N, Yoshiura K. Genome-wide linkage analysis and mutation analysis of hereditary congenital blepharoptosis in a Japanese family. J Hum Genet 53(1): 34-41, 2008.

2．学会発表

＜国際学会＞

American Psychiatric Association, 161st annual meeting, Washington DC, USA. May3-8. 2008.

Linkage analysis and mutation analysis in Paroxysmal kinesigenic choreoatetosis (PKC). Taeko Kikuchi, Naohiro Kurotaki, Akira Imamura, Koh-ichiro Yoshiura, Norio Niikawa, Hiroki Ozawa.

The European Human Genetics Conference 2008, Barcelona, Spain, May 31 - June 3, 2008.

P05.020: Functional analysis of CD96, a causative gene for a form of C (Opitz trigonocephaly) syndrome. T. Kaname, K. Yanagi, Y. Chinen, Y. Makita, N. Okamoto, K. Kurosawa, H. Maehara, Y. Fukushima, A. Bohring, J. M. Opitz, K. Yoshiura, N. Niikawa, K. Naritomi

P06.038: A strong association of axillary osmidrosis with genotype of the ABCC11 gene defining the earwax type. M. Tsuda, N. Miwa, M. Nakashima, M. Nakano, M. Nakashima, K. Yoshiura, T. Ohta, N. Niikawa.

The 2008 EAUHGS Symposium & the 8th EAUHGS Annual Meeting, Sappro, Japan, July 19, 2008.

A strong association of axillary osmidrosis with genotype of the ABCC11 gene defining the earwax type. Masayoshi Tsuda, Nobutomo Miwa, Motoi Nakano, Mitsuko Nakashima, Koh-ichiro Yoshiura, Tohru Ohta, and Norio Niikawa.

A missense mutation in alternatively spliced exon 8A encoding immunoglobulin domain IIIb of *FGFR1* causes Kallmann syndrome. Kiyonori Miura, Shoko Miura, Kentaro Yamasaki, Koh-ichiro Yoshiura, Hideaki Masuzaki

＜国内学会＞

北海道医療大学セミナー 2008年1月8日 （北海道医療大学，北海道）

唇裂・口蓋裂の原因遺伝子解析の現状と展望について　吉浦孝一郎

第十一回胎児遺伝子診断研究会 2008年2月16日，良順会館，長崎．

一般演題10：マイクロアレイを使用した全ゲノムコピー数解析による出生前診断の試み。原田直樹，佐々木健作，霜川修，川良洋城，冨士山龍伊，近藤達郎，夫　律子，松本直通，吉浦孝一郎，新川詔夫．

第8回東北出生前医学研究会 2008年2月7日（土）岩手県民情報交流センタ

ーアイーナ 8 階.

全ゲノムコピー数解析による染色体検査（Molecular karyotyping）の有用性. 霜川 修，佐々木健作，近藤達郎，松本直通，吉浦孝一郎，新川詔夫，原田直樹.

第 60 回日本産科婦人科学会総会・学術講演会 2008 年 4 月 12-15 日

P4-63 母体血漿中へ流入する胎盤由来 mRNA の網羅的遺伝子解析−妊娠高血圧症候群における臨床所見との関連性について− 三浦清徳，三浦生子，山崎健太郎，嶋田貴子，中山大介，吉浦孝一郎，新川詔夫，増崎英明

P1-145 胎盤機能の推定を目指した cDNA マイクロアレイジーンチップの作成に関する検討

三浦生子，三浦清徳，山崎健太郎，嶋田貴子，中山大介，吉浦孝一郎，新川詔夫，増崎英明

日本遺伝子診療学会 15 回大会 2008 年 8 月 2 日（土）〜3 日（日）仙台市戦災復興記念館.

マイクロアレイを使用した全ゲノムコピー数解析による染色体検査の有用性と問題点. Availability and problem of whole genomic copy number analysis with microarray. 霜川 修，佐々木健作，冨士山龍伊，近藤達郎，松本直通，吉浦孝一郎，新川詔夫，原田直樹

地域がん登録全国協議会 第17回総会研究会 2008年9月11日（良順会館，長崎市）

子宮頸部細胞診におけるベセスダシステムと HPV スクリーニングの有用性 三浦清徳，山崎健太郎，池本理恵，三浦生子，嶋田貴子，濱口大輔，小寺宏平，藤下晃，鮫島哲郎，村上誠，中山大介，吉浦孝一郎，増崎英明

長崎県のHPV typeについて 山崎健太郎，三浦清徳，池本理恵，三浦生子，嶋田貴子，濱口大輔，小寺宏平，藤下晃，鮫島哲郎，村上誠，中山大介，吉浦孝一郎，増崎英明

第 53 回日本人類遺伝学会 2008 年 9 月 27-30 日（パシフィコ横浜，横浜）

W-5: 全国スーパーサイエンスハイスクール (SSH) の共同による耳垢型対立遺伝子の全国地図作成の研究. 本多隆利，村松紋佳，田中清，長嶋哲也，小川隆，吉浦孝一郎，新川詔夫

O-044: Ophthalmoacromelic 症候群のゲノムワイド連鎖解析. 浜之上はるか，Megarbane Andre，當間隆也，三宅紀子，才津浩智，堺温哉，三浦生子，吉浦孝一郎，平原史樹，松本直通

O-046: 軟口蓋裂のゲノムワイド連鎖解析. 津田雅由，中島光子，平野明喜，三古谷 忠，山田崇弘，吉浦孝一郎

O-047: 爪から抽出したゲノム DNA を用いた Affymetrix 10K GeneChip による家族性脳動脈奇形の連鎖解析. 及川将弘，国場英雄，近藤達郎，永安 武，吉浦孝一郎

O-149: 大規模 SNP タイピングにおける爪 DNA の有用性の検討. 中島光子, 津田雅由, 木下晃, 新川詔夫, 吉浦孝一郎

O-172: C20orf133 は歌舞伎メーキャップ症候群の責任遺伝子ではない. 国場英雄, 津田雅由, 中島光子, 三浦生子, 近藤達郎, 松本正, 森内浩幸, 大橋博文, 黒澤健司, 外木秀文, 福嶋義光, 成富研二, 三宅紀子, 松本直通, 木下晃, 吉浦孝一郎, 新川詔夫

P-013: 羊水検査で検出した稀な 9q 近位部重複異形を有する３家系. 霜川修, 佐々木健作, 坂井和裕, 長田久夫, 佐久本薫, 近藤達郎, 松本直通, 吉浦孝一郎, 新川詔夫, 原田直樹

P-096: 古人骨における耳垢遺伝子解析の試み（続報）. 佐伯和信, 吉浦孝一郎, 新川詔夫, 岡本啓圭史, 分部哲秋

O-052: 癒着胎盤の評価に母体血漿中 cell-free placental mRNA 定量化は有用か？三浦清徳, 山崎健太郎, 三浦生子, 嶋田貴子, 吉田敦, 中山大介, 吉浦孝一郎, 新川詔夫, 増崎英明

第10回 甲信越・北陸出生前診断研究会 2008年10月4日（土） KKR甲府ニュー芙蓉.

全ゲノムコピー数解析による染色体検査（Molecular karyotyping）の有用性. 霜川 修, 佐々木健作, 近藤達郎, 松本直通, 吉浦孝一郎, 新川詔夫, 原田直樹.

