UUB を伴う，肋骨癒合・肋骨欠損が存在する，広範囲な肋骨異常，などが大きく関与していた．この結果を加味して，手術時期を判断する目的で４段階の重症度分類を提唱した.

謝　辞

本研究においては多くの施設の医師の方々から症例の提供と協力を頂いたことを心から感謝します．特に共同演者以外で順天堂大学米澤郁穂先生，滋賀小児医療センター太田英吾先生，神奈川こども医療センター上杉昌章先生，には症例についての協力を賜り深く感謝します.

文　献

1）Campbell RM, Smith MD：Thoracic Insufficiency Syndrome and Exotic Scoliosis. J Bone Joint Surg. 2007；89-A Supplement 1：108-122
2）Campbell RM：Spine Deformities in Rare Congenital Syndromes：Clinical Issues. Spine. 2009；34：1815-1827
3）Ramirez N, Cornier AS, Campbell RM, et al：Natural History of Thoracic Insufficiency Syndrome：A Spondylothoracic Dysplasia Perspective. J Bone & Joint Surg. 2007；89-A：2663-2675
4）Tsirikos AL, McMaster MJ：Congenital Anomalies of the Ribs and Chest Wall Associated with Congenital deformities of the Spine. J Bone & Joint Surg. 2005；87-A：2523-2536
5）川上紀明，辻　太一，他：肋骨異常を伴う先天性側弯症の自然経過．J Spine Res. 2011；2：1750-1754

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Pure Duplication of 19p13.3

Aki Ishikawa,[1] Keisuke Enomoto,[1] Makiko Tominaga,[1] Toshiyuki Saito,[2] Jun-ichi Nagai,[2] Noritaka Furuya,[1] Kentaro Ueno,[3] Hideaki Ueda,[3] Mitsuo Masuno,[4] and Kenji Kurosawa[1,5]*

[1]Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan

[2]Department of Clinical Laboratory, Kanagawa Children's Medical Center, Yokohama, Japan

[3]Department of Pediatric Cardiology, Kanagawa Children's Medical Center, Yokohama, Japan

[4]Genetic Counseling Program, Graduate School of Health and Welfare, Kawasaki University of Medical Welfare, Kurashiki, Japan

[5]Institute for Clinical Research, Kanagawa Children's Medical Center, Yokohama, Japan

Manuscript Received: 10 June 2012; Manuscript Accepted: 15 April 2013

Chromosomal abnormalities involving 19p13.3 have rarely been described in the published literature. Here, we report on a girl with a pure terminal duplication of 6.1 Mb on 19p13.3, caused by an unbalanced translocation der(19)t(10;19)(qter;p13.3)dn. Her phenotype included severe psychomotor developmental delay, skeletal malformations, and a distinctive facial appearance, similar to that of a patient previously reported by Lybaek et al. [Lybaek et al. (2009); Eur J Hum Genet 17:904–910]. These results suggest that a duplication of >3 Mb at the terminus of 19p13.3 might represent a distinct chromosomal syndrome.

Key words: 19p13.3 duplication; array CGH; developmental delay; subtelomere

How to Cite this Article:
Ishikawa A, Enomoto K, Tominaga M, Saito T, Nagai J, Furuya N, Ueno K, Ueda H, Masuno M, Kurosawa K. 2013. Pure duplication of 19p13.3.
Am J Med Genet Part A 161A:2300–2304.

INTRODUCTION

Chromosome 19 is more gene-dense than any other human chromosome. Non-mosaic 19p trisomy is a rare chromosomal aberration, of which only 9 occurrences have been reported to date [Byrne et al., 1980; Salbert et al., 1992; Stratton et al., 1995; Andries et al., 2002; Puvabanditsin et al., 2009; Lybaek et al., 2009; Descartes et al., 2011; Siggberg et al., 2011; Lehman et al., 2012]. More specifically, pure and non-mosaic trisomy of 19p has been reported in only five of these patients [Stratton et al., 1995; Andries et al., 2002; Lybaek et al., 2009; Siggberg et al., 2011; Lehman et al., 2012].

Here, we report on a 3-year-old girl with pure terminal duplication of 19p13.3, confirmed using FISH and array CGH. She had multiple malformations, including a complex congenital heart defect, a distinctive facial appearance, and severe developmental delay. Taken together, our findings, along with a review of the literature, allow clarification of a more precise and comprehensive phenotype–genotype correlation for pure 19p duplication.

CLINICAL REPORT

The proposita is the first child of healthy unrelated parents with unremarkable family history. At the time of delivery, the mother was 36 years old, and the father was 27 years old. The pregnancy was complicated by intrauterine growth retardation first noted at 27 weeks. The infant was delivered at 35 weeks of gestation by cesarean due to fetal distress. Her birth weight was 1,216 g; length, 36.5 cm; and occipitofrontal circumference (OFC), 28.0 cm. Her Apgar scores were 4 at 1 min, and 9 at 5 min. Because of her very low birth weight and respiratory failure, she was admitted to a neonatal intensive care unit. Initial physical examination showed a distinctive facial appearance with micrognathia, low-set ears, and a prominent occiput. An echocardiogram revealed a complete atrioventricular septal defect of Rastelli A type, severe pulmonary hypertension, and mitral valve dysplasia.

At the age of 8 months, catheter examination demonstrated that an operative procedure was not indicated for her heart defects; conservative treatment with beraprost sodium and bosentan hydrate, in addition to oxygen supplementation, was adopted for heart failure and severe pulmonary hypertension. From the age of 1 year and 8 months, sildenafil citrate was also added to her treatment. At this age, she had marked cardiac failure and had experienced several episodes of recurrent respiratory infection.

Grant sponsor: Research on Measures for Intractable Diseases Project, The Ministry of Health, Labour and Welfare, Japan.
*Correspondence to:
Kenji Kurosawa, M.D., Ph.D., Division of Medical Genetics, Kanagawa Children's Medical Center, 2-138-4 Mutsukawa, Minami-ku, Yokohama 232-8555, Japan. E-mail: kkurosawa@kcmc.jp
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 29 July 2013
DOI 10.1002/ajmg.a.36041

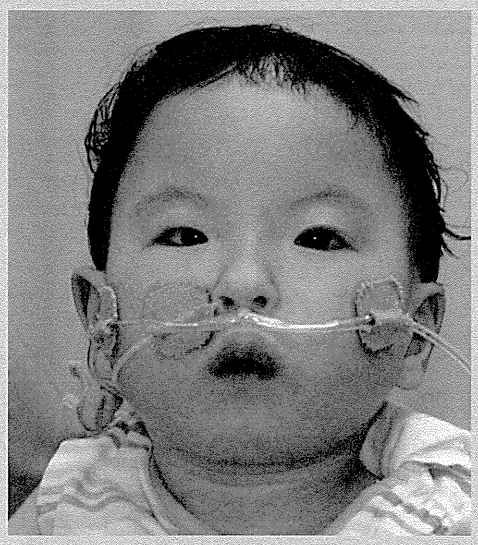

FIG. 1. Patient at the age of 3 years, showing strabismus, short palpebral fissures, hypoplastic nasal alae, low-set ears, and microstomia.

On examination at the age of 3 years, her weight was 7,680 g (−3.4 SD); height, 75 cm (−5.0 SD); and OFC, 42 cm (−4.0 SD) (Fig. 1). Her facial appearance showed strabismus, short and downslanting palpebral fissures, microcephaly, hypoplastic nasal alae, sparse scalp hair, and eyebrows, low-set ears, a short philtrum, protruding upper lip, and microstomia with micrognathia. Orthopedic examination showed kyphoscoliosis and dislocation of bilateral hip joints. Her development was severely delayed. She could roll over and required gavage feeding. Her heart failure progressed, and died at age 4 years. Postmortem examination revealed an ectopic left kidney in front of the vertebrae.

