研究分担者：大橋博文（埼玉県立小児医療センター遺伝科）
研究協力者：清水健司（埼玉県立小児医療センター遺伝科）

ウォルフヒルシュホーン症候群の日本人症例における欠失領域と表現型の相関解析を行った．ゲノムアレイ解析を実施した 22 例のうち，15 例につき臨床症状を検討した．4p の欠失サイズは 2.0Mb～29.42Mb（中央値 8.77Mb）と幅があり，単純欠失 18 例，不均衡転座 3 例，派生 4 番染色体 1 例でその割合は従来の報告と類似していた．さらに欠失サイズにより，1：5Mb 以下，2：5～15Mb，3：15Mb 以上，の 3 群に分類し臨床症状との相関を検討した．腎臓，眼，骨格症状などの身体合併症は欠失サイズが大きいほど頻度，重症度ともに増加傾向であった．けいれんの発症頻度は 12/15(80%)であり，うち 83%(10/12)に重責もしくは群発を認めたが，多くは 3 歳前後で改善傾向になっており，また臭化ナトリウム/カリウムが奏功する例を複数認めた．ウォルフヒルシュホーン症候群を含むゲノムコピー数異常を伴う希少な奇形症候群患者における早期のゲノムアレイ解析が，その後の合併症管理の有用な情報につながるよう各疾患ごとに症例の蓄積が重要である．そのためにも，患者・家族への情報提供や継続的支援体制が欠かせないと考え，集団外来を企画し，勉強会として継続的な情報提供を実施した．

研究分担者：川目裕（お茶の水女子大学大学院遺伝カウンセリングコース）

ウォルフヒルシュホーン症候群（Wolf-Hirschhorn syndrome: WHS）の欠失のタイプと頻度を把握するために，日本小児遺伝学会「Dysmorphology の夕べ」実行委員会の協力のもとに，その欠失のタイプの調査と患者数の推定を行った．今回報告のあった 65 例のうち，端部欠失は，49 例（75%），転座その他の構造異常 16 例（25%）であった．発生頻度の推定は，2005 年から 2009 年の出生別症例数から，各施設を WHS とダウン症候群が同じ割合で受診すると仮定し，ダウン症候群の頻度を 1/800 出生として推定をおこなった．その間に出生した WHS は，ダウン症候群に比して平均 2.6%（0.8%から 4.7%，中央値 2.65%）であった．従って WHS の出生率は，約 31,000 出生にひとり（1/17,000 から 1/100,000 出生）と推測された．

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
涌井敬子，福嶋義光	Ⅰ．染色体検査・遺伝子関連検査総論，Ⅱ．染色体検査（第13章）	金井正光	臨床検査法提要改訂第33版	金原出版	東京	2010	1113-1164
福嶋義光	ミラー・ディーカー症候群	井村裕夫	症候群ハンドブック	中山書店	東京	2011	661

雑誌（英文）

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Narumi Y, Kosho T, Tsuruta G, Shiohara M, Shimazaki E, Mori T, Shimizu A, Igawa Y, Nishizawa S, Takagi K, Kawamura R, Wakui k, Fukushima Y	Genital abnormalities in Pallister-Hall syndrome: Report of two patients and review of the literature	Am J Med Genet	152A	3143-3147	2010
Hayashi S, Imoto I, Aizu Y, Okamoto N, Mizuno S, Kurosawa K, Okamoto N, Honda S, Araki S, Mizutani S, Numabe H, Saitoh S, Kosho T, Fukushima Y, Mitsubuchi H, Endo F, Chinen Y, Kosaki R, Okuyama T, Ohki H, Yoshihashi H, Ono M, Takada F, Ono H, Yagi M, Matsumoto H, Makita Y, Hata A, Inazawa J	Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies	J Hum Genet	56(2)	110-124	2011
Nishimura-Tadaki A, Wada T, Bano G, Gough K, Warner J, Kosho T, Ando N, Hamanoue H, Sakakibara H, Nishimura G, Tsurusaki Y, Doi H, Miyake N, Wakui K, Saitsu H, Fukushima Y, Hirahara F, Matsumot	Breakpoint determination of X;autosome balanced translocations in four patients with premature ovarian failure	J Hum Genet	56(2)	156-160	2011
Narumi Y, Shiohara M, Wakui K, Hama A, Kojima S, Yoshikawa K, Amano Y, Kosho T, Fukushima Y	Myelodysplastic syndrome in a child with 15q24 deletion syndrome	Am J Med Genet A	158A	412-416	2011

Motobayashi M, Nishimura-Tadaki A, Inaba Y, Kosho T, Miyatake S, Niimi T, Nishimura T, Wakui K, Fukushima Y, Matsumoto, N, Koike K,	Neurodevelopmental features in 2q23.1 microdeletion syndrome: Report of a new patient with intractable seizures and review of literature	Am J Med Genet A	158(A)	861-868	2012

雑誌（和文）

福嶋義光	診療のための遺伝医学関連ガイドライン（特集：臨床遺伝学の進歩と日常診療）	日本医師会雑誌	139(3)	604	2010
福嶋義光	遺伝子解析（特集：産婦人科に関わる法と倫理の現状）	産婦人科の実際	59	2185-2190	2010
福嶋義光	遺伝子診療学とは. 遺伝子診療学（第２版）遺伝子診断の進歩とゲノム治療の展望	日本臨床	68	1-3	2010
古庄知己	奇形・染色体異常の遺伝カウンセリング	小児科診療 小児の治療指針	２０１０増刊号	907-909	2010
福嶋義光	臨床遺伝医療	BIO Clinica	26	271-275	2011

書　籍

ミラー・ディーカー症候群　Miller-Dieker syndrome

【ICD-10】Q87.8

■**疫学**　100万人出生に対し，10～40人と推定されている．

■**発症に関わる遺伝子**　*LIS1*を含む17番染色体短腕部分欠失（17p13.3欠失）

■**診断**　CTあるいはMRIで滑脳症が認められた場合に疑いがもたれ，染色体検査（G分染法）あるいはFISH法で，17p13.3欠失が認められることにより確定診断がなされる．

■**治療**　患児の状態を良好に保つため，成長，栄養，呼吸状態，発達，けいれんについて定期的に評価を行い，それぞれの症状に対する対症療法を適切に行うことが重要である．栄養状態の改善のためには，チューブ栄養や胃瘻造設が考慮される．けいれんに対しては適切な抗けいれん薬を投与する．

■**遺伝カウンセリング**　約80%の症例は突然変異によるものであり，次子の再発率は低いが，約20%は両親のどちらかに17番染色体短腕と他の染色体との相互転座があり，17p13.3欠失が生じたものであり，次子の再発率は無視できない．また，相互転座を有する親の同胞も同じ相互転座を有している可能性があるので，適切な遺伝カウンセリングの場を提供する必要がある．

■**関連語・同義語**　滑脳症，LIS1関連滑脳症，subcortical band heterotopia

■**解説**　重度の滑脳症を特徴とする先天奇形症候群である．重度の精神運動発達遅滞があり，特徴的な顔貌（前頭突出，短鼻，上向き鼻孔，小下顎など）や，その他の内臓奇形（臍帯ヘルニア，先天性心疾患など）を伴う．新生児期には，特に大きな問題に気づかれないこともあるが，次第に哺乳障害，筋緊張低下，発達遅滞，小頭症，けいれんがみられるようになる．けいれんは生後6か月以内に始まることが多い．

■**所見**　全身症状：重度の精神運動発達遅滞，筋緊張低下，哺乳障害，体重増加不良，成長障害
中枢神経：滑脳症，小頭症，けいれん
顔面：特徴的顔貌（前頭突出，短鼻，上向き鼻孔，小下顎など）
胸部：先天性心疾患
腹部：臍帯ヘルニア　（福嶋義光）

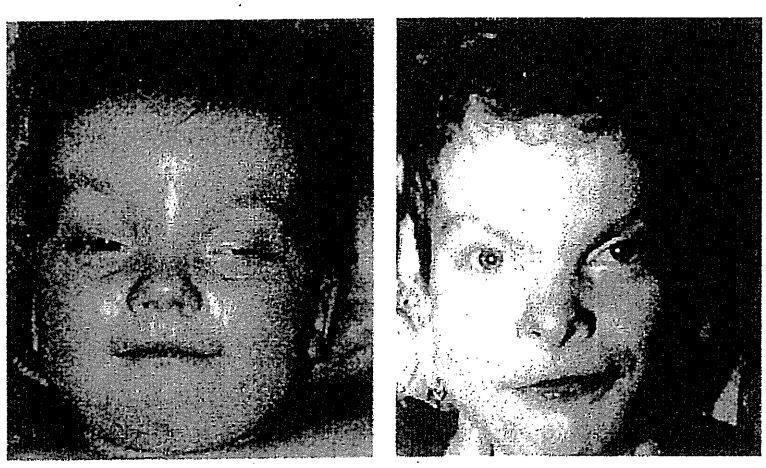

図1　Miller-Dieker症候群の顔貌（文献1）

【文献】1) Dobyns WB, Das S: IS1-Associated Lissencephaly/Subcortical Band Heterotopia. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle 2009; 1993-2006.
2) Available at http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=chrom17-lis. Accessed August 30, 2010.