第 15 回遺伝性疾患に関する出生前診断研究会 2008 年 10 月 19 日（大分県医師会館, 大分県）

絨毛性疾患合併妊娠を疑われた 1 例 松本由有子, 山崎健太郎, 三浦清徳, 中山大介, 吉浦孝一郎, 増崎英明

第 47 回日本臨床細胞学会秋季大会 2008 年 11 月 14-15 日（グランドプリンスホテル新高輪, 東京都）

長崎県におけるHPV typing検査について 荒木裕之, 山崎健太郎, 三浦清徳, 池本理恵, 濱口大輔, 藤下晃, 鮫島哲郎, 村上誠, 吉浦孝一郎, 増崎英明

HPV感染と細胞診判定の経時的変化について 山崎健太郎, 三浦清徳, 池本理恵, 三浦生子, 嶋田貴子, 藤下晃, 鮫島哲郎, 村上誠, 吉浦孝一郎, 増崎英明

妊婦の子宮頸部細胞診におけるベセスダシステムとHPVスクリーニングの有用性について 三浦清徳, 山崎健太郎, 池本理恵, 嶋田貴子, 濱口大輔, 藤下晃, 鮫島哲郎, 村上誠, 吉浦孝一郎, 増崎英明

第31回日本分子生物学会年会・第81回日本生化学会大会 合同大会 2008年12月9日（火）～12日（金）, 神戸ポートアイランド, 神戸.

1P-0232: Biomedical characterization of a genetic polymorphism of human ABCC11 as a determinant of earwax type. 豊田優, 櫻井亜季, 中島正洋, 中川 大, 吉浦孝一郎, 新川詔夫, 石川智久

4P-1146: ハイスループット遺伝子スキャンニングへ向けたPCR-高解像度融解曲線分析法の至適条件検討とFGD1遺伝子変異スクリーニング. 要匡, 柳　久美子, 知念安紹, 前原博樹, 福嶋義光, 吉浦孝一郎, 新川詔夫, 成富研二

H. 知的財産権の出願・登録状況

平成20年の出願はありません。

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

本年の書籍刊行物はありません

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sato D, Kawara H, Shimokawa O, Harada N, Tonoki H, Takahashi N, Imai Y, Kimura H, Matsumoto N, Ariga T, NiikawaN, Yoshiura K.	A Down syndrome girl with partial trisomy for 21pter-q22.13: A clue to narrow the Down syndrome critical region.	Am J Med Genet A.	146A(1)	124-127	2008
Wu LQ, Long Z, Liang DS, Harada N, Pan Q, Yoshiura K, Xia K, Dai HP, Niikawa N, Xia JH	Pre- and postnatal overgrowth in a patient with proximal 4p deletion.	Am J Med Genet A.	146A(6)	791-794	2008
Kuniba H, Tsuda M, Nakashima M, Miura S, Miyake N, Kondoh T, Matsumoto T, Moriuchi H, Ohashi H, Kurosawa K, Tonoki H, Nagai T, Okamoto N, Kato M, Fukushima Y, Naritomi K, Matsumoto N, Kinoshita A, Yoshiura K, Niikawa N.	Lack of *C20orf133* and *FLRT3* mutations in 43 patients with Kabuki syndrome in Japan.	J Med Genet	45(7)	479-480	2008
Kuniba H, Sato D, Yoshiura K, Ohashi H, Kurosawa K, Miyake N, Kondoh T, Matsumoto T, Nagai T, Okamoto N, Fukushima Y, Naritomi K, Matsumoto N, Niikawa N.	No mutation in RAS-MAPK pathway genes in 30 patients with Kabuki syndrome.	Am J Med Genet A.	146A(14)	1893-1896	2008

Miura K, Miura S, Yamasaki K, Yoshida A, Yoshiura K, Nakayama D, Niikawa N, Masuzaki H.	Increased level of cell-free placental mRNA in a subgroup of placenta previa that needs hysterectomy.	Prenat Diagn	28(9)	805-809	2008
Nakashima M, Tsuda M, Kishino T, Kondoh S, Kinoshita A, Shimokawa O, Niikawa N, Yoshiura K.	The accuracy of SNP genotyping using genomic DNA extracted from finger nail: Comparison with blood.	Clin Chem	54(10)	1746-1748	2008
Nakashima M, Nakano M, Hirano A, Kishino T, Kondoh S, Miwa N, Niikawa N, Yoshiura K.	Genome-wide linkage analysis and mutation analysis of hereditary congenital blepharoptosis in a Japanese family.	J Hum Genet	53(1)	34-41	2008
Oishi H, Moriyama S, Kotera K, Miura K, Masuzaki H.	First case of liposarcoma arising from the fallopian tube: A case report and review of the literature.	The Journal of Obstetrics and Gynaecology Research	34 (4 pt 2)	713-716	2008
Shimada T, Miura K, Gotoh H, Nakayama D, Masuzaki H.	Management of prenatal ovarian cysts.	Early Hum Dev	84(6)	417-420	2008
Hamada T, Hirose R, Kosaka T, Taniguchi K, Noguchi M, Kihara T, Egashira M, Tagawa M, Miura K, Masuzaki H, Tajima Y, Hayashi T, Kanematsu T.	Giant cystic meconium peritonitis associated with a cloacal anomaly: case report.	Journal of Pediatric Surgery	43(3)	E21-E23	2008
Yoshida S, Miura K, Yamasaki K, Miura S, Shimada T, Tanigawa T, Yoshida A, Nakayama D, Masuzaki H.	Does increased nuchal translucency indicate a fetal abnormality? : A retrospective study to clarify the clinical significance of nuchal translucency in Japan.	J Hum Genet	53	688-693	2008

Yoshida A, Miura K, Nakayama D, Masuzaki H.	Correlation between preeclampsia and prevalence of polymorphism of angiotensinogen, methyleneteterahydrofolate reductase and factor V, prothrombin genes among Japanese women.	Act Med Nagasaki	53	37-41	2008
Khan KN, Kitajima M, Hiraki K, Fujishita A, Sekine I, Ishimaru T, Masuzaki H.	Immunopathogenesis of pelvic endometriosis: role of hepatocyte growth factor, macrophages and ovarian steroids.	Am J Reprod Immunol	60	383-404	2008
Khan KN, Kitajima M, Imamura T, Hiraki K, Fujishita A, Sekine I, Ishimaru T, Masuzaki H.	Toll-like receptor 4-mediated growth of endometriosis by human heat-shock protein 70.	Hum Reprod	23	2210-2219	2008
三浦清徳，新川詔夫	Wolf-Hirschhorn 症候群と猫なき症候群 21 トリソミー症候群，18 トリソミー症候群，13 トリソミー症候群，Turner 症候群	別冊領域別症候群シリーズ No7.『循環器症候群（IV）』	第 2 版（日本臨床）	332-335 336-341	2008
三浦清徳，増崎英明	不妊治療と多胎/不妊治療と双胎妊娠発生機序	臨床婦人科産科	62 巻第 3 号	247-253	2008
三浦生子，三浦清徳，吉田敦，山崎健太郎，増崎英明	母体血漿中に流入する胎盤由来 mRNA の同定とその臨床的意義	産婦人科の実際	57 巻第 8 号	1315-1320	2008
三浦清徳，増崎英明	Assisted Reproductive Technology におけるキメラ発生の危険性	哺乳動物卵子学会誌	25 巻	206-212	2008
三浦清徳，増崎英明	周産期医療における胎児・胎盤由来 cell-free DNA/mRNA の臨床的意義とその応用	医学のあゆみ	225 巻第 9 号	963-969	2008

山崎健太郎，三浦清徳，三浦生子，吉田敦，平木宏一，中山大介，増崎英明	Discordant twins の病態に関する検討	産婦人科の実際	57 巻第 10 号	1575-1581	2008
長谷川史郎，増崎英明	出生前診断をめぐる諸問題一座長のまとめ	日本周産期新生児学会誌	44 巻	877	2008
吉田敦，増崎英明	癒着胎盤の術前診断に関する検討	産婦人科の実際	57 巻第 12 号	2021-2026	2008
吉田敦，増崎英明	妊娠高血圧症候群の発症および重症化の予知	産婦人科の実際	57 巻第 6 号	1021-1026	2008
牛嶋公生，和氣徳夫，小林裕明，蜂須賀　徹，土岐尚之，増崎英明，小寺宏平，瓦林達比古，江本　精，嘉村　敏治	婦人科癌化学療法時の悪心・嘔吐に対するインジセロトン塩酸塩の有効性および安全性	癌と化学療法	35	1169-1173	2008