MATERIALS AND METHODS

Written informed consent was obtained from the parents of the patient, and the study was performed in accordance with the Kanagawa Children's Medical Center Review Board and Ethics Committee.

An initial FISH analysis for patients with developmental delay/intellectual disability (DD/ID) and/or multiple congenital anomalies (MCA) was carried out using subtelomeric probes (Vysis, Downers Grove, IL) according to the standard protocol. Further FISH analysis for determining the breakpoint on 19p13.3 was carried out using bacterial artificial chromosome (BAC) clones that had been selected from the May 2004 (NCBI35/hg17). Human assembly of the UCSC Genome Browser (http://genome.ucsc.edu/). A centromeric probe specific for chromosome 10 was used to confirm chromosome 10. The BAC clones were labeled by nick translation according to the manufacturer's instructions (Vysis, Downers Grove, IL). Hybridization, post-hybridization washing, and counterstaining were performed according to standard procedures. Slides were analyzed using a completely motorized epifluorescence microscope (Leica DMRXA2; Leica Microsystems Imaging Solutions, Cambridge, UK) equipped with a CCD camera. Both the camera and microscope were controlled with Leica CW4000 M-FISH software [Yamamoto et al., 2009].

Array comparative genomic hybridization (array-CGH) was performed using the Agilent SurePrint G3 Human CGH Microarray Kit 8 × 60K (Agilent Technologies, Inc., Santa Clara, CA). The total genomic DNA of the patient was prepared using standard techniques. The results were analyzed using Agilent Genomic Workbench software. Only experiments having a derivative log ratio (DLR) spread value <0.30 were considered.

RESULTS

The complete subtelomere probe set analysis detected an additional signal for 19pter on the terminal of the long arm in group C chromosomes in the patient. Based on the results of the G-banding patterns and FISH with a centromeric probe, the derivative chromosome was determined to be chromosome 10 (Fig. 2a,b). However, the 10qter probe signal was retained in the derivative chromosome (data not shown). To characterize the size of the deletion, we further applied FISH analysis using the BAC clones that mapped to the region. This revealed that the breakpoint was 6.1 Mb from 19pter (Table I). Subsequent array-CGH analysis revealed a 19p13.3 duplication of approximately 6.1 Mb (chr19: 327,273–6,106,229), which was consistent with the FISH results (Fig. 2c). No other genomic imbalances were identified on the array analysis. FISH analysis with relevant BAC clones indicated that the duplication was absent in both parents, and therefore had occurred de novo.

DISCUSSION

Reports of abnormalities of the short arm chromosome 19 are rare; to date, only nine patients with non-mosaic duplication of 19p have been reported. Of these, four involved translocation of other chromosomes [Byrne et al., 1980; Salbert et al., 1992; Puvabanditsin et al., 2009; Descartes et al., 2011], and only five patients had a pure partial duplication of 19p [Stratton et al., 1995; Andries et al., 2002; Lybaek et al., 2009; Siggberg et al., 2011; Lehman et al., 2012] (Fig. 3., Table II). This report is, to our knowledge, only the second report of a pure terminal duplication of 19p13.3.

Array-CGH and FISH analysis refined the breakpoint at 6.1 Mb from 19pter. Three patients harboring a duplication of more than 1 Mb at 19p13.3 have been recorded on the DECIPHER database (https://decipher.sanger.ac.uk/), but no individual with a duplication of more than 3 Mb is recorded therein. Fourteen patients having a duplication of a fragment of 19p13.3 have been reported in the database of International Standards for Cytogenetic Arrays Consortium (ISCA). The phenotypical manifestations of these patients consist of multiple congenital abnormalities and seizures. However, the detailed phenotypic features of the patients were not available. Although the phenotype deriving from duplication of a limited region of 19pter is not always recognizable [Andries

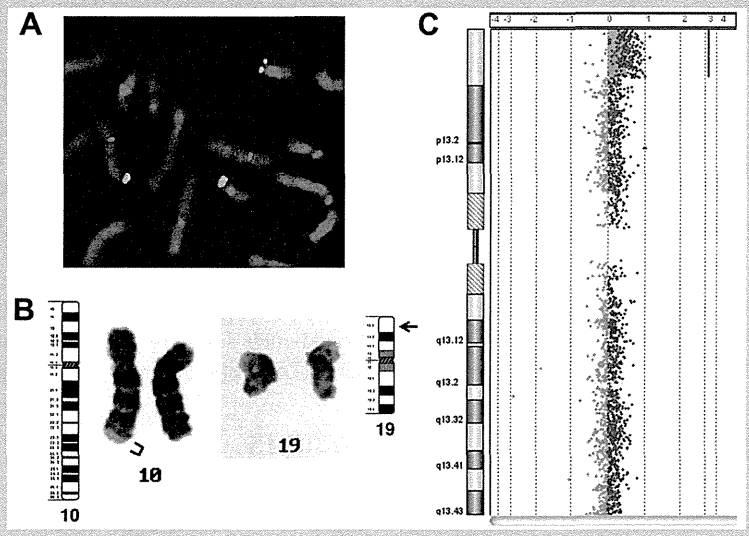

FIG. 2. FISH and array-CGH characterization of 19p13.3 terminal duplication. A: FISH image showing an additional signal at 10qter. BAC probe RP11-43H17 from the duplicated region of 19p13.3 is labeled in green, and chromosome 10 centromeric probe (Vysis, CEP10) is labeled in red, as a control. B: G-banded metaphase chromosomes, showing der(10)t(10;19)(qter;p13.3). C: Array-CGH showing duplication of 19p13.3. The region extends to position 6,106,229 according to UCSC human genome assembly build 19.

et al., 2002], the present case presented with severe psychomotor disability, no verbal language use, a distinctive facial appearance, and skeletal features including small hands and feet and bilateral hip dysplasia. These phenotypic features, especially the characteristic facial appearance, were also shared by the patient described by Lybaek et al. [2009]. The patient had a small mouth, short philtrum, full cheek, short palpebral fissures, and the extreme precocious puberty before the age of 5 months. They demonstrated that only about 25% of the duplicated 215 genes presented the overall expression pattern by more than 1.3-fold, and suggested no genes might explain the precocious puberty characterized in their patient. However, our present patient had no symptom of early puberty observed by the age of 4 years.

TABLE I. FISH Results Around the Breakpoint of the Translocation

BAC clones	Position from 19pter[a]	FISH results
RP11-1051P16	3,421,215–3,617,048	×3
RP11-43H17	4,318,718–4,491,568	×3
RP11-348B12	4,960,407–5,144,570	×3
RP11-294F21	5,854,144–6,041,711	×3
RP11-576B17	6,172,183–6,249,454	×3
RP11-114A7	6,199,888–6,359,433	×2
RP11-30F17	6,351,112–6,519,252	×2
RP11-459P1	6,396,557–6,544,479	×2
RP11-526C20	6,450,800–6,626,432	×2
RP11-222E10	6,560,463–6,759,394	×2
RP11-441C15	7,891,868–8,086,997	×2

[a]Positions of the BAC clones were based on the May 2004 (NCBI35/hg17) Human Assembly of the UCSC Genome Browser (http://genome.ucsc.edu/).