雑　誌（英文）

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Genital Abnormalities in Pallister–Hall Syndrome: Report of Two Patients and Review of the Literature

Yoko Narumi,[1]* Tomoki Kosho,[1] Goro Tsuruta,[2] Masaaki Shiohara,[2] Ei Shimazaki,[3] Tetsuo Mori,[3] Ayako Shimizu,[2] Yasuhiko Igawa,[4] Shuji Nishizawa,[5] Kimiyo Takagi,[6] Rie Kawamura,[1] Keiko Wakui,[1] and Yoshimitsu Fukushima[1]

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

[2]Department of Pediatrics, Shinshu University School of Medicine, Matsumoto, Japan

[3]Department of Pediatrics, Nagano National Hospital, Ueda, Japan

[4]Department of Continence Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

[5]Department of Urology, Nagano Children's Hospital, Azumino, Japan

[6]Department of Obstetrics, Nagano Children's Hospital, Azumino, Japan

Received 5 March 2010; Accepted 25 July 2010

We describe two patients with Pallister–Hall syndrome (PHS) with genital abnormalities: a female with hydrometrocolpos secondary to vaginal atresia and a male with micropenis, hypoplastic scrotum, and bilateral cryptorchidism. Nonsense mutations in *GLI3* were identified in both patients. Clinical and molecular findings of 12 previously reported patients who had *GLI3* mutations and genital abnormalities were reviewed. Genital features in the male patients included hypospadias, micropenis, and bifid or hypoplastic scrotum, whereas all the females had hydrometrocolpos and/or vaginal atresia. No hotspot for *GLI3* mutations has been found. The urogenital and anorectal abnormalities associated with PHS might be related to dysregulation of SHH signaling caused by *GLI3* mutations rather than hormonal aberrations. We recommend that clinical investigations of genital abnormalities are considered in patients with PHS, even those without hypopituitarism. © 2010 Wiley-Liss, Inc.

Key words: Pallister–Hall syndrome; genital abnormality; hydrometrocolpos; micropenis; *GLI3*; sonic hedgehog signaling

How to Cite this Article:
Narumi Y, Kosho T, Tsuruta G, Shiohara M, Shimazaki E, Mori T, Shimizu A, Igawa Y, Nishizawa S, Takagi K, Kawamura R, Wakui K, Fukushima Y. 2010. Genital abnormalities in Pallister–Hall syndrome: Report of two patients and review of the literature.
Am J Med Genet Part A 152A:3143–3147.

INTRODUCTION

Pallister–Hall syndrome (PHS) [OMIM#146510] is a rare autosomal dominant disorder, characterized by hypothalamic hamartoma, central or postaxial polydactyly, bifid epiglottis, and various visceral anomalies [Hall et al., 1980]. The disorder is caused by mutations in *GLI3*, a gene encoding a zinc finger transcription factor that regulates downstream target genes in the sonic hedgehog (SHH) signaling pathway [Kang et al., 1997]. PHS is caused by nonsense, frameshift, and single splice mutations in the middle third of *GLI3*, which includes exons 13, 14, and part of 15 [Johnston et al., 2005]. PHS is hypothesized to be caused by *GLI3* transcription factor repressor activity [Shin et al., 1999; Tsanev et al., 2009].

The male patients reported by Hall et al. [1980] had micropenis, hypospadias, and cryptorchidism; however, the genital abnormalities associated with this disorder have not been reviewed. Previously, only 12 patients with PHS and *GLI3* mutations have been reported to have genital abnormalities (Table I) [Topf et al., 1993; Verloes et al., 1995; Zucchini et al., 1998; Fujiwara et al., 1999; Stroh et al., 1999; Stoll et al., 2001; Ng et al., 2004; Johnston et al., 2005; McCann et al., 2006; Kos et al., 2008]. Here, we report two patients with genital abnormalities and *GLI3* mutations, thereby providing further evidence of genital abnormalities as a feature of PHS.

Grant sponsor: Ministry of Education, Culture, Sports, Science and Technology, Japan; Grant Number: 22790972.
*Correspondence to:
Yoko Narumi, M.D., Ph.D., Department of Medical Genetics, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan.
E-mail: ynarumi@shinshu-u.ac.jp
Published online 24 November 2010 in Wiley Online Library (wileyonlinelibrary.com)
DOI 10.1002/ajmg.a.33720

TABLE I. Characteristics of 14 Patients With *GLI3* Mutation-Positive Pallister–Hall Syndrome and Genital Abnormalities

	1	2	3	4	5	6	7	8	9	10	11	12	Patient 1	Patient 2
Sex	Male	Male	Male	Male	Female	Female	Female	Female	Female	Unknown	Unknown	Unknown	Female	Male
Age	9y	10w	8y	19mo	12y7mo	3mo	2y5mo	2y	20mo	NI	NI	NI	3y	10y
GLI3 mutation														
Exon	13	13	15	13	14	14	15	15	NI	14	15	15	14	15
Nucleotide	c.2023delG	c.2062G > T	c.3386_3387 delTT	+	c.2146C > T	c.2149C > T	c.2567C > A	c.3439G > T	+	c.2139delC	c.2351_2355 del5	c.2628delC	c.2169T > G	c.2454C > A
Amino acids	p.E675S fsX17	p.E688X	p.F1129X	NI	p.Q716X	p.Q717X	p.S856X	p.E147X	NI	p.C713fs X713	p.K784_Q785 delinsSfsX15	p.S877A fsX13	p.Y723X	p.C818X
Genital abnormalities	Hypospadias	Genital hypoplasia	Hypospadias	Micropenis, bifid scrotum, hypospadias	HMC, vaginal atresia, urogenital sinus	HMC, vaginal atresia	HMC	Bifid uterus, vaginal atresia	HMC, vagina-bladder fistula	Genital hypoplasia	Genital hypoplasia	Genital hypoplasia	HMC' vaginal atresia	Micropenis, hypoplastic scrotum, cryptorchidism
Urological abnormalities	—	—	—	—	—	Small kidney	VUR	*1	Hydronephrosis' urethral atresia	—	—	—	—	Small kidney
Anorectal abnormalities	Imperforate anus	—	HD, imperforate anus	Imperforate anus	—	—	Imperforate anus	Imperforate anus, rectoperineal fistula	—	—	—	—	—	—
Endocrine abnormalities	PP	—	PHP, PP	—	GHD	GHD	GHD	—	PP	GHD	GHD	GHD	GHD, primary hypothyroidism	PHP
References	Topf et al. [1993]; Kang et al. [1997]	Stroh et al. [1999]; Johnston et al. [2005]	Ng et al. [2004]	Stoll et al. [2001]	Zucchini et al. [1998]; Johnston et al. [2005]	Kos et al. [2008]	McCann et al. [2006]; Johnston et al. [2005]	Verloes et al. [1995]; Radhakrishna et al. [1999]	Fujiwara et al. [1999]	Johnston et al. [2005]	Johnston et al. [2005]	Johnston et al. [2005]	Present report	Present report

y, year; mo, month; w, week; HMC, hydrometrocolpos; VUR, vesicoureteral reflux; HD, Hirschsprung disease; GHD, growth hormone deficiency; PP, precocious puberty; PHP, panhypopituitarism; NI, no information available.
*1: Leaflet ectopic kidneys, duplicated right renal pelvis, ectopic uretheral meatus, vesicoureteric reflux, bladder neck deformation, and patent urachus.

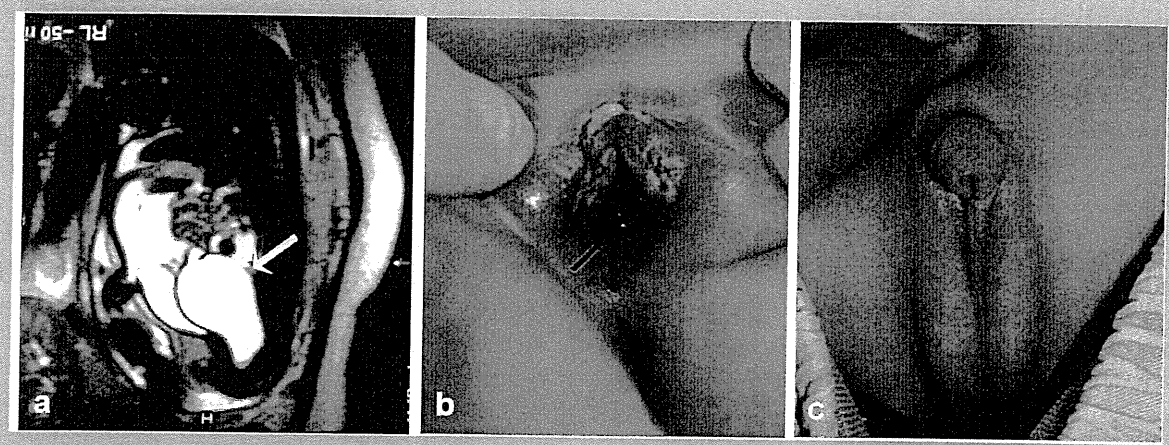

FIG. 1. a: Fetal MRI at 31 weeks of gestation in Patient 1. Dilation of the vagina and uterine cavity was noted (white arrow). b: External genitalia in Patient 1 at birth. The vaginal opening was not identified (black arrow). c: External genitalia in Patient 2 at age 4 years. A micropenis with a hypoplastic scrotum was present.