研究成果の刊行物・別冊

American Journal of Medical Genetics Part A 146A:124–127 (2008)

Research Letter

A Girl With Down Syndrome and Partial Trisomy for 21pter-q22.13:

A Clue to Narrow the Down Syndrome Critical Region

Daisuke Sato,[1,2,3] Hiroki Kawara,[4] Osamu Shimokawa,[1,3,4] Naoki Harada,[3,4] Hidefumi Tonoki,[2,5,6] Nobuhiro Takahashi,[5] Yumi Imai,[6] Hiromi Kimura,[6] Naomichi Matsumoto,[3,7] Tadashi Ariga,[2] Norio Niikawa,[1,3] and Koh-ichiro Yoshiura[1,3]*

[1]Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[2]Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[3]Solution Oriented Research of Science and Technology (SORST), Japan Science and Technology Agency (JST), Kawaguchi, Japan
[4]Kyushu Medical Science, Nagasaki, Japan
[5]Department of Pediatrics, Caress Alliance Medical Corporation, Tenshi Hospital, Sapporo, Japan
[6]Section of Clinical Genetics, Caress Alliance Medical Corporation, Sapporo, Japan
[7]Department of Human Genetics, Yokohama City Graduate School of Medicine, Yokohama, Japan

Received 28 January 2007; Accepted 26 May 2007

How to cite this article: Sato D, Kawara H, Shimokawa O, Harada N, Tonoki H, Takahashi N, Imai Y, Kimura H, Matsumoto N, Ariga T, Niikawa N, Yoshiura K. 2008. A girl with Down syndrome and partial trisomy for 21pter-q22.13: A clue to narrow the Down syndrome critical region. Am J Med Genet Part A 146A:124–127.

To the Editor:

Down syndrome (DS) is the most common multiple congenital anomaly syndrome with mental retardation. The majority of patients with DS have full trisomy for chromosome 21, but rare patients have partial trisomy 21, indicating the presence of a DS critical region (DSCR) that contains genes contributing to cognitive defects and/or other DS features [Antonarakis et al., 2004]. The DSCR has been narrowed down to a region within 21q22, about 5.4 Mb in length [Delabar et al., 1993; Arron et al., 2006]. Here, we describe a girl with DS and a novel karyotype that may narrow the DSCR down to 2.3 Mb.

The Japanese girl was born with a birthweight of 2,964 g (−0.3 SD), length of 46.8 cm (−1.1 SD) and OFC of 33.0 cm (mean) after 38 weeks gestation to a 22-year-old mother and a 30-year-old father. Diagnosis of DS was made soon after birth. Echocardiogram showed PDA. Although further growth was within the normal range, developmental delay was severe. Physical examinations at age 16 months showed the following abnormalities: brachycephaly with flat occiput, epicanthus, strabismus, upslanting palpebral fissures, small nose with low nasal bridge, upturned nostrils, open mouth, protruding tongue, short neck (Fig. 1a,b), short and broad hands with short fifth fingers, congenital heart defect with heart murmur, joint hyperflexibility and muscular hypotonia. These are 13 of 25 signs and satisfied the criterion for a clinical diagnosis of DS according to the Jackson score [Jackson et al., 1976].

After written informed-consent was obtained from her parents, and the study protocol was approved by the Committee for Genetic Testing and Counseling, Tenshi Hospital, cytogenetic studies were performed. A 550-band-level G-banding analysis on her metaphase chromosomes from cultured peripheral blood lymphocytes revealed an isodicentric chromosome 21, 46,XX,idic(21)(q22) (Fig. 1c). Centromere-dot (Cd) banding revealed that one side of the centromere dots of the isochromosome was separated (data not shown). This finding indicated that one of the centromeres was inactivated; hence, the isochromosome was segregating stably [Maraschio et al., 1980]. Spectral karyotyping (SKY) analysis disclosed a more complex abnormality, that is, 46,XX,t(13;21),idic(21)(q22). This karyotype was confirmed by conventional fluorescence in situ hybridization (FISH) using whole-chromosome-painting probes for chromosomes 13 and 21 (Vysis, Downers Grove, IL; Fig. 1d). Additional FISH analysis

*Correspondence to: Koh-ichiro Yoshiura, M.D., Ph.D., Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Sakamoto 1-12-4, Nagasaki 852-8523, Japan.
E-mail: kyoshi@net.nagasaki-u.ac.jp

DOI 10.1002/ajmg.a.31974

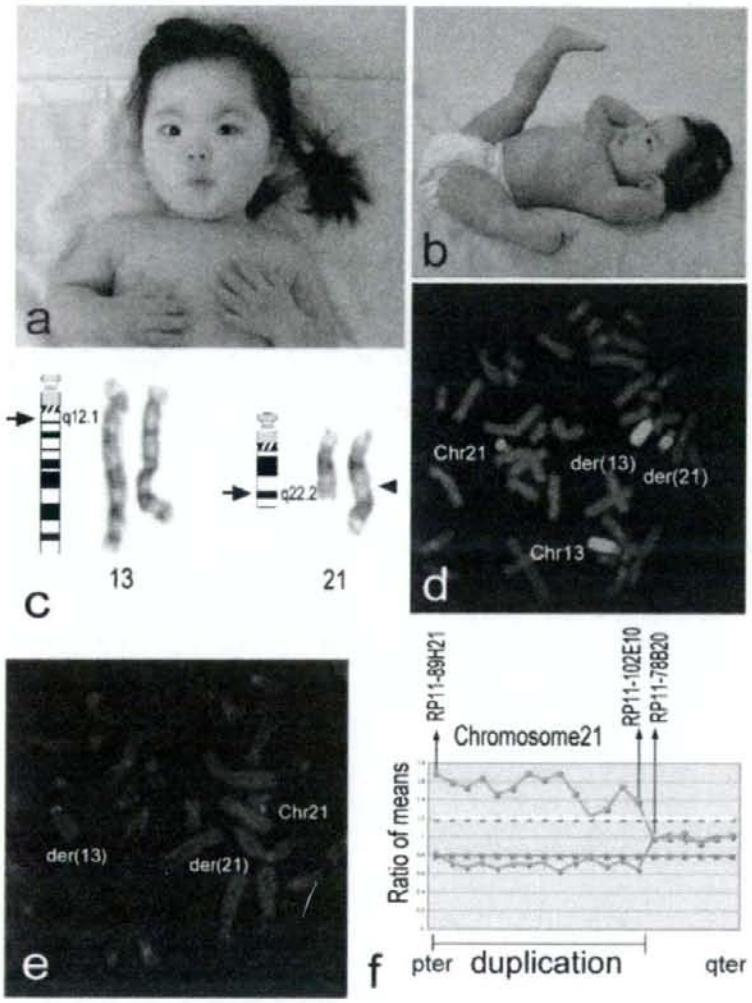

FIG. 1. **a**,**b**: The patient at age 16 months (a and b). **c**: G-banded partial karyotype of the patient. Arrows indicate the breakpoints of an inserted chromosome 13 and an isodicentric chromosome 21. **d**: FISH with chromosome-specific painting probes for chromosomes 13 (red) and 21 (green), showing an insertion of a chromosome 21-derived segment into a pericentromeric region of 13q. **e**: FISH using BAC-clone probes, RP11-1012D8 (green) and RP11-124E9 (red). Green signals appear on both der(13) and der(21) as well as normal chromosome 21, while red signals are seen on normal chromosome 21 and der(13), but not observed on der(21). **f**: Array CGH analysis demonstrates a duplication of a part of chromosome 21. The proximal and distal end clones within the duplication region were RP11-89H21 (21q11.2) and RP11-102E10 (21q22.13), respectively. The breakpoint of der (21) was suggested to be located between RP11-102E10 and RP11-78B20.