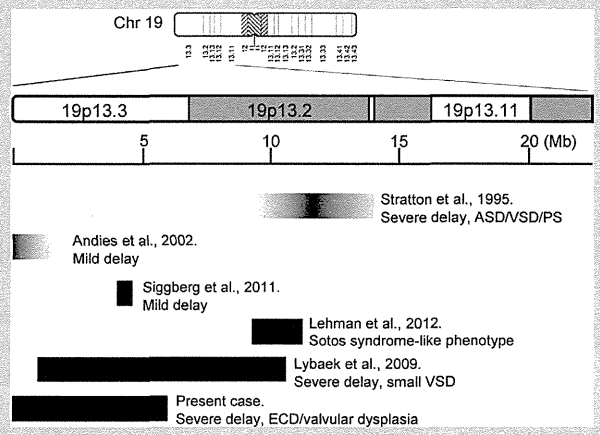

FIG. 3. Schematic representation of the microduplication on 19p13.3. The dark horizontal bars indicate the range of the duplication in reported patients. The duplicated regions in the patients reported by Stratton et al. [1995] and Andries et al. [2002] were ascertained from the respective reports.

TABLE II. Summary of Clinical Features in Five Individuals With Pure Microduplication at 19p13

	Stratton et al. [1995]	Andries et al. [2002]	Siggberg et al. [2011]	Lehman et al. [2012]	Lybaek et al. [2009]	Present patient
Age, sex	9 months, F	20 months, F	9 yrs, M	1–74 yrs, M/F	2 ½ yrs, F	3yrs, F
Karyotype	dup(19) (p13.2p13.13)	der(14)t (14;19) (q32.3;p13.3)	dup(19) (p13.3p13.3)	dup(19) (p13.2p13.2)	ins(19) (q13.3p13.2-p13.3)	der(10)t (10;19) (qter;p13.3)
Duplication	p13.2–p13.13	pter-p13.3	p13.3, 0.81 Mb	p13.2. 1.9 Mb	p13.3–p13.2, 8.9 Mb	p13.3, 6.10 Mb
(from pter)	?	?	(3.927–4.471 Mb)	(9.109–11.068 Mb)	(1.4–10.3 Mb)	(–6.106 Mb)
Pattern	Interstitial	Terminal	Interstitial	Interstitial	Interstitial	Terminal
Gestational age	Term	41 wks	Term	Term	35 wks	35 wks
Birth weight	2,730 g		2,730 g	4,550 g	1,790 g	1,216 g
Growth retardation	+	–	+	–	+	+ Severe
Development	Delay	Mild delay	Mild delay	Mild delay	Severe delay	Severe delay
Cardiovascular	PS, ASD, VSD	–	–	–	small VSD	ECD, PH, valvular dysplasia
Others	Strabismus, nail hypoplasia	Sparse hair, low-set ears, short nose	Amblyopia	Sotos syndrome-like	Severe eating problem, congenital hip dysplasia	Strabismus, renal aplasia (L), vertebral defects, nail hypoplasia, dislocation of hip joint

Thus, a duplication of >3 Mb of the terminal region of 19p13.3 might contribute to a more severe phenotype than do smaller duplications, and this phenotype might be characteristic of this chromosomal aberration.

Accurate assessment of the duplication size enabled us to evaluate the genes located within the duplicated region, which presumably contribute to the phenotypes. The duplicated region contains approximately 150 RefSeq genes and 130 OMIM genes, 18 of which have known disease associations. However, this case demonstrates that evaluation of the gene content of a chromosomal region is not sufficient to assess the pathogenicity of a gene duplication. Additional reports of individuals with this chromosomal aberration are required to demonstrate genotype–phenotype correlation in 19p duplication.

ACKNOWLEDGMENTS

We thank the patient and her family for making this study possible. The authors thank Dr. Yoshikazu Kuroki (Kanagawa Children's Medical Center, Yokohama, Japan) for his valuable comments. This research was supported in part by a "Research on Measures for Intractable Diseases" project: Matching funds subsidy from the Ministry of Health, Labor and Welfare, Japan.

REFERENCES

Andries SA, Sartenaer D, Rack K, Rombout S, Tuerlinckx D, Gillerot Y, van Maldergem L. 2002. Pure terminal duplication of the short arm of chromosome 19 in a boy with mild microcephaly. J Med Genet 39:e60.

Byrne JLB, Korn GA, Dev VG, Bunch GM, Brooks K, Friedman JM, Harrod MJE. 1980. Partial trisomy 19p. Am J Hum Genet 32:64A.

Descartes M, Mikhail FM, Franklin JC, McGrath TM, Bebin M. 2011. Monosomy 1p36.3 and trisomy 19p13.3 in a child with periventricular nodular heterotopias. Pediatr Neurol 45:274–278.

Lehman AM, du Souich C, Chai D, Eydoux P, Huang JL, Fok AK, Avila L, Swingland J, Delaney AD, McGillivray B, Goldowitz D, Argiropoulos B, Kobor MS, Boerkoel CF. 2012. 19p13.2 microduplication causes a Sotos syndrome-like phenotype and alters gene expression. Clin Genet 81: 56–63.

Lybaek H, Orstavik KH, Prescott T, Hovland R, Breilid H, Stansberg C, Steen VM, Houge G. 2009. An 8.9 Mb 19p13 duplication associated with precocious puberty and a sporadic 3.9Mn 2q23.2q24.1 deletion containing *NR4A2* in mentally retarded members of a family with an intrachromosomal 19p-into-19q between-arm insertion. Eur J Hum Genet 17:904–910.

Puvabanditsin S, Garrow E, Brandsma E, Savla J, Kunjumon B, Gadi I. 2009. Partial trisomy 19p13.3 and partial monosomy 1p36.3: Clinical report and a literature review. Am J Med Genet Part A 149A:1782–1785.

Salbert BA, Solomon M, Spence JE, Jackson-Cook C, Brown J, Bodurtha J. 1992. Partial trisomy 19p: Case report and natural history. Clin Genet 41:143–146.

Siggberg L, Olsen P, Kanto-Salonen K, Knuutila S. 2011. 19p13.3 aberrations are associated with dysmorphic features and deviant psychomotor development. Cytogenet Genome Res 132:8–15.

Stratton RF, DuPont BR, Olsen AS, Fertitta A, Hoyer M, Moore CM. 1995. Interstitial duplication 19p. Am J Med Genet 57:562–564.

Yamamoto K, Yoshihashi H, Furuya N, Adachi M, Ito S, Tanaka Y, Masuno M, Chiyo H, Kurosawa K. 2009. Further delineation of 9q22 deletion syndrome associated with basal cell nevus (Gorlin) syndrome: Report of two cases and review of the literature. Congenit Anom (Kyoto) 49:8–14.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Surgical Intervention for Esophageal Atresia in Patients With Trisomy 18

Eriko Nishi,[1,2] Shigeru Takamizawa,[3] Kenji Iio,[4] Yasumasa Yamada,[5] Katsumi Yoshizawa,[3] Tomoko Hatata,[3] Takehiko Hiroma,[6] Seiji Mizuno,[7] Hiroshi Kawame,[8] Yoshimitsu Fukushima,[2] Tomohiko Nakamura,[6] and Tomoki Kosho[1,2]*

[1]Division of Medical Genetics, Nagano Children's Hospital, Azumino, Japan

[2]Department of Medical Genetics, Shinshu University Graduate School of Medicine, Matsumoto, Japan

[3]Department of Surgery, Nagano Children's Hospital, Azumino, Japan

[4]Department of Surgery, Central Hospital, Aichi Human Service Center, Kasugai, Japan

[5]Department of Neonatology, Central Hospital, Aichi Human Service Center, Kasugai, Japan

[6]Division of Neonatology, Nagano Children's Hospital, Azumino, Japan

[7]Department of Pediatrics, Central Hospital, Aichi Human Service Center, Kasugai, Japan

[8]Department of Genetic Counseling, Graduate School of Humanities and Sciences, Ochanomizu University, Tokyo, Japan