CLINICAL REPORTS

Patient 1, now a 3-year-old Japanese girl, was born as the second child of healthy nonconsanguineous parents. At 31 weeks of gestation, a large intra-abdominal cystic lesion and ascites were detected by fetal ultrasonography. Fetal magnetic resonance imaging (MRI) showed dilation of the uterus and vagina, which was suspected to be hydrometrocolpos (Fig. 1a). At 32 weeks of gestation, she was delivered by cesarean for fetal distress. Her birth weight was 2,380 g (+1.6 SD), birth length was 40 cm (−1.0 SD), and OFC was 30.9 cm (+0.3 SD). She was treated with mechanical ventilation and nitric oxide inhalation therapy for respiratory insufficiency and persistent pulmonary hypertension due to surfactant deficiency. She was also treated with antibiotics for peritonitis. The clinical findings included a short and flat nose, a high-arched palate, bilateral postaxial polydactyly, and hypoplastic nails. She had normal major and minor labia but her vagina was blind-ended (Fig. 1b). Cranial MRI showed a large hypothalamic hamartoma and normal pituitary glands. Radiographs of the hands showed mesoaxial and postaxial polysyndactyly. No abnormalities of the epiglottis, anus, kidney, or lung were found. Serum levels of thyroid-stimulating hormone (TSH) were elevated at 13.84 μIU/ml (normal 0.2–4.0 μIU/ml). A thyrotropin-releasing hormone (TRH) loading test showed an elevated peak TSH at 41.16 μIU/ml, compatible with primary hypothyroidism, and L-thyroxine therapy was started. Serum levels of growth hormone (GH), adrenocorticotropic hormone (ACTH), cortisol, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), and free thyroxine (f-T4) were normal. The biochemical test result of fluid collected via a transabdominal vaginal catheter suggested urine constitution. The enlarged uterus and vagina was diagnosed as hydrometrocolpos communicating to the bladder, although retrograde cystography did not show a fistula between the uterus and the bladder. At 3 months of age, she underwent vaginoplasty. At age 1 year, her height was 63.5 cm (−3.0 SD). An arginine loading test showed a low peak GH level at 2.4 ng/ml, compatible with GH deficiency. GH replacement therapy was therefore started.

Patient 2 was a 10-year-old boy at the time of evaluation and of Japanese descent. Part of his medical history was described previously [Shimizu et al., 2002]. He was born at 40 weeks of gestation as the second child of healthy nonconsanguineous parents after an uneventful pregnancy. His birth weight was 3,298 g (+0.3 SD), birth length was 51 cm (+0.3 SD), and OFC was 35 cm (+0.8 SD). He had ambiguous genitalia, and showed severe hypoglycemia and hypotension on the first day of life. He was suspected of having adrenal insufficiency or pituitary dysfunction and accordingly received corticosteroid therapy. He had no known PHS-associated abnormalities of the epiglottis, anus, or lung. Endocrinologic examinations demonstrated panhypopituitarism: GH, <0.05 ng/ml; ACTH, 7.0 pg/ml; cortisol, <1.0 μg/dl; PRL, <1.0 ng/ml; TSH, 0.07 μIU/ml; f-T4, 0.51 ng/dl; LH, <0.5 mIU/ml; FSH, <0.5 mIU/ml; and testosterone, <5.0 ng/dl. Replacement of GH, glucocorticoid, mineralocorticoid, and thyroid hormone was started. Cranial MRI showed a hypothalamic hamartoma and absence of the anterior pituitary gland. A radiograph of the left hand showed Y-shaped third metacarpals.

When seen by us at age 4 years, he showed downslanting palpebral fissures, a flat nasal bridge, a high-arched palate, brachydactyly, and hypoplastic nails. His genital findings included severe micropenis, hypoplastic scrotum, and cryptorchidism (Fig. 1c). At age 8 years, he underwent orchiopexy. At age 9 years, abdominal ultrasonography showed a small left kidney and bilateral hypoplastic testes. Testosterone treatment for micropenis did not have a sufficient effect on urination in the standing position. At age 7 years, a human chorionic gonadotrophin (hCG) loading test showed no testosterone response (<0.05 ng/ml). At age 10 years, an LH-RH loading test showed no LH or FSH response (LH <0.5 mIU/ml, FSH <0.5 mIU/ml).

MUTATION ANALYSIS

After informed consent had been obtained, leukocyte genomic DNA was amplified by polymerase chain reaction (PCR) for the 15 exons and exon–intron boundaries of *GLI3* (primers and conditions are available on request). After purification, the PCR samples were directly sequenced using the ABI BigDye terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Reactions were run on an ABI 3100 semi-automated sequencing analyzer (Applied Biosystems). The DNA sequences were analyzed using FinchTV version 1.4.0 (Geospiza, Inc., Seattle, WA). This study was conducted according to the Declaration of Helsinki and was approved by the ethics committee of Shinshu University School of Medicine.

Heterozygous nonsense mutations were identified in both patients: c.2169C > G in exon 14 (p.Tyr723X) in Patient 1 and c.2454C > A in exon 15 (p.Cys818X) in Patient 2. These mutations have not been reported in the literature and were not present in either set of parents, though biologic parentage was not confirmed.

DISCUSSION

We found novel *GLI3* mutations in two PHS patients showing genital abnormalities. A female with GH deficiency had hydrometrocolpos, and a male with panhypopituitarism had severe micropenis, hypoplastic scrotum, and bilateral cryptorchidism. To date, a total of 14 patients, including the present two patients (five males, six females, three unknown), with *GLI3* mutation-positive PHS have been reported to have genital abnormalities (Table I). All patients had nonsense or frameshift mutations in exons 13, 14, or 15; however, no hotspot for *GLI3* mutations has been found. In affected males, hypospadias was observed in three patients, micropenis in two, and bifid or hypoplastic scrotum in two. Three had an anorectal abnormality (imperforate anus) and one had a urological abnormality (small kidney). In affected females, all had hydrometrocolpos and/or vaginal atresia. Two had an anorectal abnormality (imperforate anus and rectoperineal fistula) and four had various urological abnormalities, including vesicoureteric reflux in two. A male patient reported by Topf et al. [1993] had an affected father, showing polysyndactyly, hypothalamic mass, and normal genitalia. Another male patient reported by Ng et al. [2004] had an affected sister whose condition was caused by parental gonadal mosaicism, showing panhypopituitarism, precocious puberty, imperforate anus, and genu recurvatum, but no genital abnormalities. Additionally, a girl described by Roscioli et al. [2005] with the same *GLI3* nucleotide change as the siblings reported by Ng et al. [2004] had an anteriorly placed anus with stenosis and rectovaginal fistula, but no genital abnormalities. These findings suggest that genital features in patients with PHS might present with a wide range of severities among patients with the same nucleotide change.

The cloaca, a transient embryonic cavity, is subdivided into the urogenital sinus and anal canal, which subsequently differentiate into the urogenital and anorectal organs in both sexes [Haraguchi et al., 2007]. *Shh* knockout mice show anorectal malformations and a complete absence of external genitalia formation, and *Gli3* knockout mice show anal stenosis or atresia [Haraguchi et al., 2001; Mo et al., 2001; Böse et al., 2002]. Haraguchi et al. [2007] reported that *Shh* mutant embryos have hypoplasia of the external genitalia, pelvic urethra, and bladder, and they concluded that the Shh signaling pathway is important for bladder development and external genital morphogenesis. Using temporally controlled *Shh* deletion mice, Seifert et al. [2009] demonstrated that disruption of *Shh* function caused coordinated anorectal and genitourinary malformations in both males and females, including severe underdevelopment of the external genitalia, hypospadias, persistence of the embryonic cloaca during the anogenital phase (E10.5–E13), and localized defects of the external genitalia during external genital phase (E13.5–E15.5). Gli transcription factors are likely to be required for normal development of the genital tubercle, an embryonic anlage of external genitalia, which later differentiates into a penis in males and a clitoris in females [Yamada et al., 2003, 2006]. Thus, urogenital and anorectal malformations in patients with PHS might be related to dysregulation of SHH signaling caused by *GLI3* mutations.

Masculine development of the external genitalia is generally androgen dependent. The actions of androgens during external genital morphogenesis generally occur after the initial unisexual patterning of the external genital anlagen [Yamada et al., 2003, 2006]. Graham et al. [1985] speculated that micropenis and cryptorchism in male patients with PHS were caused by absent or diminished gonadotrophins during fetal development and that hypopituitarism resulted from disruption of normal relationships between the pituitary and the hypothalamus by a hypothalamic hamartoma. In our review of the literature, only two of five male patients had panhypopituitarism. Hydrometrocolpos results from failure of canalization of the hymen and developing vagina. Most cases are sporadic, however, familial occurrence and association of genetic syndromes (McKusick–Kaufman syndrome) or congenital anomalies have been reported [Nazir et al., 2006]. No female patients in our review of the literature had panhypopituitarism.

In conclusion, we describe genital abnormalities in two patients with PHS and *GLI3* mutations. The urogenital and anorectal abnormalities in patients with PHS may be related to dysregulation of SHH signaling caused by *GLI3* mutations rather than hormonal aberrations. We recommend that thorough investigations of genital abnormalities are considered in patients with PHS, even in those without hypopituitarism.