with a BAC clone (RP11-1012D8) located to 21q22.13 showed split signals both on der(13) and der(21) chromosomes as well as on normal chromosome 21. FISH using RP11-89H21 (21q11.2), RP11-166F15 (21q22.13), RP11-98O13 (21q22.13), RP11-183O20 (21q22.13), and RP11-95G19 (21q22.13) all gave signals only on both der(21) and normal chromosome 21, while signals appeared on both der(13) and normal chromosome 21 when using other BAC probes located more distantly, such as RP11-608F9 (21q22.13), RP11-749M19 (21q22.2), RP11-814F13 (21q22.3), RP11-124E9 (21q22.3), and RP11-135B17 (subtelomeric region). These analyses successfully identified the breakpoint of the isochromosome at band 21q22.13 (the UCSC genome browser, 2004 May version, http://genome.ucsc.edu/cgi-bin/hgGateway). As for the derivative chromosome 13, we could not define an insertion point precisely, because the insertion occurred closely to the centromeric region where no BAC probes were available. Within the insertion, a FISH signal for RP11-124E9 (SpectrumOrange) mapped at 21q22.3 was observed

proximally to that for RP11-1012D8 (Spectrum-Green) at 21q22.13 (Fig. 1e), demonstrating an inverted insertion on chromosome 13. All these findings indicated that the patient had partial trisomy for a 21pter-q22.13 segment, while her 21q22.13-qter region was disomic. The distal boundary of the trisomic segment lies at the region corresponding to the BAC clone, RP11-1012D8. As her parental karyotypes were normal in both Q-banding and SKY analyses, the patient's karyotype was interpreted as 46,XX,psu idic(21)(q22.13) ins(13;21)(q12.1;q22.13q22.3)dn (Fig. 2a). To know the presence of any other chromosomal aberrations, home-made whole-genome BAC-based microarray with 1.5 Mb resolution comparative genomic hybridization (array CGH) [Sato et al., 2007] was carried out using the patient's DNA. Consequently, the array CGH detected the same partial trisomy 21 (Fig. 1f), but no other deletion nor amplification in the genome.

The DSCR has always been somewhat controversial since many reports included were either premature for precise chromosome banding, include mosaic individuals, or resulted from familial translocations which may have involved undefined reciprocal deletions [Ronan et al., 2007]. Olson et al. [2004] reported that trisomy for the DSCR alone is insufficient and largely unnecessary to cause specific DS phenotypes in mice models. But, the suggestion may not be suitable for human because there are some differences between mouse and human gene content. Later, Olson et al. [2007] reported that trisomy for the DSCR is necessary for brain phenotypes of trisomic mice, thus the DSCR must have some association with the occurrence of DS. Furthermore, recent experiments using mouse models of DS suggested that a 1.5-fold increase in dosage of *DSCR1* and *DYRK1A* within the DSCR destabilizes a regulatory circuit in a cooperative way, contributing to the reduced NFATc activity and many of the characteristics of DS [Arron et al., 2006].

Interestingly, the trisomic segment of our patient partially overlaps the previously estimated DSCR at 21q22 and we have also confirmed that no known genes were disrupted at the breakpoint (Fig. 2b). With the knowledge gained from certain papers of accurately identified partial trisomy 21 that have been published in the past few years [Forster-Gibson et al., 2001; Rost et al., 2004; Kosaki et al., 2005; Kondo et al., 2006; Ronan et al., 2007], we narrow down the DSCR to a region between the proximal boundary of DS1 [Ronan et al., 2007] and the distal

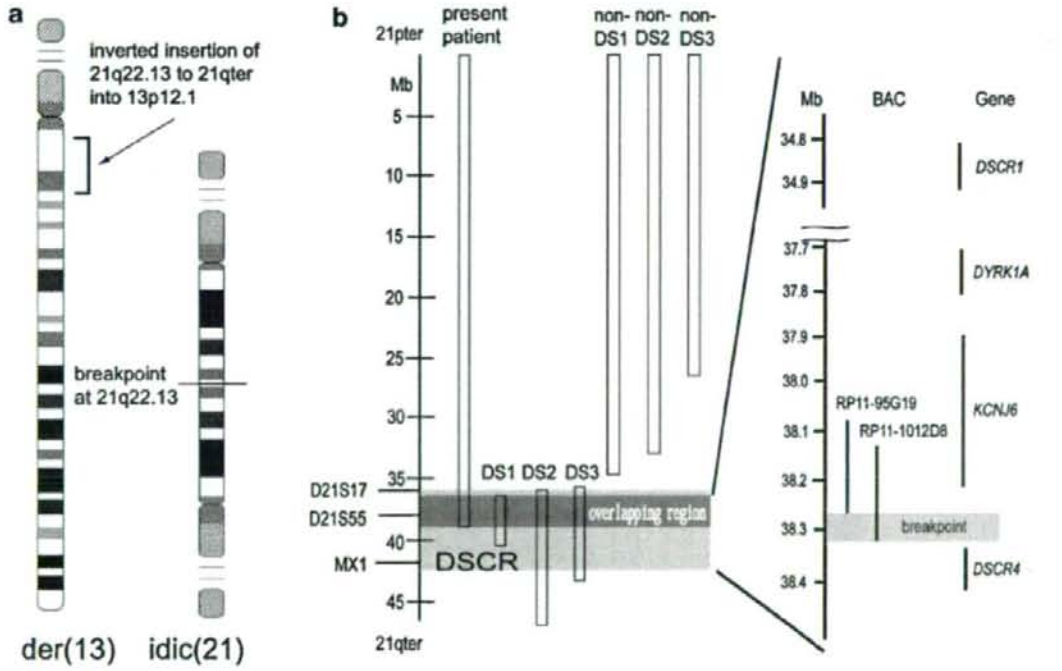

FIG. 2. **a**: Ideogram of der(13) and der(21) of the patient, showing trisomy for 21pter-q22.13 and disomy for 21q22.13-qter. **b**: Relationship between the DSCR and trisomic segments of the present DS patient and recently reported three non-DS cases; non-DS1 and DS2 [Kondo et al., 2006] and non-DS3 [Rost et al., 2004] and three other DS patients; DS1 [Ronan et al., 2007], DS2 [Forster-Gibson et al., 2001], and DS3 [Kosaki et al., 2005]. Enlargement of the overlapping region containing RP11-1012D8 with split FISH signals and its contiguous clone (RP11-95G19) without split signal. *KCNJ6* and *DSCR4* are the closest known genes flanking the breakpoint.

border of our patient, from RP11-957K9 located on 21q22.12 to RP11-1012D8 on 21q22.13, approximately 2.3 Mb in size (Fig. 2b) from the previous estimated 5.4 Mb DSCR at 21q22. [Delabar et al., 1993; Arron et al., 2006]. Since our patient manifested most of the main features of DS, we propose that the newly limited DSCR plays a role of the occurrence of DS in humans. A segment distal to the RP11-1012D8 may not attribute to the DS phenotype as far as our patient is concerned. Our data may support the studies by Arron et al. in mice. Because *DYRK1A* gene is included in the DSCR described here but *DSCR1* is not (Fig. 2b), key role of *DYRK1A* could be applied for human.

In conclusion, we have reported on a girl with DS who had a de novo 46,XX,psu idic(21)(q22.13) ins(13;21)(q12.1;q22.13q22.3) karyotype that might provide a potential clue to minimize the DSCR.