Manuscript Received: 5 April 2013; Manuscript Accepted: 16 September 2013

Trisomy 18 is a common chromosomal aberration syndrome involving growth impairment, various malformations, poor prognosis, and severe developmental delay in survivors. Although esophageal atresia (EA) with tracheoesophageal fistula (TEF) is a potentially fatal complication that can only be rescued through surgical correction, no reports have addressed the efficacy of surgical intervention for EA in patients with trisomy 18. We reviewed detailed clinical information of 24 patients with trisomy 18 and EA who were admitted to two neonatal intensive care units in Japan and underwent intensive treatment including surgical interventions from 1982 to 2009. Nine patients underwent only palliative surgery, including six who underwent only gastrostomy or both gastrostomy and jejunostomy (Group 1) and three who underwent gastrostomy and TEF division (Group 2). The other 15 patients underwent radical surgery, including 10 who underwent single-stage esophago-esophagostomy with TEF division (Group 3) and five who underwent two-stage operation (gastrostomy followed by esophago-esophagostomy with TEF division) (Group 4). No intraoperative death or anesthetic complications were noted. Enteral feeding was accomplished in 17 patients, three of whom were fed orally. Three patients could be discharged home. The 1-year survival rate was 17%: 27% in those receiving radical surgery (Groups 3 and 4); 0% in those receiving palliative surgery (Groups 1 and 2). Most causes of death were related to cardiac complications. EA is not an absolute poor prognostic factor in patients with trisomy 18 undergoing radical surgery for EA and intensive cardiac management.

Key words: trisomy 18; esophageal atresia; surgical intervention; neonatal intensive care; survival; causes of death

How to Cite this Article:
Nishi E, Takamizawa S, Iio K, Yamada Y, Yoshizawa K, Hatata T, Hiroma T, Mizuno S, Kawame H, Fukushima Y, Nakamura T, Kosho T. 2014. Surgical intervention for esophageal atresia in patients with trisomy 18.
Am J Med Genet Part A 164A:324–330.

INTRODUCTION

Trisomy 18, first described by Edwards et al. [1960], is a common chromosomal aberration syndrome. Patients with the syndrome have prenatal-onset severe growth impairment, characteristic craniofacial features, various visceral and skeletal malformations, and a reduced lifespan; survivors have severe developmental delay [Carey, 2010]. The largest and most cited population-based study

The authors have no conflict of interest to declare.
Grant sponsor: Research on Intractable Diseases, Ministry of Health, Labour and Welfare, Japan (T. Kosho); Grant sponsor: Shinshu Public Utility Foundation for Promotion of Medical Sciences, Japan (E. Nishi); Grant sponsor: Nagano Children's Hospital Research Foundation (E. Nishi).
*Correspondence to:
Tomoki Kosho, Department of Medical Genetics, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan.
E-mail: ktomoki@shinshu-u.ac.jp
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 5 December 2013
DOI 10.1002/ajmg.a.36294

[Rasmussen et al., 2003] showed a 1-year survival rate of 5–8% and median survival time of 10–14.5 days. The major causes of death were reportedly apnea and withdrawal of treatment, and the presence of a congenital heart defect was not reported to be associated with early death [Embleton et al., 1996; Rasmussen et al., 2003].

Esophageal atresia (EA) with/without tracheoesophageal fistula (TEF) is a common esophageal malformation that occurs in between 1 in 3000–4000 live births. Currently, the best treatment option for EA with TEF in patients with no other severe malformations is primary single-staged correction comprising esophago-esophagostomy and TEF division. For patients with unstable respiratory and/or cardiovascular conditions, however, the procedure should be performed in steps [Pinheiro et al., 2012]. There have been three classification systems of preoperative risks regarding EA: the Waterston classification based on birth weight, associated anomalies, and pneumonia [Waterston et al., 1962]; the Montreal classification based on mechanical ventilation and associated congenital anomalies [Poenaru et al., 1993]; and the Spitz classification based on birth weight and cardiac anomalies [Spitz et al., 1994]. A recent report by Sugio et al. [2006] showed that birth weight might no longer be a risk factor. Patients with EA were reported to have other abnormalities: cardiovascular complications (23%), musculoskeletal malformations (18%), and chromosomal aberrations (5.5%). Patients with life-threatening anomalies, including Potter syndrome, cerebral hypoplasia, and chromosomal abnormalities such as trisomy 13 or 18, as well as infants with totally uncorrectable major cardiac defects or grade IV intraventricular hemorrhage, were recommended to undergo nonoperative management [Pinheiro et al., 2012]. The accurate frequency of EA in trisomy 18 has not been determined by systematic investigation, and only an institution-based study from Japan demonstrated that a total of 33% (8/24) patients with trisomy 18 had EA, representing the most common non-cardiac visceral malformation [Kosho et al., 2006]. Although EA with TEF is a potentially fatal complication that can only be rescued through surgical correction, no reports have addressed the efficacy of surgical intervention for EA in patients with trisomy 18.

We herein describe the detailed clinical information of patients with trisomy 18 and EA who were admitted to two Japanese institutions that provided intensive treatment including surgical correction for EA in these patients.

MATERIALS AND METHODS

Patients

Patient data were collected from two institutions in Japan. Nagano Children's Hospital (NCH), established in 1993, is a tertiary hospital for sick children in Nagano Prefecture, which reports roughly 20,000 births per year. Since the obstetric department was established in 2000, pregnant women whose fetuses were found to have severe abnormalities by ultrasonography have also been referred for further evaluation, genetic counseling, and delivery. In the neonatal intensive care unit of this hospital, patients with this syndrome have been managed under the principle of providing intensive treatment based on careful discussion with the parents. The management comprises resuscitation including intratracheal intubation, appropriate respiratory support, establishment of enteral nutrition including corrective and palliative surgery for gastrointestinal malformation, and pharmacological treatment for congenital heart defects. This management was demonstrated to improve survival, with a 1-year survival rate of 25% and median survival time of 152.5 days. The common underlying factors associated with death were congenital heart defects and heart failure (96%) followed by pulmonary hypertension (78%), and the common final modes of death were sudden cardiac or cardiopulmonary arrest (26%) and progressive pulmonary hypertension-related events (26%) [Kosho et al., 2006]. The surgical strategy for EA in patients with trisomy 18 has been to perform gastrostomy soon after birth, followed by a second surgery after stabilization of the general condition (esophago-esophagostomy and TEF division from 1993 to 2003; TEF division from 2003).

The Central Hospital of Aichi Human Service Center (CHAHSC), established in 1970, is a tertiary hospital for sick children and handicapped children/adults covering the northern part of Aichi prefecture and the southern part of Gifu prefecture, which report roughly 70,000 births per year. The management principle of this hospital has been to perform intensive treatment including surgery for every patient, whether he/she has a severe disorder and/or handicap, if he/she needs the treatment or surgery for longer survival and better quality of life. The surgical strategy for EA in patients with trisomy 18 has been to perform esophago-esophagostomy with TEF division as a one-stage operation, whereas a two-stage operation comprising gastrostomy and jejunostomy followed by esophago-esophagostomy was planned in the early period.

A total of 27 patients with karyotypically confirmed full trisomy 18 and EA were admitted to the neonatal intensive care units of NCH from April 1993 to March 2008 and CHAHSC from April 1982 to March 2009. Two patients with A-type EA and one patient who died of uncontrollable respiratory failure before surgery were excluded. The other 24 patients (9 boys, 15 girls; Patients 1, 3, 5, 6, 7, 9, 20–24 from NCH, Patients 2, 4, 8, 10–19 from CHAHSC) with C-type EA who underwent surgery were included in this study (Table I).