ACKNOWLEDGMENTS

We are grateful to the patients and their families for their cooperation. This work was supported by Research on Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (22790972).

REFERENCES

Böse J, Grotewold L, Rüther U. 2002. Pallister–Hall syndrome phenotype in mice mutant for Gli3. Hum Mol Genet 11:1129–1135.

Fujiwara I, Kondo Y, Iinuma K. 1999. Oral-facial-digital syndrome with hypothalamic hamartoma, postaxial ray hypoplasia of the limbs, and vagino-cystic communication: A new variant? Am J Med Genet 83:77–81.

Graham JM Jr, Harris M, Frank JE, Little GA, Klein RZ. 1985. Congenital hypothalamic hamartoblastoma syndrome: Natural history and genetic implications. Prog Clin Biol Res 200:163–174.

Hall JG, Pallister PD, Clarren SK, Beckwith JB, Wiglesworth FW, Fraser FC, Cho S, Benke PJ, Reed SD. 1980. Congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate a.u. and postaxial polydactyly—A new syndrome? Part I: Clinical, causal, and pathogenetic considerations. Am J Med Genet 7:47–74.

Haraguchi R, Mo R, Hui C, Motoyama J, Makino S, Shiroishi T, Gaffield W, Yamada G. 2001. Unique functions of sonic hedgehog signaling during external genitalia development. Development 128:4241–4250.

Haraguchi R, Motoyama J, Sasaki H, Satoh Y, Miyagawa S, Nakagata N, Moon A, Yamada G. 2007. Molecular analysis of coordinated bladder and urogenital organ formation by Hedgehog signaling. Development 134:525–533.

Johnston JJ, Olivos-Glander I, Killoran C, Elson E, Turner JT, Peters KF, Abbott MH, Aughton DJ, Aylsworth AS, Bamshad MJ, Booth C, Curry CJ, David A, Dinulos MB, Flannery DB, Fox MA, Graham JM, Grange DK, Guttmacher AE, Hannibal MC, Henn W, Hennekam RC, Holmes LB, Hoyme HE, Leppig KA, Lin AE, Macleod P, Manchester DK, Marcelis C, Mazzanti L, McCann E, McDonald MT, Mendelsohn NJ, Moeschler JB, Moghaddam B, Neri G, Newbury-Ecob R, Pagon RA, Phillips JA, Sadler LS, Stoler JM, Tilstra D, Walsh Vockley CM, Zackai EH, Zadeh TM, Brueton L, Black GC, Biesecker LG. 2005. Molecular and clinical analyses of Greig cephalopolysyndactyly and Pallister–Hall syndromes: Robust phenotype prediction from the type and position of GLI3 mutations. Am J Hum Genet 76:609–622.

Kang S, Graham JM Jr, Olney AH, Biesecker LG. 1997. GLI3 frameshift mutations cause autosomal dominant Pallister–Hall syndrome. Nat Genet 15:266–268.

Kos S, Roth K, Korinth D, Zeilinger G, Eich G. 2008. Hydrometrocolpos, postaxial polydactyly, and hypothalamic hamartoma in a patient with confirmed Pallister–Hall syndrome: A clinical overlap with McKusick–Kaufman syndrome. Pediatr Radiol 38:902–906.

McCann E, Fryer AE, Craigie R, Baillie C, Ba'ath ME, Selby A, Biesecker LG. 2006. Genitourinary malformations as a feature of the Pallister–Hall syndrome. Clin Dysmorphol 15:75–79.

Mo R, Kim JH, Zhang J, Chiang C, Hui CC, Kim PC. 2001. Anorectal malformations caused by defects in sonic hedgehog signaling. Am J Pathol 159:765–774.

Nazir Z, Rizvi RM, Qureshi RN, Khan ZS, Khan Z. 2006. Congenital vaginal obstructions: Varied presentation and outcome. Pediatr Surg Int 22:749–753.

Ng D, Johnston JJ, Turner JT, Boudreau EA, Wiggs EA, Theodore WH, Biesecker LG. 2004. Gonadal mosaicism in severe Pallister–Hall syndrome. Am J Med Genet Part A 124A:296–302.

Radhakrishna U, Bornholdt D, Scott HS, Patel UC, Rossier C, Engel H, Bottani A, Chandal D, Blouin JL, Solanki JV, Grzeschik KH, Antonarakis SE. 1999. The phenotypic spectrum of GLI3 morphopathies includes autosomal dominant preaxial polydactyly type-IV and postaxial polydactyly type-A/B; No phenotype prediction from the position of GLI3 mutations. Am J Hum Genet 65:645–655.

Roscioli T, Kennedy D, Cui J, Fonseca B, Watson GF, Pereira J, Xie YG, Mowat D. 2005. Pallister–Hall syndrome: Unreported skeletal features of a GLI3 mutation. Am J Med Genet Part A 136A:390–394.

Seifert AW, Bouldin CM, Choi KS, Harfe BD, Cohn MJ. 2009. Multiphasic and tissue-specific roles of sonic hedgehog in cloacal septation and external genitalia development. Development 136:3949–3957.

Shimizu A, Shimazaki E, Tooyama K, Mori T, Miyairi Y, Shigeta H. 2002. An infant case of Pallister–Hall syndrome treated with hormone replacement. Clin Pediatr Endocrinol 11:29–32.

Shin SH, Kogerman P, Lindström E, Toftgård R, Biesecker LG. 1999. GLI3 mutations in human disorders mimic Drosophila cubitus interruptus protein functions and localization. Proc Natl Acad Sci 96:2880–2884.

Stoll C, De Saint Martin A, Donato L, Alembik Y, Sauvage P, Messer J. 2001. Pallister–Hall syndrome with stenosis of the cricoid cartilage and microphallus without hypopituitarism. Genet Couns 12:231–235.

Stroh B, Rimell FL, Mendelson N. 1999. Bifid epiglottis. Int J Pediatr Otorhinolaryngol 47:81–86.

Topf KF, Kletter GB, Kelch RP, Brunberg JA, Biesecker LG. 1993. Autosomal dominant transmission of the Pallister–Hall syndrome. J Pediatr 123:943–946.

Tsanev R, Tiigimägi P, Michelson P, Metsis M, Østerlund T, Kogerman P. 2009. Identification of the gene transcription repressor domain of Gli3. FEBS Lett 583:224–228.

Verloes A, David A, Ngô L, Bottani A. 1995. Stringent delineation of Pallister–Hall syndrome in two long surviving patients: Importance of radiological anomalies of the hands. J Med Genet 32:605–611.

Yamada G, Satoh Y, Baskin LS, Cunha GR. 2003. Cellular and molecular mechanisms of development of the external genitalia. Differentiation 71:445–460.

Yamada G, Suzuki K, Haraguchi R, Miyagawa S, Satoh Y, Kamimura M, Nakagata N, Kataoka H, Kuroiwa A, Chen Y. 2006. Molecular genetic cascades for external genitalia formation: An emerging organogenesis program. Dev Dyn 235:1738–1752.

Zucchini S, Mazzanti L, Ambrosetto P, Salardi S, Cacciari E. 1998. Unusual magnetic resonance imaging findings of the sellar region in subjects with hypopituitarism: Report of 4 cases. J Pediatr Endocrinol Metab 11:35–44.

Journal of Human Genetics (2011) 56, 110–124

www.nature.com/jhg

ORIGINAL ARTICLE

Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies

Shin Hayashi[1,2], Issei Imoto[1,3], Yoshinori Aizu[4], Nobuhiko Okamoto[5], Seiji Mizuno[6], Kenji Kurosawa[7], Nana Okamoto[1,8], Shozo Honda[1], Satoshi Araki[9], Shuki Mizutani[9], Hironao Numabe[10], Shinji Saitoh[11], Tomoki Kosho[12], Yoshimitsu Fukushima[12], Hiroshi Mitsubuchi[13], Fumio Endo[13], Yasutsugu Chinen[14], Rika Kosaki[15], Torayuki Okuyama[15], Hirotaka Ohki[16], Hiroshi Yoshihashi[17], Masae Ono[18], Fumio Takada[19], Hiroaki Ono[20], Mariko Yagi[21], Hiroshi Matsumoto[22], Yoshio Makita[23], Akira Hata[24] and Johji Inazawa[1,25]

Recent advances in the analysis of patients with congenital abnormalities using array-based comparative genome hybridization (aCGH) have uncovered two types of genomic copy-number variants (CNVs); pathogenic CNVs (pCNVs) relevant to congenital disorders and benign CNVs observed also in healthy populations, complicating the screening of disease-associated alterations by aCGH. To apply the aCGH technique to the diagnosis as well as investigation of multiple congenital anomalies and mental retardation (MCA/MR), we constructed a consortium with 23 medical institutes and hospitals in Japan, and recruited 536 patients with clinically uncharacterized MCA/MR, whose karyotypes were normal according to conventional cytogenetics, for two-stage screening using two types of bacterial artificial chromosome-based microarray. The first screening using a targeted array detected pCNV in 54 of 536 cases (10.1%), whereas the second screening of the 349 cases negative in the first screening using a genome wide high-density array at intervals of approximately 0.7 Mb detected pCNVs in 48 cases (13.8%), including pCNVs relevant to recently established microdeletion or microduplication syndromes, CNVs containing pathogenic genes and recurrent CNVs containing the same region among different patients. The results show the efficient application of aCGH in the clinical setting.
Journal of Human Genetics (2011) **56**, 110–124; doi:10.1038/jhg.2010.129; published online 28 October 2010