ACKNOWLEDGMENTS

Participation of the patient and her parents in this study is highly appreciated. We also thank Ms. Y. Noguchi, K. Miyazaki and A. Goto for their technical assistance.

REFERENCES

Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S. 2004. Chromosome 21 and Down syndrome: From genomics to pathophysiology. Nature Rev Genet 5:725–738.

Arron JR, Winslow MM, Polleri A, Chang CP, Wu H, Gao X, Neilson JR, Chen L, Heit JJ, Kim SK, Yamasaki N, Miyakawa T, Francke U, Graef IA, Crabtree GR. 2006. NFAT dysregulation by increased dosage of *DSCR1* and *DYRK1A* on chromosome 21. Nature 441:595–600.

Delabar JM, Theophile D, Rahmani Z, Chettouh Z, Blouin JL, Prieur M, Noel B, Sinet PM. 1993. Molecular mapping of twenty-four features of Down syndrome on chromosome 21. Eur J Hum Genet 1:114–124.

Forster-Gibson CJ, Davies J, MacKenzie JJ, Harrison K. 2001. Cryptic duplication of 21q in an individual with a clinical diagnosis of Down syndrome. Clin Genet 59:438–443.

Jackson JF, North ER III, Thomas JG. 1976. Clinical diagnosis of Down's syndrome. Clin Genet 9:483–487.

Kondo Y, Mizuno S, Ohara K, Nakamura T, Yamada K, Yamamori S, Hayakawa C, Ishii T, Yamada Y, Wakamatsu N. 2006. Two cases of partial trisomy 21(pter-q22.1) without the major features of Down syndrome. Am J Med Genet Part A 140A: 227–232.

Kosaki R, Kosaki K, Matsushima K, Mitsui N, Matsumoto N, Ohashi H. 2005. Refining chromosomal region critical for Down syndrome-related heart defects with a case of cryptic 21q22.2 duplication. Congenital Anomalies 45:62–64.

Maraschio P, Zuffardi O, Lo Curto F. 1980. Cd bands and centromeric function in dicentric chromosomes. Hum Genet 54:265–267.

Olson LE, Richtsmeier JT, Leszl J, Reeves RH. 2004. A chromosome 21 critical region does not cause specific Down syndrome phenotypes. Science 306:687–690.

Olson LE, Roper RJ, Sengstaken CL, Peterson EA, Aquino V, Galdzicki Z, Siarey R, Pletnikov M, Moran TH, Reeves RH. 2007. Trisomy for the Down syndrome critical region is necessary but not sufficient for brain phenotypes of trisomic mice. Hum Mol Genet 16:774–782.

Ronan A, Fagan K, Christie L, Conroy J, Nowak N, Turner G. 2007. Familial 4.3Mb duplication of 21q22 sheds new light on the Down syndrome critical region. J Med Genet 44:448–451.

Rost I, Fiegler H, Fauth C, Carr P, Bettecken T, Kraus J, Meyer C, Enders A, Wirtz A, Meitinger T, Carter NP, Speicher MR. 2004. Tetrasomy 21pter-q21.2 in a male infant without typical Down's syndrome dysmorphic features but moderate mental retardation. J Med Genet 41:e26.

Sato D, Shimokawa O, Harada N, Olsen OE, Hou JW, Muhlbauer W, Blinkenberg E, Okamoto N, Kinoshita A, Matsumoto N, Kondo S, Kishino T, Miwa N, Ariga T, Niikawa N, Yoshiura K. 2007. Congenital arhinia: Molecular-genetic analysis of five patients. Am J Med Genet Part A 143A:546–552.

American Journal of Medical Genetics Part A 146A:791–794 (2008)

Research Letter

Pre- and Postnatal Overgrowth in a Patient With Proximal 4p Deletion

Lingqian Wu,[1,2,3] Zhigao Long,[1] Desheng Liang,[1,2,3]* Naoki Harada,[2,3,4] Qian Pan,[1] Koh-ichiro Yoshiura,[2,3] Kun Xia,[1] Heping Dai,[1] Norio Niikawa,[2,3] and Jiahui Xia[1]

[1]National Laboratory of Medical Genetics of China, Xiangya Hospital, Central South University, Changsha, China
[2]Department of Human Genetics, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan
[3]Solution Oriented Research of Science and Technology (SORST), Japan Science and Technology Agency (JST), Kawaguchi, Japan
[4]Kyushu Medical Science, Nagasaki, Japan

Received 14 February 2007; Accepted 25 November 2007

How to cite this article: Wu L, Long Z, Liang D, Harada N, Pan Q, Yoshiura K, Xia K, Dai H, Niikawa N, Xia J. 2008. Pre- and postnatal overgrowth in a patient with proximal 4p deletion. Am J Med Genet Part A 146A:791–794.

To the Editor:

Terminal and interstitial deletions encompassing 4p16 result in Wolf–Hirschhorn syndrome (WHS) and the Pitt-Rogers-Danks syndrome (PRDS) [Wright et al., 1998]. A more proximal, interstitial deletion involving p16.1-p14 shows a distinct clinical entity without overlapping features with WHS and/or PRDS, and is characterized by long face, upslanting palpebral fissures, epicanthal folds, large lax lips, high-arched palate, micrognathia, prominent nose, tall and thin body habitus, broad hands and feet, and varying degrees of mental retardation [White et al., 1995; Tonk et al., 2003]. At least 22 cases of 4p16.1–p12 deletion have been reported [reviewed by Tonk et al., 2003], 17 of whom had a 4p16.1–p14 deletion with a common clinical profile [Romain et al., 1985; Fryns et al., 1989; Davies et al., 1990; Ishikawa et al., 1990; Chitayat et al., 1995; White et al., 1995; Innes et al., 1999; Tonk et al., 2003] (Table I). Here we report a girl with mental retardation, overgrowth and mild facial anomalies, who has a de novo 46,XX,del(4)(p16.1p15.2). A well-proportioned overgrowth pattern in our patient seems distinctive in comparison to reported features of patients with the proximal 4p deletion syndrome.

The patient, a 24-year-old female Han Chinese, was born at full-term to a 26-year-old G1P1 mother who reported an unremarkable pregnancy and nondiabetic history. Consanguinity of the parents was denied. Family history was negative for tall habitus: the body weight/height of her father, mother and a sister was 67 kg/175 cm, 49 kg/165 cm, and 43 kg/160 cm, respectively. Birth weight of the patient was 3,550 g (75th centile), length 61 cm (>97th centile) and OFC 37 cm (>97th centile). She has always been taller than the Chinese age-cohorts since birth. She raised her head at age 6 months, spoke at 15 months and walked at 3 years. She was diagnosed in her early childhood to have mental retardation by a local pediatrician and never attended school except for kindergarten. She has been able to care for herself since she was a teenager. On physical examination at age 23 years, her height was 181 cm (>97th centile), weight 74 kg (>97th centile) and OFC 58 cm, facial length 19 cm, and she had the following facial abnormalities: square-jawed face, epicanthal folds, prominent nose with overhanging tip, short philtrum, high-arched palate and hypoplastic earlobes (Fig. 1a,b). She has a tall, thickset and proportionate habitus without broad hands and feet. Her carpal bone age was advanced during her childhood and adolescence, but the recent radiographic findings at age 24 years were normal. Her first menstruation appeared at age 12 years, then it came regularly, and her secondary sexual characteristics developed normally. Psychometric testing showed moderate mental retardation with estimated IQ of 50, with poorer performance in calculations. Clinical manifestations of the patient including her facial gestalt did not fit to those for any of generalized overgrowth syndromes, such

Lingqian Wu and Zhigao Long contributed equally to this work.

Grant sponsor: NSFC, China; Grant number: 30571021; Grant sponsor: SORST, Japan Science and Technology Agency (JST).