Methods

From the medical records of NCH and CHAHSC, we collected detailed clinical data about the surgical methods and courses of EA in the 24 patients including eight who were described in our previous study [Kosho et al., 2006]. In addition, their perinatal conditions and interventions, other medical complications and treatments, and prognosis including survival and discharge were reviewed. We classified the patients into four groups (Table I): Group 1 (Patients 1–6) underwent gastrostomy with/without jejunostomy; Group 2 (Patients 7–9) underwent gastrostomy and TEF division; Group 3 (Patients 10–19) underwent esophago-esophagostomy with TEF division as one operation; and Group 4 (Patients 20–24) underwent gastrostomy followed by esophago-esophagostomy with TEF division.

TABLE I. Clinical Information of Patients With Trisomy 18 Undergoing Surgery for Esophageal Atresia

		Perinatal conditions							Complications				Intervention				Prognosis				
													Surgery for esophageal atresia		Crdiovascular	Respiratory					
Patient	Sex	Gestational age (weeks/days)	Birth weight (g)	Apgar score (1/5 min)	Prenatal diagnosis by amniocentesis	Polyhydramnios	Cesarean section	Resuscitation by intubation	Congenital heart defects	Respiratory complications	Gastrointestinal complications	Urogenital system, Seizure	Methods (age [days] at surgery)	Complications	Cardiac intervention	IMV/extubation (day) or TS	Enteral/oral feeding	Discharge (days)	Survival (days)	Underlying factors associated with death	Final cause of death
Group 1: Gastrostomy+Jejunostomy																					
1	M	31/4	1,017	2/2	−	+	+	+	AVSD, DORV	TA, DE, LH		HU, RD	GS (0)		DO, NG	+/−	−		1	CHD, HF, TA, LH, RsF	RsF
2	M	34/1	1,420		−		−	+	VSD, PDA			HK, IH	GS+JS (1)		D	+/−	+		9	CHD, HF	SCA
3	F	39/3	1,956	2/4	−	+	+	−	ASD, VSD, PDA	RTI			GS (0)	Bleeding	D, DO	+/−	−		12	CHD, PH, HF	Aspiration pneumonia
4	F	35/1	1,464	−/5	−	+	+	+	VSD	PnT	GER	HN	GS+JS (1)		None	+/−	+		20	CHD, PH, Hemorrhagic tendency	PHE, RsF
5	M	36/0	1,220	4/7	−	+	+	+	VSD, PDA		Microileum	HN	GS (0)		D, DG, DO	+/−	−		41	CHD, PH, HF, Malnutrition	HF, PHE
6	M	41/5	1,990	1/5	+	+	−	+	PDA, ASD			Sz	GS (0)		D	+/−	−		133	CHD, PH, HF	HF
Group 2: Gastrostomy+Tracheoesophageal fistula division																					
7	M	34/5	1,515	1/6	−	+	+	+	VSD, PDA				GS (2), TEFD (29)	ChT	D, DG, DO	+/−	+		47	CHD, PH, HF	HF
8	F	35/6	1,152	7/9	+	+	+	−	VSD, ASD, PDA			Sz	GS+TEFD (5)	ChT	D	+/−	+		106	CHD, CLD, PH	HF
9	F	35/2	1,412	5/9	+	+	+	−	AVSD, PDA	Tracheomalacia			GS (0), TEFD (29)		D, NG, PGI2	+/−	+		172	CHD, PH, HF	HF
Group 3: Esophago-esophagostomy+Tracheoesophageal fistula division																					
10	F	37/4	1,776	−/5	−	+	−	+	ASD, VSD, PDA				EES+TEFD (1)		DO	+/−	−		2	CHD, PPHN, HF	SCA
11	F	36/0	1,510	−/5	−		+	+	CoA, VSD, MS, AS			PK, RnF	EES+TEFD (3)		D, PGE1, DO	+/−	+		17	CHD, HF, PK	HF, RnF
12	F	39/4	1,840	5/8	−	+	+	−	VSD, PS	RTI	GER		GS+EES+TEFD (1)		D, DG, DO	+/−	+		17	CHD, RTI, PHE	HF, PHE
13	M	33/5	1,364	8/8	−	+	+	−	ASD, VSD, PDA	RTI		HN, RnF	EES+TEFD (0)		D, DO, PDA ligation	+/−	−		18	CHD, HF	HF, RsF
14	F	41/1	2,320	−/9	−		−	−	VSD, TR			RnF	EES+TEFD (2)		D, DO	+/−	−		23	CHD, PH, HF, RsF	HF
15	M	35/0	938		−	+	−	+	VSD, PH				EES+TEFD (0)		D, DG	+/−	+		27	CHD, PH	SCA
16	F	40/0	1,670	7/8	−	+	+	−	VSD, ASD, PDA	RTI	GER	HK	EES+TEFD (1)		D, DG	+/−	+		70	CHD, PH, RTI	HF
17	M	35/1	1,560	1/4	−	+	−	+	VSD, PDA	RTI	Enteritis Hypertrophic pyloric stenosis		EES+TEFD (2)		D, DO	+/−	+		202	CHD, PH, CLD	HF
18	F	36/0	1,488	5/9	−		+	+	VSD, ASD, PS			Sz	EES+TEFD (1)		D	+/−	+/+		236	CHD, PH	HF
19	F	37/1	1,759	4/7	−	+	+	−	ASD, VSD				EES+TEFD (1)	PnT	D, DG	+/+ (7)	+/+	+ (73)	694	CHD, PH	HF
Group 4: Gastrostomy followed by Esophago-esophagostomy + Tracheoesophageal fistula division																					
20	M	35/4	1,310	7/8	−	+	+	+	VSD, ASD			Sz	GS (0), EES+TEFD (14)	Mediastinitis	D, DO	+/−	+		32	CHD, PH, HF, RsF, Mediastinitis	HF, RsF
21	F	36/4	1,804	1/1	−	+	−	+	VSD, PDA		GER	Sz	GS (1), EES+TEFD (93)	Atelectasis	D, DO, NG	+/+ (125)	+	+ (137)	210	CHD, CLD, PH	SCA
22	M	37/4	1,747	2/3	−	+	+	−	VSD	RTI	AM	HN, Sz	GS (0), EES+TEFD (3)		D, DO, NG	+/TS	+		518	CHD, PH	PH crisis
23	F	36/1	1,422	8/9	−	+	−	−	PDA, VSD (closed)	RTI	GER, AM	Sz	GS (0), EES+TEFD (17)		D	+/−	+		580	RnF, Malnutrition	RnF
24	F	35/1	1,420	5/8	−	+	+	+	PA, VSD, PDA			VUR	GS (1), EES+TEFD (6)	TEF recanalization	D, DG, PGE1	+/TS	+/+	+ (947)	1,786	CHD, PH, HF, RsF	Tube trouble

M, male; F, female; AM, anorectal malformation; AS, aortic stenosis; ASD, atrial septal defect; AVSD, atrioventricular defect; ChT, chylothorax; CHD, congenital heart defects; CLD, chronic lung disease; CoA, coarctation of aorta; D, diuretics; DE, diaphragmatic eventration; DG, digoxin; DO, dopamine and/or dobutamine; DORV, double outlet right ventricular; EA, esophageal atresia; EES, esophago-esophagostomy; GER, gastroesophageal reflux; GS, gastrostomy; HF, heart failure; HK, horseshoe kidney; HN, hydronephrosis; HU, hydroureter; IH, inguinal hernia; IMV, intermittent mandatory ventilation; JS, jejunostomy; LH, lung hypoplasia; MS, mitral valve stenosis; NG, nitroglycerin; PA, pulmonary atresia; PDA, patent ductus arteriosus; PGE1, prostaglandin E1; PGI2, prostaglandin I2; PH, pulmonary hypertension; PHE, pulmonary hemorrhage; PK, polycystic kidney; PnT, pneumothorax; PPHN, persistent pulmonary hypertension of the newborn; PS, pulmonary stenosis; RD, renal dysplasia; RnF, renal failure; RsF, respiratory failure; RTI, respiratory truct infection; SCA, sudden cardiac or cardiopulmonary arrest; Sz, Seizure; TA, tracheoatresia; TEFD, tracheoesophageal fistura division; TOF, tetralogy of fallot; TR, tricuspid valve regurgitaion; TS, tracheostomy; VSD, ventricular septal defect; VUR, vesicoureteral reflux.