Keywords: array-CGH; congenital anomaly; mental retardation; screening

INTRODUCTION

Mental retardation (MR) or developmental delay is estimated to affect 2–3% of the population.[1] However, in a significant proportion of cases, the etiology remains uncertain. Hunter[2] reviewed 411 clinical cases of MR and reported that a specific genetic/syndrome diagnosis was carried out in 19.9% of them. Patients with MR often have congenital anomalies, and more than three minor anomalies can b useful in the diagnosis of syndromic MR.[2,3] Although chromosom aberrations are well-known causes of MR, their frequency determine by conventional karyotyping has been reported to range from 7.9 t 36% in patients with MR.[4–8] Although the diagnostic yield depen on the population of each study or clinical conditions, such studi

[1]Department of Molecular Cytogenetics, Medical Research Institute and School of Biomedical Science, Tokyo Medical and Dental University, Tokyo, Japan; [2]Hard Tissue Genom Research Center, Tokyo Medical and Dental University, Tokyo, Japan; [3]Department of Human Genetics and Public Health Graduate School of Medical Science, The University Tokushima, Tokushima, Japan; [4]Division of Advanced Technology and Development, BML, Saitama, Japan; [5]Department of Medical Genetics, Osaka Medical Center ar Research Institute for Maternal and Child Health, Osaka, Japan; [6]Department of Pediatrics, Central Hospital, Aichi Human Service Center, Kasugai, Japan; [7]Division of Medic Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; [8]Department of Maxillofacial Orthognathics, Graduate School, Tokyo Medical and Dental University, Toky Japan; [9]Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University Graduate School, Tokyo, Japan; [10]Department of Medical Genetics, Kyo University Hospital, Kyoto, Japan; [11]Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [12]Department of Medical Genetics, Shinsh University School of Medicine, Matsumoto, Japan; [13]Department of Pediatrics, Kumamoto University Graduate School of Medical Science, Kumamoto, Japan; [14]Department Pediatrics, University of the Ryukyu School of Medicine, Okinawa, Japan; [15]Department of Clinical Genetics and Molecular Medicine, National Center for Child Health ar Development, Tokyo, Japan; [16]The Division of Cardiology, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan; [17]The Division of Medical Genetics, Tokyo Metropolit Children's Medical Center, Tokyo, Japan; [18]Department of Pediatrics, Tokyo Teishin Hospital, Tokyo, Japan; [19]Department of Medical Genetics, Kitasato University Gradua School of Medical Sciences, Sagamihara, Japan; [20]Department of Pediatrics, Hiroshima Prefectural Hospital, Hiroshima, Japan; [21]Department of Pediatrics, Kobe Univers Graduate School of Medicine, Kobe, Japan; [22]Department of Pediatrics, National Defense Medical College, Saitama, Japan; [23]Education Center, Asahikawa Medical Colleg Asahikawa, Japan; [24]Department of Public Health, Chiba University Graduate School of Medicine, Chiba, Japan and [25]Global Center of Excellence (GCOE) Program f 'International Research Center for Molecular Science in Tooth and Bone Diseases', Tokyo Medical and Dental University, Tokyo, Japan
Correspondence: Professor J Inazawa, Department of Molecular Cytogenetics, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-k Tokyo 113-8510, Japan.
E-mail: johinaz.cgen@mri.tmd.ac.jp
Received 20 August 2010; revised 25 September 2010; accepted 30 September 2010; published online 28 October 2010

suggest that at least three quarters of patients with MR are undiagnosed by clinical dysmorphic features and karyotyping.

In the past two decades, a number of rapidly developed cytogenetic and molecular approaches have been applied to the screening or diagnosis of various congenital disorders including MR, congenital anomalies, recurrent abortion and cancer pathogenesis. Among them, array-based comparative genome hybridization (aCGH) is used to detect copy-number changes rapidly in a genome-wide manner and with high resolution. The target and resolution of aCGH depend on the type and/or design of mounted probes, and many types of microarray have been used for the screening of patients with MR and other congenital disorders: bacterial artificial chromosome (BAC)-based arrays covering whole genomes,[9,10] BAC arrays covering chromosome X,[11,12] a BAC array covering all subtelomeric regions,[13] oligonucleotide arrays covering whole genomes,[14,15] an oligonucleotide array for clinical diagnosis[16] and a single nucleotide polymorphism array covering the whole genome.[17] Because genome-wide aCGH has led to an appreciation of widespread copy-number variants (CNVs) not only in affected patients but also in healthy populations,[18–20] clinical cytogenetists need to discriminate between CNVs likely to be pathogenic (pathogenic CNVs, pCNVs) and CNVs less likely to be relevant to a patient's clinical phenotypes (benign CNVs, bCNVs).[21] The detection of more CNVs along with higher-resolution microarrays needs more chances to assess detected CNVs, resulting in more confusion in a clinical setting.

We have applied aCGH to the diagnosis and investigation of patients with multiple congenital anomalies and MR (MCA/MR) of unknown etiology. We constructed a consortium with 23 medical institutes and hospitals in Japan, and recruited 536 clinically uncharacterized patients with a normal karyotype in conventional cytogenetic tests. Two-stage screening of copy-number changes was performed using two types of BAC-based microarray. The first screening was performed by a targeted array and the second screening was performed by an array covering the whole genome. In this study, we diagnosed well-known genomic disorders effectively in the first screening, assessed the pathogenicity of detected CNVs to investigate an etiology in the second screening and discussed the clinical significance of aCGH in the screening of congenital disorders.

MATERIALS AND METHODS

Subjects

We constructed a consortium of 23 medical institutes and hospitals in Japan, and recruited 536 Japanese patients with MCA/MR of unknown etiology from July 2005 to January 2010. All the patients were physically examined by an expert in medical genetics or a dysmorphologist. All showed a normal karyotype by conventional approximately 400–550 bands-level G-banding karyotyping. Genomic DNA and metaphase chromosomes were prepared from peripheral blood lymphocytes using standard methods. Genomic DNA from a lymphoblastoid cell line of one healthy man and one healthy woman were used as a normal control for male and female cases, respectively. All samples were obtained with prior written informed consent from the parents and approval by the local ethics committee and all the institutions involved in this project. For subjects in whom CNV was detected in the first or second screening, we tried to analyze their parents as many as possible using aCGH or fluorescence *in situ* hybridization (FISH).

Array-CGH analysis

Among our recently constructed in-house BAC-based arrays,[22] we used two arrays for this two-stage survey. In the first screening we applied a targeting array, 'MCG Genome Disorder Array' (GDA). Initially GDA version 2, which contains 550 BACs corresponding to subtelomeric regions of all chromosomes except 13p, 14p, 15p, 21p and 22p and causative regions of about 30 diseases already reported, was applied for 396 cases and then GDA version 3, which contains 660 BACs corresponding to those of GDA version 2 and pericentromeric regions of all chromosomes, was applied for 140 cases. This means that a CNV detected by GDA is certainly relevant to the patient's phenotypes. Subsequently in the second screening we applied 'MCG Whole Genome Array-4500' (WGA-4500) that covers all 24 human chromosomes with 4523 BACs at intervals of approximately 0.7 Mb to analyze subjects in whom no CNV was detected in the first screening. WGA-4500 contains no BACs spotted on GDA. If necessary, we also used 'MCG X-tiling array' (X-array) containing 1001 BAC/PACs throughout X chromosome other than pseudoautosomal regions.[12] The array-CGH analysis was performed as previously described.[12,23]

For several subjects we applied an oligonucleotide array (Agilent Human Genome CGH Microarray 244K; Agilent Technologies, Santa Clara, CA, USA) to confirm the boundaries of CNV identified by our in-house BAC arrays. DNA labeling, hybridization and washing of the array were performed according to the directions provided by the manufacturer. The hybridized arrays were scanned using an Agilent scanner (G2565BA), and the CGH Analytics program version 3.4.40 (Agilent Technologies) was used to analyze copy-number alterations after data extraction, filtering and normalization by Feature Extraction software (Agilent Technologies).

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization was performed as described elsewhere[23] using BACs located around the region of interest as probes.

RESULTS

CNVs detected in the first screening

In the first screening, of 536 cases subjected to our GDA analysis, 54 (10.1%) were determined to have CNV (Figure 1; Tables 1 and 2).

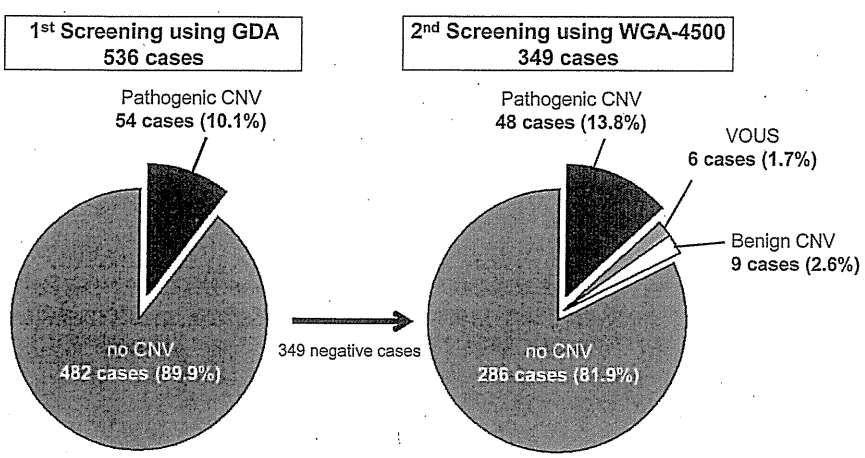

Figure 1 Percentages of each screening in the current study.