*Correspondence to: Dr. Desheng Liang, National Laboratory of Medical Genetics, Xiangya Hospital, Central South University, 110 Xiangya Road, Changsha, Hunan 410078, China.
E-mail: liangdesheng@cnlmg.com

DOI 10.1002/ajmg.a.32221

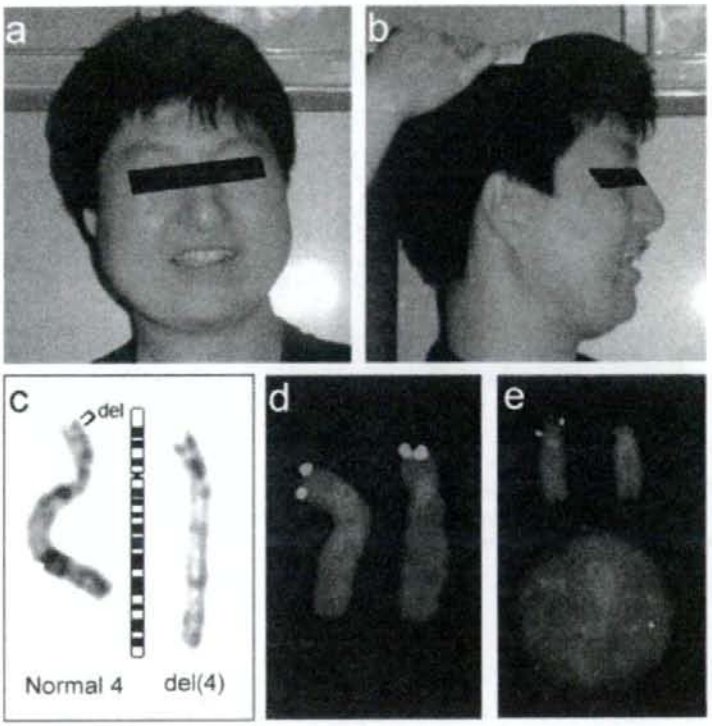

FIG. 1. A girl with a proximal 4p deletion. **a,b**: Facial appearance at age 23 years, showing well-developed, square-jawed face with hypoplastic earlobe. **c**: GTG-banded partial karyotype, showing del(4)(p16.1p15.2). **d,e**: FISH analysis using a BAC clone RP11-1150D2, showing signals in both normal and derivative chromosomes 4, and using a BAC clone RP11-29N16, showing a signal only in normal 4p and only one signal in an interphase cell.

as Weaver, Sotos, Simpson-Golabi-Behmel, Seip-Berardinelli, Perlman, Nevo, MOMO, Marshall-Smith, Beckwith-Wiedemann or Bannayan-Riley-Ruvalcaba syndromes [Douglas et al., 2003]. Thus, it is most likely that her overgrowth is constitutional and associated with a chromosomal deletion below.

High-resolution GTL-banding showed a 46,XX, del(4)(p16.1p15.2) karyotype (Fig. 1c). Fluorescence in situ hybridization (FISH) analysis with 11 BAC clones mapped to 4pter–4p14 [Kondoh et al., 2003] revealed that GS-36p21, RP11-1150D2 (Fig. 1d), 261G12 and 24K3 were retained, but RP11-29N16 (Fig. 1e), 77N9, 46O17, 79N22, 116N19, 192P23, and 106M4 were deleted. These results indicated that the WHS critical region is not deleted and that the proximal and distal deletion breakpoints are located in the regions between UCSC coordinate chromosome 4 nucleotide 24,549,727 and 24,551,523 and between nucleotide 6,504,169 and 6,504,249, respectively. Therefore, the deletion is assigned to an 18-Mb region (nt. 6,504,169–24,551,523) at 4p16.1–p15.2. The karyotypes of her father, mother and sister were normal.

Chitayat et al. [1995] reported three cases of the proximal 4p deletion syndrome and proposed 4p15.33–p15.2 as the minimal deleted segment for this syndrome, which was later supported by Innes et al. [1999]. However, our patient has a deletion encompassing the 4p16.1–p15.2 region, and there have been five reported cases of a deletion similar to our patients [Davies et al., 1990; White et al., 1995; Innes et al., 1999; Tonk et al., 2003]. All of these five cases shared several clinical features that include a long face, epicanthal folds, distinctive nose, thick lower lip, tall and thin habitus and moderate mental retardation. Thus, Tonk et al. [2003] suggested that the critical region for the proximal 4p deletion syndrome can be narrowed to a region from 4p16.1 to p15.2. As all reported cases that had a 4p16.1–p15.1 deletion manifested all typical features of this syndrome (Table I), the critical region should be confined to 4p16.1–p15.1. By a review of breakpoints of 4p16.1–p14 deletions in reported patients (Table I), we found that the tall habitus is most likely attributed to 4p16.1–p15.32 deletion, probably implying the presence of a negative control mechanism against tall status or overgrowth.

Overgrowth and other features in our patient merit comments. According to the information from Table II, 82% (14/17) of patients with a 4p16.1–p14

TABLE I. Clinical Features of 18 Reported Cases of 4p16.1–4p14 Deletion

	Reported patients with deletion [references]								
	4p16.1–p15.2, present case	4p16.1–p15.2, 5 cases [1–4]	4p16.1–p15.1, 4 cases [2]	4p16.1–p14, 1 case [2]	4p15.33–15.2, 3 cases [5,6]	4p15.32–p14, 2 cases [2,7]	4p15.32–p15.2, 1 case [8]	4p15.2–14, 1 case [5]
Long face	−	+ + − + −	+ + +	+	+ + +	+ −	?	+
Upslanted fissures	−	+ + ? + −	+ + +		− ? ?	+ +	?	?
Epicanthal folds	+	+ + ? + −	+ + +	+	− + −	+ +	−	+
Distinctive nose	+	+ + − − −	+ + +	+	− − +	− −	?	−
High or cleft palate	+	− − ? + −	+ + +	+	− + +	− −	+	+
Thick lower lip	−	+ + ?+ +	+ + +		− + +	− +	+	−
Micrognathia	− (macrognathia)	− − − + −	+ + +	+	− + +	+ −	+	+
Broad, short neck	−	− − − + −	−	+	− ? ?	− +	?	?
Broad hands and feet	−	− − ? + −	+ + +	+	− + −	− −	?	+
Tall, thin habitus	Tall but symmetric	+ + + + +	+ + +	+	+ − +	+ +	−	−
Mental retardation								
Mild	−	+ +	−	−	+	−	+	
Mederate	+	+ +	+ + +	−	+ +	+ +		+
Severe	−	+ −	+	−	−			

1, White et al. [1995]; 2, Tonk et al. [2003]; 3, Innes et al. [1999]; 4, Davies et al. [1990]; 5, Chitayat et al. [1995]; 6, Romain et al. [1985]; 7, Fryns et al. [1989]; 8, Ishikawa et al. [1990]; ?, no available data.

deletion had a tall, thin stature, while the habitus of our patient was tall and well-proportioned, rather than thin, and began prenatally. In addition, her face was not long but her jaw was well developed. These features seem distinctive among the reported manifestations for the proximal 4p deletion syndrome, and might be attributed to possibly different extent of deletion between our and other patients with del(4)(p16.1p15.2), although our findings remain inconclusive as this is a single case and a possible role of genetic background within the family cannot be ruled out. Based on a familial transmission and reproductive fitness of the syndrome reported previously, as well as a possible reproductive capacity in our patient, we must exercise a caution when counseling patients with this condition [Tonk et al., 2003].