RESULTS

Perinatal Conditions and Interventions

Three patients were prenatally diagnosed with trisomy 18 by amniocentesis. A total of 67% (16/24) of patients were delivered by cesarean, which was selective in six and emergent in eight. Common indications for the cesarean were fetal distress in six, intrauterine growth retardation with polyhydramnios in three, a previous cesarean in one, and breech presentation in one. A total of 58% (14/24) of patients underwent resuscitation by intratracheal intubation. The mean gestational age was 36 weeks and 3 days (range, 31 weeks and 4 days to 41 weeks and 5 days). The mean birth weight was 1,544 g (range, 1,017–1,990 g). The mean Apgar score was 4.0 (range, 1–8) at 1 min and 6.0 (range, 1–9) at 5 min.

Surgery for EA and Surgical Complications

A total of 37% (9/24) of patients (Groups 1 and 2) underwent only palliative surgery. Group 1 (n = 6) underwent only gastrostomy or gastrostomy and jejunostomy on days 0–1. Group 2 (n = 3) underwent gastrostomy on days 0–5 and TEF division on days 5–29.

A total of 63% (15/24) of patients (Groups 3 and 4) underwent radical surgery. Group 3 (n = 10) underwent primary esophago-esophagostomy with TEF division on days 0–3. Group 4 (n = 5) underwent gastrostomy on days 0–1 followed by esophago-esophagostomy with TEF division on days 3–93.

Major surgical complications included hemorrhage (Patient 3), chylothorax (Patients 7 and 8), pneumothorax (Patient 19), mediastinitis (Patient 20), respiratory tract infection and atelectasis (Patient 21), and recanalization of the TEF due to insufficient sutures, requiring reoperation (Patient 24). No intraoperative death or anesthetic complications were noted.

Structural Defects and Medical Complications

All patients had congenital heart defects including ventricular septal defect (VSD), patent ductus arteriosus (PDA), atrial septal defect (ASD), atrioventricular defect, double outlet right ventricle, pulmonary stenosis, coarctation of the aorta, mitral valve stenosis, aortic stenosis, and tricuspid valve regurgitation.

Excluding EA with TEF, noncardiac defects or complications included respiratory abnormalities in 10 patients (42%), such as lung hypoplasia, tracheomalacia, and respiratory tract infection; renal abnormalities in 10 (42%), such as hydroureter, renal dysplasia, horseshoe kidney, polycystic kidney, and renal failure; gastrointestinal abnormalities in 10 (42%), such as gastroesophageal reflux, hypertrophic pyloric stenosis, and anorectal malformation; and seizures in 8 (33%).

Patients 22 and 24 underwent tracheostomy for persistent respiratory failure for the purpose of discharge. Patient 18 underwent Ramstedt procedure for hypertrophic pyloric stenosis. Patient 22 underwent colostomy for anorectal malformation.

Treatment and Courses of Cardiac Defects

A total of 96% (23/24) of patients received cardiovascular drugs. Diuretics (furosemide with/without spironolactone) and dopamine with/without dobutamine pressors were commonly used for heart failure. Prostaglandin E1 was administered to two patients with PDA-dependent congenital heart defects. Nitroglycerin was given to four patients with severe persistent pulmonary hypertension of the newborn. Patient 13 underwent PDA ligation. Patient 8 underwent pulmonary artery banding for a large left-to-right shunt by ASD, VSD, and PDA, but the banding had to be released during the same operation because of worsening of pulmonary hypertension.

Enteral Feeding

A total of 71% (17/24) of patients underwent enteral feeding: 33% in Group 1, 100% in Group 2, 70% in Group 3, and 100% in Group 4. A total of 12.5% (3/24) of patients underwent oral feeding: 20% in Group 3 and 20% in Group 4.

Prognosis

A total of 12.5% (3/24) of patients were discharged home. All the patients had died at the time of this study. Survival rates at 1 day, 1 week, 1 month, and 1 year of age were 100%, 92%, 58%, and 17%, respectively. The overall median survival time was 44 days (range, 1–1,786 days): 88 days in girls and 36.5 days in boys. The median survival time in Groups 1, 2, 3, and 4 was 16 days (range, 1–133 days), 106 days (range, 47–172 days), 25 days, (range, 2–694 days), and 518 days (range, 32–1786 days), respectively. A survival curve for each group is shown in Figure 1A.

Cause of Death

Cause of death was classified into underlying factors associated with death and final mode of death, as described by Kosho et al. [2006] and Kaneko et al. [2008]. The most frequent underlying factors associated with death were congenital heart defects and heart failure in 23 patients (96%), followed by pulmonary hypertension in 18 patients (78%). The most frequent final mode of death was heart failure in 14 patients (58%), followed by respiratory failure and/or pulmonary hemorrhage in five (20%) and sudden cardiac or cardiopulmonary arrest in four (17%).

DISCUSSION

This is the first series to describe the efficacy of surgical intervention for EA with TEF in patients with trisomy 18. Even the natural history of these patients has not been elucidated. A recent support group-based study from Japan [Kosho et al., 2013] described nine patients with EA, with the rate of being offered intensive treatment as 29% (2/7), that of receiving IMV as 57% (4/7), and that of undergoing surgery as 22% (2/9). Survival rate at age 1 year was 0%, and the median survival time was 15.5 days (range, 0–88 days) and was 4 days (range, 0–32 days) without surgical intervention. Statistical analysis showed the presence of EA to be a significant factor associated with shorter survival (<1 year). Our current study shows the survival rate at age 1 year to be 17% and the median survival time to be 44 days. It is, therefore, no doubt that surgical intervention, probably coupled with intensive neonatal treatment,

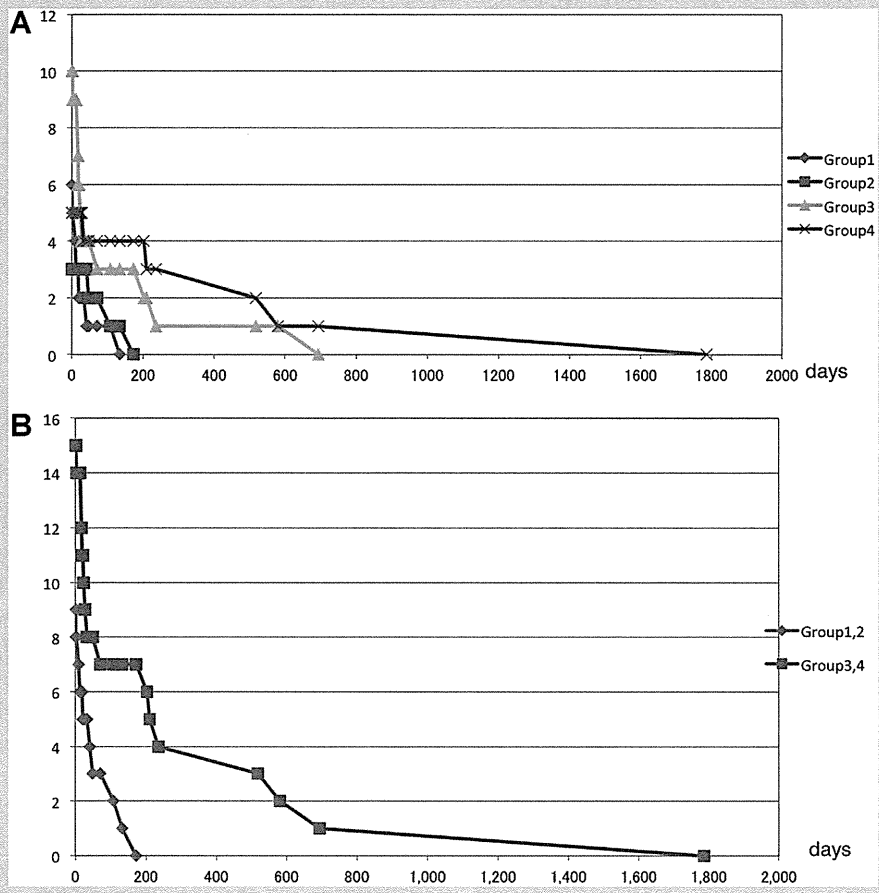

FIG. 1. Survival curves. A vertical axis shows numbers of survivors. A transverse axis shows days after birth. A: Survival curves for Group 1, 2, 3, and 4. B: Survival curves for Groups 1 and 2 (palliative surgery group) and Groups 3 and 4 (radical surgery group).

would contribute to longer survival in patients with trisomy 18 and EA.