Table 1 A total of 40 cases with CNV at subtelomeric region(s) among 54 positive cases in the first screening

	Position where CNV detected				
Gender	*Loss*	*Gain*	*Corresponding disorder*[a]	*OMIM or citation*	*Parental analysis*[b]
M	1p36.33		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1q44		Chromosome 1q43-q44 deletion syndrome	#612337	
F	2q37.3		2q37 monosomy[c]	Shrimpton *et al.*[24]	
F	2q37.3		2q37 monosomy[c]	Shrimpton *et al.*[24]	
M	3q29		Chromosome 3q29 deletion syndrome	#609425	
F	5p15.33p15.32		Cri-du-chat syndrome	#123450	
M	5q35.2q35.3		Chromosome 5q subtelomeric deletion syndrome	Rauch *et al.*[25]	
F	6p25.3		Chromosome 6pter-p24 deletion syndrome	#612582	
M	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	
F	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	
M	9p24.3p24.2		Chromosome 9p deletion syndrome	#158170	
F	9q34.3		Kleefstra syndrome	#610253	
F	10q26.3		Chromosome 10q26 deletion syndrome	#609625	
F	16p13.3		Chromosome 16p13.3 deletion syndrome	#610543	
F	22q13.31		Chromosome 22q13 deletion syndrome	#606232	
M	22q13.31q13.33		Chromosome 22q13 deletion syndrome	#606232	
M		15q26.3	15q overgrowth syndrome[c]	Tatton-Brown *et al.*[27]	
F		15q26.3	15q overgrowth syndrome[c]	Tatton-Brown *et al.*[27]	
M		21q22.13q22.3	Down's syndrome (partial trisomy 21)	#190685	
M		Xp22.33	A few cases have been reported; e.g. V5-130 in Lu *et al.*[28]		
M		Xq28	Chromosome Xq28 duplication syndrome	#300815	
F	1q44		Chromosome 1q43-q44 deletion syndrome	#612337	
		8p23.2p23.3			
M	3p26.3		3p deletion syndrome[d]	Fernandez *et al.*[29]	
		12p13.33p11.22			
F	3p26.3		3p deletion syndrome[d]	Fernandez *et al.*[29]	
		16p13.3	Chromosome 16p13.3 duplication syndrome	#613458	
F	4q35.2		4q– syndrome[d]	Jones *et al.*[30]	
		7q36.3			
M	5p15.33		Cri-du-chat syndrome	#123450	
		20p13			
M	5p15.33p15.32		Cri-du-chat syndrome	#123450	
		2p25.3			
F	6q27		6q terminal deletion syndrome[d]	Striano *et al.*[31]	
		11q25			
F	6q27		6q terminal deletion syndrome[d]	Striano *et al.*[31]	
		8q24.3			
M	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	*dn*
		1q44			
M	9p24.3p24.2		Chromosome 9p deletion syndrome	#158170	
		7q36.3			
F	10p15.3p15.2		Chromosome 10p terminal deletion[d]	Lindstrand *et al.*[32]	*pat*
		7p22.3p22.2			
M	10p15.3		Chromosome 10p terminal deletion[d]	Lindstrand *et al.*[32]	
		2p25.3			
M	10q26.3		Chromosome 10q26 deletion syndrome	#609625	
		2q37.3	Distal trisomy 2q[d]	Elbracht *et al.*[33]	
M	18q23		Chromosome 18q deletion syndrome	#601808	
		7q36.3			
F	22q13.31q13.33		Chromosome 22q13.3 deletion syndrome	#606232	*pat*
		17q25.3	One case was reported	Lukusa *et al.*[34]	
M	Xp22.33/Yp11.32		Contiguous gene–deletion syndrome on Xp22.3[d]	Fukami *et al.*[35]	
		Xq27.3q28	Chromosome Xq28 duplication syndrome	#300815	

Abbreviations: F, female; CNV, copy-number variant; M, male; OMIM, Online Mendelian Inheritance in Man; *dn, de novo* CNV observed in neither of the parents.
[a]The name of disorder is based on entry names of OMIM, expect for entry names in DECIPHER and description in each cited article.
[b]*pat*, father had a balanced translocation involved in corresponding subtelomeric regions.
[c]Entry names in DECIPHER.
[d]Description in each cited article.

All the CNVs detected in the first screening were confirmed by FISH. Among the positive cases, in 24 cases one CNV was detected. All the CNVs corresponded to well-established syndromes or already described disorders (Table 1). In 16 cases two CNVs, one deletion and one duplication, were detected at two subtelomeric regions, indicating that one of parents might be a carrier with reciprocal translocation involved in corresponding subtelomeric regions, and at least either of the two CNVs corresponded to the disorders. We also performed parental analysis by FISH for three cases whose parental samples were available, and confirmed that in two cases the subtelomeric aberrations were inherited from paternal balanced translocation and in one case the subtelomeric aberrations were *de novo* (Table 1). In the other 14 cases, CNVs (25.9%) were detected in regions corresponding to known disorders (Table 2).

Table 2 Other cases among 54 positive cases in the first screening

	Position where CNV detected			
Gender	*Gain*	*Loss*	*Corresponding disorder*	*OMIM*
F		4p16.3	Ring chromosome	
		4q35.2		
M		3q22.323	BPES	#110100
M		2q22.3	ZFHX1B region	*605802
M		4q22.1	Synuclein (SNCA) region	*163890
F		7p21.1	Craniosynostosis, type 1	#123100
F		7q11.23	Williams syndrome	#194050
F		8q23.3q24.11	Langer–Giedion syndrome	#150230
M	15q11.2q13.1		Prader–Willi/Angelman	#176270/ #105830
F		17p11.2	Smith–Magenis syndrome	#182290
M		17q11.2	Neurofibromatosis, type I	+162200
M	22q11.21		DiGeorge syndrome	#188400
F		22q11.21	DiGeorge syndrome	#188400
F	Xp22.31		Kallmann syndrome 1	+308700
F	Whole X		Mosaicism	

Abbreviations: CNV, copy-number variant; F, female; M, male; OMIM, Online Mendelian Inheritance in Man.

CNVs detected in the second screening and assessment of the CNVs

Cases were subject to the second screening in the order of subjects detected no CNV in the first screening, and until now we have analyzed 349 of 482 negative cases in the first screening. In advance, we excluded highly frequent CNVs observed in healthy individuals and/or in multiple patients showing disparate phenotypes from the present results based on an internal database, which contained all results of aCGH analysis we have performed using WGA-4500, or other available online databases; for example, Database of Genomic Variant (http://projects.tcag.ca/variation/). As a result, we detected 66 CNVs in 63 cases (Figure 1; Table 3). Among them, three patients (cases 36, 42 and 44) showed two CNVs. All the CNVs detected in the second screening were confirmed by other cytogenetic methods including FISH and/or X-array. For 60 cases, we performed FISH for confirmation and to determine the size of each CNV. For five cases, cases 13, 36, 48, 57 and 63, with CNVs on the X chromosome, we used the X-array instead of FISH. For cases 4, 6, 16–19 and 34, we also used Agilent Human Genome CGH Microarray 244K to determine the refined sizes of CNVs. The maximum and minimum sizes of each CNV determined by these analyses are described in Table 3.

Well-documented pCNVs emerged in the second screening

CNVs identified for recently established syndromes. We assessed the pathogenicity of the detected CNVs in several aspects (Figure 2).[21,37,38] First, in nine cases, we identified well-documented pCNVs, which are responsible for syndromes recently established. A heterozygous deletion at 1q41–q42.11 in case 2 was identical to patients in the first report of 1q41q42 microdeletion syndrome.[39] Likewise a CNV in case 3 was identical to chromosome 1q43–q44 deletion syndrome (OMIM: #612337),[40] a CNV in case 4 was identical to 2q23.1 microdeletion syndrome,[41] a CNV in case 5 was identical to 14q12 microdeletion syndrome[42] and a CNV in case 6 was identical to chromosome 15q26-qter deletion syndrome (Drayer's syndrome) (OMIM: #612626).[43] Cases 7, 8 and 9 involved CNVs of different sizes at 16p12.1-p11.2, the region responsible for 16p11.2-p12.2 microdeletion syndrome.[44,45] Although an interstitial deletion at 1p36.23-p36.22 observed in case 1 partially overlapped with a causative region of chromosome 1p36 deletion syndrome (OMIM: #607872), the region deleted was identical to a proximal interstitial 1p36 deletion that was recently reported.[46] Because patients with the proximal 1p36 deletion including case 1 demonstrated different clinical characteristics from cases of typical chromosome 1p36 deletion syndrome, in the near term their clinical features should be redefined as an independent syndrome.[46]