According to information from the Gene Predictions in the UCSC database, there are several candidate genes in the deleted segment that may explain the overgrowth in the present patient. Among them, the *SLC2A9* and *BAPX1* genes may play a role in the development and survival of chondrocytes in cartilage matrices and in skeletal development, respectively. The *FGFBP1* gene encoding fibroblast growth factor binding protein 1 may have a function similar to that of *FGFR3*, the gene for fibroblast growth factor receptor 3, which regulates endochondral ossification [Deng et al., 1996]. Moreover, the *PPARGC1A* gene (chr4: 23,402,742–23,500,798) that is located near the proximal breakpoint of the 18-Mb deleted region in our patient may involve in regulating cellular cholesterol homoeostasis and development of obesity.

REFERENCES

Chitayat D, Ruvalcaba RHA, Babul R, Teshima IE, Posnick JC, Vekemans MJJ, Scarpelli H, Thuline H. 1995. Syndrome of proximal interstitial deletion 4p15: Report of three cases and review of the literature. Am J Med Genet 55:147–154.

Davies J, Voullaire L, Bankier A. 1990. Interstitial deletion of the band 4p15.3 defined by sequential replication banding. Ann Genet 33:92–95.

Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P. 1996. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell 84:911–921.

Douglas J, Hanks S, Temple I, Davies S, Murray A, Upadhyaya M, Tomkins S, Hughes H, Cole T, Rahman N. 2003. NSD1 mutations are a major cause of Sotos syndrome and occur in some cases of Weaver syndrome but are rare in other overgrowth phenotypes. Am J Hum Genet 72:132–143.

Fryns JP, Yang-Aisheng, Kleczkowska A, Lemmens F, Vandecasseye W, van den Berghe H. 1989. Interstitial deletion of the short arm of chromosome 4—A phenotype distinct from the Wolff-Hirschhorn syndrome. Ann Genet 32:59–61.

Innes AM, Chudley AE, Carson NL, Dawson AJ. 1999. Interstitial 4p deletion in a child with an Angelman syndrome-like phenotype. Clin Genet 56:238–241.

Ishikawa T, Sumi S, Fujimoto S, Shima Y, Wada Y. 1990. Interstitial deletion of the short arm of chromosome 4 in a boy with mild

psychomotor retardation and dysmorphism. Clin Genet 38: 314–317.

Kondoh Y, Toma T, Ohashi H, Harada N, Yoshiura K, Ohta T, Kishino T, Niikawa N, Matsumoto N. 2003. Inv dup del(4)(:p14->p16.3::p16.3->qter) with manifestations of partial duplication 4p and Wolf-Hirschhorn syndrome. Am J Med Genet Part A 120A:123–126.

Romain DR, Columbano-Green LM, Parfitt RG, Chapman CJ, Smythe RH, Gebbie OB. 1985. A complex structural rearrangement of chromosome 4 in a woman without phenotypic features of Wolff-Hirschhorn syndrome. Clin Genet 28:166–172.

Tonk VS, Jalal SM, Gonzalez J, Kennedy A, Velagaleti GV. 2003. Familial interstitial deletion of chromosome 4 (p15.2p16.1). Ann Genet 46:453–458.

White DM, Pillers D-AM, Reiss JA, Brown MG, Magenis RE. 1995. Interstitial deletions of the short arm of chromosome 4 in patients with a similar combination of multiple minor anomalies and mental retardation. Am J Med Genet 57:588–597.

Wright TJ, Clemens M, Quarrell O, Altherr MR. 1998. Wolff-Hirschhorn and Pitt-Rogers-Danks syndromes caused by overlapping 4p deletions. Am J Med Genet 75: 345–350.

CORRESPONDENCE

Lack of *C20orf133* and *FLRT3* mutations in 43 patients with Kabuki syndrome in Japan

Kabuki syndrome (KS, OMIM 147920), also known as Niikawa–Kuroki syndrome, is a multiple congenital anomalies/mental retardation (MCA/MR) syndrome characterised by a peculiar facial appearance, skeletal abnormalities, joint hypermobility, dermatoglyphic abnormalities, postnatal growth retardation, recurrent otitis media and occasional visceral anomalies. Although some studies have ruled out several loci from the candidacy for KS, any putative disease gene loci or candidate genes remain unidentified.

In a recent issue of the journal, Maas *et al*[1] reported that exon 5 of the *C20orf133* gene at 20p12.1 was disrupted by a 250 kb de novo microdeletion in a patient with KS; they also screened for mutations in *C20orf133* and *FLRT3* (a nested gene located within intron 3 of *C20orf133*) in 19 additional patients with KS, but failed to detect such mutations or deletions in any of them. It remains unclear whether the two genes are responsible for the pathogenesis of KS, and if so, how frequently the deletion at the locus is found in KS patients. Herein we describe the results of a deletion assay for the exon 5 in *C20orf133* and a mutation analysis of *C20orf133* and *FLRT3* among 43 patients with KS in Japan. In addition, we also show the results of a copy number analysis at 20p12.1 by Human Mapping 250K Nsp Array among 18 patients with KS in Japan.

Ethics approval for this study was obtained from the Committee for the Ethical Issues on Human Genome and Gene Analysis in Nagasaki University. The subjects studied consisted of 43 patients (20 girls and 23 boys) with KS from Japan for mutation analysis and a deletion assay, and of 18 patients (nine girls and nine boys) with KS for copy number analysis. Genomic DNA was isolated by the standard method. Genomic sequences were retrieved from the UCSC genome browser. GenBank accession numbers of NCBI of *C20orf133* and *FLRT3* are NM_080676 and NM_198391, respectively.

C20orf133 and *FLRT3* were directly sequenced. Polymerase chain reaction (PCR) primers used for the two genes were those described by Maas *et al*,[1] but as some primers did not work in our laboratory, we newly designed the primes. Direct sequencing of *C20orf133* and *FLRT3* did not show any pathogenic nucleotide changes in the 43 patients. Observed five nucleotide changes in the patients were all found in normal Japanese individuals as well—that is, a first substitution, c.173C>T (p.T58I) in *C20orf133*, already registered in the database of single nucleotide polymorphisms (SNPs) as rs2990505, was identified in 14 patients (12 heterozygous and two homozygous status). A second substitution, c.1069T>C (p.S357P) in *C20orf133*, not registered in the database, was observed in 10 patients (heterozygous status) and in 35 of 159 normal Japanese individuals (29 heterozygous and six homozygous status). A third substitution, g.14257801T>C in exon 2 (5'UTR exon) of *FLRT3*, found in nine patients (heterozygous status), was registered as rs761998. A fourth substitution, c.765A>G (p.Q255Q) in *FLRT3*, not registered in the database, was found in one patient (heterozygous status) and in three of 137 normal Japanese individuals (heterozygous status). The last nucleotide change, heterozygous deletions of three nucleotides, g.14257934_14257936delCAG in exon 2 (5'UTR exon) of *FLRT3*, not registered in dbSNP, was found in nine patients, and in four of 81 normal Japanese individuals.

Deletion assay involving exon 5 of *C20orf133* was performed by quantitative real-time PCR on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, California, USA). *ALB* gene was chosen as a reference gene,[3] which had no copy number polymorphism (CNP) in the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation/). Primers and fluorogenic probes were designed with the assistance of Primer Express v1.5 (Applied Biosystems). Primer sequences are available on request. The quantitative PCR indicated that none of the 43 patients had any copy number changes involving *C20orf133* exon 5. The average quotient (SD) of the target/reference genes in patients was 1.090 (0.124) with a range (SD) of 0.934–1.291 (0.025–0.426), and the control persons as well (data not shown).