The data in the current study were obtained from two children's hospitals in Japan, where surgeons and neonatologists proposed the most effective treatment (surgical procedure, respiratory support, mainly pharmacological cardiovascular support, and other neonatal intensive care) that they considered when they saw each patient, for the purpose of establishment of enteral feeding, discharge, and longer survival. All the parents consented the proposals and no patients had withdrawal care or comfort care in this study period. NCH proposed a two-stage operation with the first procedure as gastrostomy and the second as esophago-esophagostomy with TEF division from 1993 to 2003 and only TEF division from 2003. CHAHSC proposed a two-stage operation with gastrostomy and jejunostomy followed by esophago-esophagostomy with TEF division in the early period and then a one-stage operation with gastrostomy and esophago-esophagostomy with TEF division. As a result, intervention for EA was retrospectively classified into four types (Group 1–4). Thus, the classification would reflect not only the severities of non-EA complications including congenital heart defects accompanied by heart failure and pulmonary hypertension but also surgical strategy for each patient depending on the hospital and the period, irrespective of severity of non-EA complications.

Patients included in each group are characterized as follows. There were only two patients (Patients 1 and 10) who could indeed be judged as "lethal." They could not survive past the first operation because of uncontrollable respiratory failure due to pulmonary hypoplasia in Patient 1, and sudden cardiac arrest due to primary pulmonary hypertension in Patient 10. *Group 1*: Patients in Group 1 only had the first palliative operation (gastrostomy with/without jejunostomy), and died before the second radical operation because of progressive heart failure and/or pulmonary hypertension due to large left-to-right shunts. *Group 2*: Two patients in Group 2, both in NCH from 2003, underwent gastrostomy and TEF division in two stages according to the institutional strategy. Patient 8 from CHAHSC underwent gastrostomy and TEF division in one stage because esophago-esophagostomy was not available due to the long gap between the upper and lower esophagus. All three patients died of progressive heart failure and/or pulmonary hypertension due to large left-to-right shunts. *Group 3*: Nine patients in Group 3 survived past the one-stage radical operation of esophago-esoph-

agostomy with TEF division. Five of them died within 30 days after the operation (progressive heart failure and/or pulmonary hypertension due to large left-to-right shunts in four and heart failure and renal failure due to coarctation of the aorta in one). The other four patients who survived past the neonatal period finally died of progressive heart failure and/or pulmonary hypertension due to large left-to-right shunts. Thus, the differences between the five non-survivors and the four survivors might be related mainly to their cardiovascular conditions, namely, differences in the severities of original cardiac lesions in view of developing heart failure and pulmonary hypertension and/or differences in intra- and post-operative cardiac management. *Group 4:* Three patients in Group 4 survived past 1 year, and two could be discharged home. Deaths of the four patients in Group 4 were associated with cardiac problems. Patient 20 might have survived longer if his postoperative course had not been complicated by mediastinitis.

Patients in Group 4 showed the longest survival with the median survival time as 518 days (range, 32–1786 days), followed by those in Group 2 with the median survival time as 106 days (range, 47–172 days), those in Group 3 with the median survival time as 25 days (range, 2–694 days), and those in Group 1 with the median survival time as 16 days (range, 1–133 days). We compare those who had radical surgery (Groups 3 and 4) with those who didn't (Groups 1 and 2). Survival rate at age 1 year was 27% (4/15) in Groups 3 and 4 and 0% (0/9) in Groups 1 and 2, and the median survival time was 56 days in Groups 3 and 4 and 31 days in Groups 1 and 2 (Fig. 1B). Most importantly, patients with trisomy 18 and EA could not survive long without radical surgery for EA. Factors in prognostic difference between patients in Group 3 (one-stage operation) and those in Group 4 (two-stage operation) is discussed as follows: firstly, patients in Group 3 might have severer non-EA complications, especially congenital heart defects accompanied by heart failure and pulmonary hypertension. However, no apparent difference of non-EA complications was noted (Table I), except Patient 10 who had fatal pulmonary hypertension leading to sudden death on the next day of radical surgery. Secondly, a one-stage operation on the 0–3 days after birth might be too invasive for potentially unstable cardiopulmonary status, especially persistent pulmonary hypertension, in any patients with trisomy 18 complicated by typical left-to-right shunts. The inter-operative period between the first gastrostomy and the second esophago-esophagostomy with TEF division might have been meaningful in careful assessment of patients' physical conditions (reduction of pulmonary hypertension could be expected) and appropriate treatment for patients with unstable cardiopulmonary conditions.

Management of neonates with trisomy 18 has long been discussed from an ethical point of view. Traditional ways of managing patients with this syndrome had been a noninterventional approach, meaning avoidance of emergency surgery [Bos et al., 1992; Paris et al., 1992], labeling this condition as "lethal" or these patients as "hopeless" beings. For the last two decades, however, trends in neonatal intensive care have resulted in the attachment of greater importance to parental decision-making, seeking the "best interest of the child" [Carey, 2010]. Currently, a balanced approach is recommended when counseling families of neonates with this syndrome, comprising the presentation of accurate figures for survival; avoidance of language that assumes outcome such as "lethal," "hopeless," or "incompatible with life"; accurate communication of developmental outcomes that does not presuppose a family's perception of quality of life; and recognition of the family's choice, whether it be comfort care or interventions [Carey, 2012]. In Japan, trisomy 18 had been classified, together with trisomy 13, into a condition in which no additional treatments were considered, but ongoing life-supporting procedures or routine care (temperature control, enteral nutrition, skin care, and love) were not withdrawn [Nishida et al., 1987]. This categorization had a considerable influence on the field of neonatology in Japan, but no legal or social obligation. Thus, babies with trisomy 18 have actually been managed according to an individual policy at each hospital [Kosho, 2008]. The categorization had a harmful effect on physicians in terms of inflexible and paternalistic attitudes toward parents of neonates with severe disorders/disabilities, especially trisomy 18 and trisomy 13. Thus, in 2004, a research project founded by the Ministry of Health, Labour and Welfare, Japan proposed guidelines entitled "Guidelines for Healthcare Providers and Parents to Follow in Determining the Medical Care," which presented a general principle of coping with families of neonates with severe disorders/disabilities, stressing the importance of frank discussion and equal communication between medical staff members and families to seek the "best interests of the babies" [Kosho, 2008]. An increasing number of hospitals have followed the guideline, and important evidences about specific intensive treatments for patients with trisomy 18 have been published recently from single or multiple institutions in Japan: cardiac surgery [Kaneko et al., 2008, 2009; Kobayashi et al., 2010; Maeda et al., 2011] and treatment of seizures [Kumada et al., 2010, 2013]. A recent support group-based study from Japan showed that children with trisomy 18 could live longer and be discharged home through standard intensive treatment such as cesarean and respiratory support, achieve slow but constant psychomotor maturation if they survive, and interact with their families; and that the parents could adapt well [Kosho et al., 2013]. Positive parental feelings have also been demonstrated in several studies from US [Walker et al., 2008; Bruns, 2010; Janvier et al., 2012]. Based on these findings, an intensive approach in the care of children with trisomy 18, adjusted to individual physical conditions and considering parental feelings, can be justified [Kosho et al., 2013]. Two-stage operation would be preferable in management of EA in patients with trisomy 18 in that the inter-operative period could be spent for frank discussion with the parents in view of considerable informed consent seeking "the best interest of the child".