CNVs containing pathogenic gene(s). In four cases we identified pCNVs that contained a gene(s) probably responsible for phenotypes. In case 10, the CNV had a deletion harboring *GLI3* (OMIM: *165240) accounting for Greig cephalopolysyndactyly syndrome (GCS; OMIM: 175700).[47] Although phenotypes of the patient, for example, pre-axial polydactyly of the hands and feet, were consistent with GCS, his severe and atypical features of GCS, for example, MR or microcephaly, might be affected by other contiguous genes contained in the deletion.[48] Heterozygous deletions of *BMP4* (OMIM: *112262) in case 11 and *CASK* (OMIM: *300172) in case 13 have been reported previously.[49,50] In case 12, the CNV contained *YWHAE* (OMIM: *605066) whose haploinsufficiency would be involved in MR and mild CNS dysmorphology of the patient because a previous report demonstrated that haploinsufficiency of *ywhae* caused a defect of neuronal migration in mice[51] and a recent report also described a microdeletion of *YWHAE* in a patient with brain malformation.[52]

Recurrent CNVs in the same regions. We also considered recurrent CNVs in the same region as pathogenic; three pairs of patients had overlapping CNVs, which have never been reported previously. Case 16 had a 3.3-Mb heterozygous deletion at 10q24.31–q25.1 and case 17 had a 2.0-Mb deletion at 10q24.32–q25.1. The clinical and genetic information will be reported elsewhere. Likewise, cases 14 and 15 also had an overlapping CNV at 6q12–q14.1 and 6q14.1, and cases 18 and 19 had an overlapping CNV at 10p12.1–p11.23. Hereafter, more additional cases with the recurrent CNV would assist in defining new syndromes.

CNVs reported as pathogenic in previous studies. Five cases were applicable to these criteria. A deletion at 3p21.2 in case 20 overlapped with that in one case recently reported.[53] The following four cases had CNVs reported as pathogenic in recent studies: a CNV at 7p22.1 in case 21 overlapped with that of patient 6545 in a study by Friedman *et al.*,[14] a CNV at 14q11.2 in case 22 overlapped with those of patients 8326 and 5566 in Friedman *et al.*,[14] a CNV at 17q24.1–q24.2 in case 23 overlapped with that in patient 99 in Buysse *et al.*[54] and a CNV at 19p13.2 in case 24 overlapped with case P11 in Fan *et al.*[55]

Large or gene-rich CNVs, or CNVs containing morbid OMIM genes. In cases inapplicable to the above criteria, we assessed CNVs

Table 3 Sixty-three cases with CNV in the 2nd screening

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
1	M	MCA/MR		del	1p36.23p36.22	arr cgh 1p36.23p36.22 (RP11-81J7→RP11-199O1)x1	ish del(1)(p36.23p36.22) (RP11-462M3+, RP11-106A3-, RP11-28P4+)dn	8 585 127	8 890 860	10 561 097	11 143 717	1 670 237	2 558 590	*dn*	32	P	
2	M	MCA/MR		del	1q41q42.11	arr cgh 1q41 (RP11-135J2→RP11-239E10)x1	ish del(1)(q41q42.11) (RP11-706L9+, RP11-224O19-, RP11-367O4-)dn	215 986 492	216 532 600	221 534 398	222 467 931	5 001 798	6 481 439	*dn*	35	P	
3	F	MCA/MR	Epilepsy	del	1q44	arr cgh 1q44 (RP11-156E8)x1	ish del(1)(q44) (RP11-56O19+, RP11-156E8-)	241 996 973	243 177 632	243 251 660	244 141 010	74 028	2 144 037		11	P	
4	F	MCA/MR		del	2q22	arr cgh 2q23.1 (RP11-72H23)x1	ish del(2)(q23.1) (RP11-375H16-)	147 651 472	147 688 255	149 855 826	149 879 891	2 167 571	2 228 419		7	P	
5	F	MCA/MR		del	14q12q13.2	arr cgh 14q12q13.2 (RP11-369O9→RP11-26M6)x1	ish del(14)(q13.2) (RP11-831F6-)	28 768 137	29 297 829	34 689 412	35 489 337	5 391 583	6 721 200		25	P	
6	M	MCA/MR	CHD	del	15q26.2	arr cgh 15q26.2q26.3 (RP11-79C10→RP11-80F4)x1	ish del(15)(q26.2) (RP11-308P12-)	93 199 415	93 214 053	96 928 421	96 942 334	3 714 368	3 742 919		6	P	
7	M	MCA/MR	CHD	del	16p12.1p11.2	arr cgh 16p12.1p11.2 (RP11-309I14→RP11-150K5)x1	ish del(16)(p11.2) (RP11-75J11-)dn	25 795 340	27 008 538	29 825 404	31 443 492	2 816 866	5 648 152	*dn*	138	P	
8	M	MCA/MR	CHD	del	16p11.2	arr cgh 16p12.1p11.2 (RP11-360L15→RP11-150K5)x1	ish del(16)(p11.2) (RP11-360L15-, RP11-388M20+, RP11-75J11+)dn	27 184 508	28 873 631	29 825 404	31 443 492	951 773	4 258 984	*dn*	134	P	
9	F	MCA/MR		del	16p11.2	arr cgh 16p11.2 (RP11-368N21→RP11-499D5)x1	ish del(16)(p11.2) (RP11-388M20-, RP11-75J11-)	28 873 841	29 408 698	32 773 200	34 476 095	3 364 502	5 602 254		125	P	
10	M	MCA/MR		del	7p14.2p13	arr cgh 7p14.2p13 (RP11-138E20→RP11-52M17)x1	ish del(7)(p14.1p13) (RP11-258I11+, RP11-2J17-, RP11-346F12-)dn	35 621 006	36 470 190	44 657 334	45 508 196	8 187 144	9 887 190	*dn*	70	P	*GLI3*
11	F	MCA/MR	Corneal opacity	del	14q22.1q22.3	arr cgh 14q22.1q22.3 (RP11-122A4→RP11-172G1)x1	ish del(14)(q22.1) (RP11-122A4-, RP11-316L15+)dn	51 964 774	51 983 834	54 730 496	55 054 754	2 746 662	3 089 980	*dn*	18	P	*BMP4*
12	M	MCA/MR	Idiopathic leukodystrophy	del	17q13.3	arr cgh 17p13.3 (RP11-294J5→RP11-357O7)x1	ish del(17)(p13.3) (RP11-4F24-, RP11-26N6+)dn	1 008 128	1 146 211	2 077 151	2 026 967	930 940	1 018 839	*dn*	22	P	*YWHAE*
13	M	MCA/MR		del	Xp11.4p11.3	arr cgh Xp11.3p11.4 (RP11-1069J5→RP11-245M24)x1	ish del(X)(p11.4p11.3) (RP11-95C16-, RP11-829C10-)dn	41 392 291	41 385 453	45 419 624	45 495 709	4 034 171	4 103 418	*dn*	9	P	*CASK*

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
14	M	MCA/MR		del	6q12q14.1	arr cgh 6q12q14.2(RP11-502L6→RP11-232L4)x1	ish del(6)(q13)(RP11-28P18-)dn	69 029 871	69 731 888	83 926 178	85 101 718	14 194 290	16 071 847	*dn*	56	P	
15	M	ZLS		del	6q14.1	arr cgh 6q14.1(RP11-343P23→RP11-217L13)x1	ish del(6)(q14.1)(RP11-5N7-,RP11-990K4-,RP11-1I6+)	75 484 004	76 145 436	79 474 428	79 851 528	3 328 992	4 367 524		10	P	
16	F	MCA/MR	CHD	del	10p12.1p11.23	arr cgh 10p12.1p11.23(RP11-89D1→91A23)x1	ish del(10)(p12.1p11.23)(RP11-164A7-,RP11-110B21-)	27 045 285	27 054 002	29 057 401	29 088 950	2 003 399	2 043 665		18	P	
17	M	MCA/MR		del	10p12.1p11.23	arr cgh 10p12.1p11.23(RP11-218D6→RP11-RP11-181I11)x1	ish del(10)(p11.23)(RP11-15H10-)	28 121 596	28 131 608	30 559 024	30 577 807	2 427 416	2 456 211		12	P	
18	M	MCA/MR	CHD	del	10q24.31q25.1	arr cgh 10q24.31q25.1(RP11-108L7→RP11-108L7)x1	ish del(10)(q24.33)(RP11-416N2-)dn	102 560 783	102 568 462	105 914 057	105 929 608	3 345 595	3 368 825	*dn*	66	P	
19	M	MCA/MR		del	10q24.32q25.1	arr cgh 10q24.32q25.1(RP11-21N23→RP11-99N20)x1	ish del(10)(q24.33)(RP11-416N2-)dn	103 917 900	103 928 189	106 005 827	106 011 522	2 077 638	2 093 622	*dn*	41	P	
20	F	MCA/MR		del	3p21.31p21.2	arr cgh 3p21.31p21.2(RP11-24F11→RP11-89F17)x1	ish del(3)(p21.31)(RP11-3B7-)	46 150 261	46 359 965	51 390 597	52 571 544	5 030 632	6 421 283		175	P	
21	M	MCA/MR		del	7p22.1	arr cgh 7p22.1(RP11-90J23→RP11-2K20)x1	ish del(7)(p22.1)(RP11-2K20-)dn	3 185 609	5 892 225	6 233 987	6 409 277	341 762	3 223 668	*dn*	28	P	
22	F	MCA/MR	Corneal opacity, CHD	dup	14q11.2	arr cgh 14q11.2(RP11-152G22→RP11-84D12)x3	ish dup(14)(q11.2)(RP11-152G22++)	20 070 731	20 306 624	20 534 929	21 264 945	228 305	1 194 214		>30	P	
23	M	MCA/MR		del	17q24.1q24.2	arr cgh 17q24.1q24.2(RP11-89L7→RP11-79K13)x1	ish del(17)(q24.1q24.2)(RP11-93E5-,RP11-89L7-,RP11-79K13-)	60 576 365	60 936 391	64 592 701	64 587 782	3 656 310	4 011 417		29	P	
24	M	SMS susp.		del	19p13.2	arr cgh 19p13.2(RP11-197O4→RP11-164D24)x1	ish del(19)(p13.2)(91O21-)	9 248 377	10 248 853	11 968 772	12 553 279	1 719 919	3 304 902	*dn*		P	
25	M	MCA/MR	Epilepsy	dup	2q11.2q13	arr cgh 2q11.2q13(RP11-90G13→RP11-79K7)x3	ish dup(2)(q11.2)(RP11-542D13++)	88 273 220	91 696 986	109 869 691	112 714 666	18 172 705	24 441 446		>30	P	
26	M	MCA/MR	CHD	dup	4p16.1	arr cgh 4p16.1(RP11-171I9)x3	ish dup(4)(p16.1)(RP11-301J10++)	8 202 790	8 520 479	9 793 705	10 638 054	1 273 226	2 435 264		17	P	