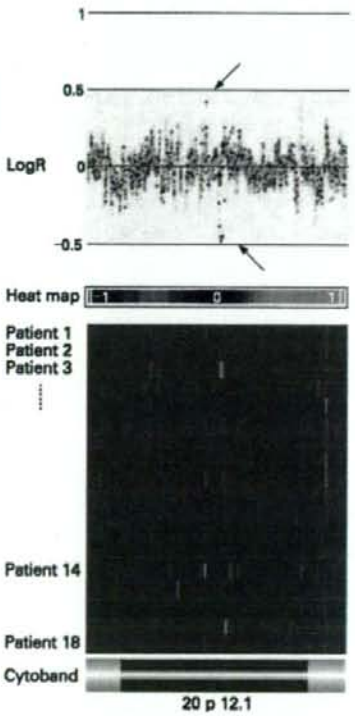

Figure 1 Copy number analysis at 20p12.1 among 18 patients with Kabuki syndrome. Each patient in log2 ratio plot is shown by a different colour. A bar of Heat Map representing gain for red and loss for green is divided in each patient. Deletion at intron 5 of *C20orf 133* is indicated in patient 3. Duplication at intron 4 of *C20orf133* is suggested in patient 14 (arrows in logR plot; deep green or deep red in HeatMap).

According to the DGV (last updated 29 Nov 29 2007), a ~368 kb deletion (chromosome 20, nucleotide numbers (nt) 14,606,364–14,974,100 bp), involving exon 5 of *C20orf133* (nt 14,613,489–14,613,605), has been reported in one of 506 unrelated healthy Northern German and 270 HapMap individuals (registered as Variation_9315, a normal loss).[4] Thus, it is possible that the 250 kb deletion at 20p12.1 in a KS patient reported by Maas *et al*[1] was a rare copy number variation. However, some CNPs may possibly play more important roles in human phenotypic variations than SNPs.[4 5] For example, Balikova *et al* reported a unique novel syndrome caused by the amplification of large genomic regions, ~750-kb at cytoband 4p16, known to be copy number variation.[6] Therefore, we must be careful to check copy number changes for an MCA/MR syndrome.

To search particular microdeletion/duplication at cytoband 20p12.1, we performed copy number analysis among 18 patients with KS by DNA oligomicroarray hybridisation using the GeneChip Human Mapping 250 K Nsp Array (Affymetrix, Santa Clara, California, USA). Data at the target region were analysed using GTYPE (GeneChip Genotyping Analysis Software), CNAT (GeneChip Chromosome Copy Number Analysis Tool) and Partek Genomic Suite v6.3 (Partek Inc, St Louis, Missouri, USA). Two copy number changes were found among 18 patients. Neither of them was registered in the DGV, but they were less likely to be pathogenic because both of them were within intronic sequences—that is, a ~100 kb deletion within intron 5 of *C20orf133* was detected in one patient (patient 3 in fig 1). Its physical positions and log2 ratios were: chromosome 20, nt 15,000,514 bp (logR −0.4136), 15,014,439 (−0.4586), 15,034,442 (−0.4831), 15,066,513 (−0.4519), and 15,102,706 (−0.4330). In another patient (patient 14 in fig 1), a ~30 kb region was suggested as duplication and the region was located within intron 4 of the *C20orf133*. The physical positions and log2 ratios were: chromosome

20, nt 14,527,943 bp (LogR 0.4113), 14,553,038 (0.4443), 14,557,957 (0.4204) and 14,563,924 (0.3878). The other 16 patients with KS did not show significant copy number changes at the region. Thus, particular copy number changes at the region were not detected in these patients with KS.

In summary, we performed a mutation analysis for *C20orf133* and *FLRT3*, a deletion assay for exon 5 of *C20orf133* in 48 patients with KS and a copy number analysis by DNA oligomicroarray among the 18 patients with KS in Japan. These studies did not reveal pathogenic alterations in the patients. Therefore, our findings unfortunately could not support the working hypothesis that the *C20orf133* and/or *FLRT3* were the causative gene in most Japanese KS patients.

H Kuniba,[1,2,13] M Tsuda,[1,13] M Nakashima,[1,13] S Miura,[1,13] N Miyake,[2,13] T Kondoh,[2] T Matsumoto,[2] H Moriuchi,[2] H Ohashi,[3,13] K Kurosawa,[4] H Tonoki,[5] T Nagai,[6,13] N Okamoto,[7] M Kato,[8] Y Fukushima,[9,13] K Naritomi,[10,13] N Matsumoto,[11,13] A Kinoshita,[1,13] K-i Yoshiura,[1,13] N Niikawa[1,12,13]

[1] Departments of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [2] Departments of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [3] Division of Medical Genetics, Saitama Children's Medical Center, Iwatsuki, Japan; [4] Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; [5] Department of Pediatrics, Tenshi Hospital, Sapporo, Japan; [6] Department of Pediatrics, Dokkyo University School of Medicine Koshigaya Hospital, Koshigaya, Japan; [7] Department of Planning and Research, Osaka Medical Center and Research Institute for Maternal and Child health, Osaka, Japan; [8] Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan; [9] Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto, Japan; [10] Department of Medical Genetics, University of the Ryukyus, Nishihara, Japan; [11] Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan; [12] Research Institute of Personalized Health Sciences, Health Sciences University of Hokkaido, Tobetsu, Japan; [13] Solution Oriented Research for Science and Technology (SORST), Japan Science and Technology Agency (JST), Tokyo, Japan

Correspondence to: Dr K-i Yoshiura, Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Sakamoto 1-12-4, Nagasaki 852-8523, Japan; kyoshi@nagasaki-u.ac.jp

Acknowledgements: We are very grateful to the patients and their parents for their participation in this research. We also thank Ms Yasuko Noguchi, Ms Ayano Goto, and Ms Miho Ohga for their technical assistance. NN was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Sports, Culture, Science and Technology of Japan, and was supported by SORST from Japan Science and Technology Agency (JST) (Nos. 17019055 and 19390095, respectively). KY was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare.

Competing interests: None declared.

Ethics approval: Ethics approval for this study was obtained from the Committee for the Ethical Issues on Human Genome and Gene Analysis in Nagasaki University.

Received 23 February 2008
Revised 5 March 2008
Accepted 6 March 2008

J Med Genet 2008;**45**:479–480.
doi:10.1136/jmg.2008.058503

REFERENCES

1. **Maas NM,** Van de Putte T, Melotte C, Francis A, Schrander-Stumpel CT, Sanlaville D, Genevieve D, Lyonnet S, Dimitrov B, Devriendt K, Fryns JP, Vermeesch JR. The C20orf133 gene is disrupted in a patient with Kabuki syndrome. *J Med Genet* 2007;**44**:562–9.
2. **Laurendeau I,** Bahuau M, Vodovar N, Larramendy C, Olivi M, Bieche I, Vidaud M, Vidaud D. TaqMan PCR-based gene dosage assay for predictive testing in individuals from a cancer family with INK4 locus haploinsufficiency. *Clin Chem* 1999;**45**:982–6.
3. **Pinto D,** Marshall C, Feuk L, Scherer SW. Copy-number variation in control population cohorts. *Hum Mol Genet* 2007;**16**:R168–73.
4. **Redon R,** Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, González JR, Gratacòs M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. Global variation in copy number in the human genome. *Nature* 2006;**444**:444–54.
5. **Stranger BE,** Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, Redon R, Bird CP, de Grassi A, Lee C, Tyler-Smith C, Carter N, Scherer SW, Tavaré S, Deloukas P, Hurles ME, Dermitzakis ET. Relative impact of nucleotide and copy number variation on gene expression phenotypes. *Science* 2007;**315**:848–53.
6. **Balikova I,** Martens K, Melotte C, Amyere M, Van Vooren S, Moreau Y, Vetrie D, Fiegler H, Carter NP, Liehr T, Vikkula M, Matthijs G, Fryns JP, Casteels I, Devriendt K, Vermeesch JR. Autosomal-dominant microtia linked to five tandem copies of a copy-number-variable region at chromosome 4p16. *Am J Hum Genet* 2008;**82**:181–7.