This study has several limitations. First, the number of patients included is small. Second, patient grouping/classification according to the intervention-type is retrospective, not prospective with appropriate randomization as discussed above. Third, the period during which the patients included in this study spans over 20 years. During these years, there could have been considerable changes in the systems or management of the neonatal intensive care units or in the surgical techniques or devices. These limitations are inevitable in discussing management of rare diseases, but could be critical for meaningful generalization. For the readers to interpret the data fairly, we present the detailed clinical background of each patient in Table I. Also, we thoroughly describe how patients received each

intervention for EA and carefully discuss relationship between intervention and prognosis.

In conclusion, EA with TEF would not be an absolute poor prognostic factor in patients with trisomy 18 under a medical environment where radical surgery including esophago-esophagostomy and TEF division and concurrent intensive cardiac management are available. Such an intensive approach could be justified based on increasing evidences about efficacy of intensive treatment, slow but constant development in survivors, and positive parental feelings. Currently, the authors propose a two-stage operation (gastrostomy followed by esophago-esophagostomy and TEF division) in that the inter-operative period could be meaningful in careful assessment of patients' physical conditions, appropriate treatment for patients with unstable cardiopulmonary conditions, and frank discussion with the parents in view of considerable informed consent seeking "the best interest of the child." This information is crucial when counseling parents whose child is prenatally or postnatally diagnosed with trisomy 18 with EA and who are considering the options regarding intensive treatment of their child.

ACKNOWLEDGMENTS

We thank all medical staff members who provided support to the patients and their families in Nagano Children's Hospital and Central Hospital, Aichi Human Service Center. We express particular respect for Dr. Ishizone, former manager of the Department of Surgery and ex-director of Nagano Children's Hospital, and Prof. Tamura, former manager of the Department of Neonatology and ex-director of Nagano Children's Hospital, who established a unique baby- and family-centered intensive care system.

REFERENCES

Bos AP, Broers CJ, Hazebroek FW, van Hemel JO, Tibboel D, Wesby-van Swaay E, Molenaar JC. 1992. Avoidance of emergency surgery in newborn infants with trisomy 18. Lancet 339:913–915.

Bruns DA. 2010. Neonatal experiences of newborns with full trisomy 18. Adv Neonatal Care 10:25–31.

Carey JC. 2010. Trisomy 18 and trisomy 13 syndromes. In: Cassidy SB, Allenson JE, editors. Management of genetic syndromes, 3rd edition. New York: Wiley-Liss. pp 807–823.

Carey JC. 2012. Perspectives on the care and management of infants with trisomy 18 and trisomy 13: Striving for balance. Curr Opin Pediatr 24:672–678.

Edwards JH, Harnden DG, Cameron AH, Crosse VM, Wolff OH. 1960. A new trisomic syndrome. Lancet 1:787–789.

Embleton ND, Wyllie JP, Wright MJ, Burn J, Hunter S. 1996. Natural history of trisomy 18. Arch Dis Child Fetal Neonatal Ed 75:F38–F41.

Janvier A, Farlow B, Wilfond BS. 2012. The experience of families with children with trisomy 13 and 18 in social networks. Pediatrics 130:293–298.

Kaneko Y, Kobayashi J, Yamamoto Y, Yoda H, Kanetaka Y, Nakajima Y, Endo D, Tsuchiya K, Sato H, Kawakami T. 2008. Intensive cardiac management in patients with trisomy 13 or trisomy 18. Am J Med Genet Part A 146A:1372–1380.

Kaneko Y, Kobayashi J, Achiwa I, Yoda H, Tsuchiya K, Nakajima Y, Endo D, Sato H, Kawakami T. 2009. Cardiac surgery in patients with trisomy 18. Pediatr Cardiol 30:729–734.

Kobayashi J, Kaneko Y, Yamamoto Y, Yoda H, Tsuchiya K. 2010. Radical surgery for a ventricular septal defect associated with trisomy 18. Gen Thorac Cardiovasc Surg 58:223–227.

Kosho T. 2008. Invited comment: Care of children with trisomy 18 in Japan. Am J Med Genet Part A 146A:1369–1371.

Kosho T, Nakamura T, Kawame H, Baba A, Tamura M, Fukushima Y. 2006. Neonatal management of trisomy 18: Clinical details of 24 patients receiving intensive treatment. Am J Med Genet Part A 140A:937–944.

Kosho T, Kuniba H, Tanikawa Y, Hashimoto Y, Sakurai H. 2013. Natural history and parental experience of children with trisomy 18 based on a questionnaire given to a Japanese trisomy 18 parental support group. Am J Med Genet Part A 161A:1531–1542.

Kumada T, Nishi R, Higashi T, Oda N, Fujii T. 2010. Epileptic apnea in a trisomy 18 infant. Pediatr Neurol 42:61–64.

Kumada T, Maihara T, Higuchi Y, Nishida Y, Taniguchi Y, Fujii T. 2013. Epilepsy in children with trisomy 18 Am J Med Genet Part A 161A:696–701.

Maeda J, Yamagishi H, Furutani Y, Kamisago M, Waragai T, Oana S, Kajino H, Matsuura H, Mori K, Matsuoka R, Nakanishi T. 2011. The impact of cardiac surgery in patients with trisomy 18 and trisomy 13 in Japan. Am J Med Genet Part A 155A:2641–2646.

Nishida H, Yamada T, Arai T, Nose K, Yamaguchi K, Sakamoto S. 1987. Medical decision making in neonatal medicine. J Jpn Soc Perinat Neonat Med 23:337–341 (in Japanese).

Paris JJ, Weiss AH, Soifer S. 1992. Ethical issues in the use of life-prolonging interventions for an infant with trisomy 18. J Perinatol 12:366–368.

Pinheiro PF, Simões e Silva AC, Pereira RM. 2012. Current knowledge on esophageal atresia. World J Gastroenterol 18:3662–3672.

Poenaru D, Laberge JM, Neilson IR, Guttman FM. 1993. A new prognostic classification for esophageal atresia. Surgery 113:426–432.

Rasmussen SA, Wong LYC, Yang QY, May KM, Friedman JM. 2003. Population-based analysis of mortality in trisomy 13 and trisomy 18. Pediatrics 111:777–784.

Spitz L, Kiely EM, Morecroft JA, Drake DP. 1994. Oesophageal atresia: At-risk groups for the 1990s. J Pediatr Surg 29:723–725.

Sugio K, Koshinaga T, Hoshino M, Inoue M, Goto H, Ikeda T, Hagiwara N. 2006. Study of 24 cases with congenital esophageal atresia: What are the risk factors? Pediatr Int 48:616–621.

Walker LV, Miller VJ, Dalton VK. 2008. The health-care experiences of families given the prenatal diagnosis of trisomy 18. J Perinatol 28:12–19.

Waterston DJ, Carter RE, Aberdeen E. 1962. Oesophageal atresia: Tracheo-oesophageal fistula. A study of survival in 218 infants. Lancet 1:819–822.