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
27	F	MCA/MR		del	7q22.1q22.2	arr cgh 7q22.1q22.2 (RP11-10D8→ RP11-72J24)x1	ish del(7)(q22.1q22.2) (RP11-124G15+,RP11-188E1-,RP11-95P19-)	97 314 215	98 261 079	105 604 920	106 451 506	7 343 841	9 137 291		135	P	
28	F	MCA/MR	Epilepsy	del	12q13.13	arr cgh 12q13.13 (RP11-74I8→ RP11-624J6)x1	ish del(12)(q13.13) (RP11-624J6-)	50 987 232	51 016 427	51 956 291	52 180 088	939 864	1 192 856		44	P	
29	M	MCA/MR		dup	16q22.3	arr cgh 16q22.3 (RP11-90L19→ RP11-89K4)x3	ish dup(16)(q22.3) (RP11-115E3++, RP11-90L19++)	70 355 260	70 848 592	72 328 913	73 785 124	1 480 321	3 429 864		25	P	
30	M	RTS susp.		dup	16q24.1	arr cgh 16q24.1 (RP11-140K16→ RP11-442O1)x3	ish dup(16)(q24.1) (RP11-770B4++, RP11-140K16++)	82 699 729	82 797 548	83 749 375	84 123 857	951 827	1 424 128		16	P	
31	M	MCA/MR	Epilepsy	del	2q24.2q24.3	arr cgh 2q24.2 (RP11-89L13→ RP11-79L13)x1	ish del(2)(q24.2) (RP11-638N12-)	160 407 234	161 072 815	162 883 584	166 923 475	1 810 769	6 516 241		28	P	*TBR1*
32	M	MCA/MR		del	3p26.2	arr cgh 3p26.2 (RP11-32F23)x1	ish del(3)(p26.2) (RP11-32F23-)	3 943 353	4 016 797	4 198 468	4 329 970	181 671	386 617		2	P	*SUMF1*
33	M	MCA/MR	IgA deficiency	del	7q21.11	arr cgh 7q21.11 (RP11-22M18)x1	ish del(7)(q21.11) (RP11-115M2+, RP11-353O4-, RP11-22M18-)	83 597 839	83 601 541	84 549 609	84 788 160	948 068	1 190 321		3	P	*SEMA3A*
34	M	MCA/MR		dup	14q32.2	arr cgh 14q32.2 (RP11-128L1)x3	ish dup(14)(q32.2) (RP11-177F8++)	99 330 486	99 337 358	99 841 558	99 845 472	504 200	514 986		7	P	*EML1, YY1*
35	M	MCA/MR	Epilepsy	dup	16p13.3	arr cgh 16p13.3 (RP11-349I11)x3	ish dup(16)(p13.3) (RP11-349I11++)	4 851 459	5 678 447	5 906 909	6 165 923	228 462	1 314 464		9	P	*A2BP1*
36	M	MCA/MR		dup	Xp22.2p22.13	arr cgh Xp22.2p22.13 (RP11-2K15→ RP11-115I10)x3	not performed (X-tiling array)	16 874 735	16 952 121	17 596 600	17 638 351	644 479	763 616		2	P	
				dup	Xp21.3	arr cgh Xp21.3 (RP11-438J7)x3	not performed (X-tiling array)	28 704 076	28 704 076	28 868 075	28 868 075	163 999	163 999		1	P	*IL1RAPL1*
37	F	MCA/MR		del	1p34.3	arr cgh 1p34.3 (RP11-89N10→ RP11-416A14)x1	ish del(1)(p34.2) (RP11-195A8+, RP11-166F21-)dn	37 830 131	38 338 265	39 466 349	39 583 645	1 128 084	1 753 514	*dn*	7	P	
38	M	MCA/MR	Hyper IgE	dup	1q25.2	arr cgh 1q25.2 (RP11-177A2→ RP11-152A16)x3	ish dup(1)(1q25.2) (RP11-177A2++, RP11-152A16++)	177 088 480	177 196 858	177 535 659	177 859 828	338 801	771 348	*dn*	9	P	
39	M	MCA/MR		del	2p24.1p23.3	arr cgh 2p24.1p23.3 (RP11-80H16→ RP11-88F6)x1	ish del(2)(p23.3) (RP11-88F6-, RP11-373D23+)dn	20 037 821	23 094 244	26 815 794	28 414 457	3 721 550	8 376 636	*dn*	86	P	
40	F	MCA/MR	CHD	del	3p26.1p25.3	arr cgh 3p26.1p25.3 (RP11-128A5→ RP11-402P11)x1	ish del(3)(p26.1p25.3) (RP11-936E1-, RP11-402P11-, RP11-1079H21+) dn	8 190 557	8 497 949	9 930 973	10 026 217	1 433 024	1 835 660	*dn*	18	P	

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
41	M	MCA/MR		del	3p22.1p21.31	arr cgh 3p22.1p21.31 (RP11-241P3→ RP11-88B8)x1	ish del(3)(p22.1) (RP11-61H16+, RP11-241P3-, RP11-78O10+)dn	41 365 663	42 284 365	48 177 538	49 198 542	5 893 173	7 832 879	*dn*	123	P	
42	M	MCA/MR	Corneal opacity	del	3p14.3p14.2	arr cgh 3p14.3p14.2 (RP11-80H18→ RP11-79J9)x1	ish del(3)(p14.2) (RP11-79J19-, RP11-230A22+)mat	57 370 434	58 149 199	58 742 633	58 887 574	593 434	1 517 140	*mat*	11	B	
				del	8q21.11q21.13	arr cgh 8q21.11q21.13 (RP11-225J6→ RP11-214E11)x1	ish del(8) (q21.11q21.13) (RP11-225J6-, RP11-48B3+)dn	75 722 961	75 821 163	81 110 557	81 493 446	5 289 394	5 770 485	*dn*	12	P	
43	M	MCA/MR		del	3q26.31q26.33	arr cgh 3q26.31-q26.33 (RP11-292L5→ RP11-355N16)x1	ish del(3)(q26.32) (RP11-300L9+, RP11-105L6-)dn	175 650 310	176 531 688	180 613 203	181 653 281	4 081 515	6 002 971	*dn*	12	P	
44	M	MCA/MR	CHD	del	13q13.2q13.3	arr cgh 13q13.2 (RP11-269G10→ 90F5)x1	ish del(13)(q13.2) (RP11-142E9+, RP11-381E21-, RP11-98D3+)dn	33 451 136	33 895 560	34 813 379	34 909 905	917 819	1 458 769	*dn*	1	P	
				del	22q11.21	arr cgh 22q11.21 (RP11-155F20→ 54C2)x1	ish del(22)(q11.21) (RP11-155F20-, RP11-590C5-, RP11-54C2-)pat	19 310 307	19 310 307	19 590 642	19 590 642	280 335	280 335	*pat*	15	B	
45	F	aRS		del	18q21.2	arr cgh 18q21.2 (RP11-89B14)x1	ish del(18)(q21.2) (RP11-159D14+, RP11-186B13-, RP11-111C17-)dn	48 218 621	49 166 752	51 288 665	51 861 143	2 121 913	3 642 522	*dn*	9	P	
46	M	MCA/MR		dup	19p13.3	arr cgh 19p13.3 (RP11-49M3→ RP11-268O21)x3		1 095 485	2 418 857	3 499 581	4 460 252	1 080 724	3 364 767	*dn*	113	P	
47	F	MCA/MR	Autism	del	19p13.3	arr cgh 19p13.3 (RP11-30F17→ RP11-330I7)x1	ish del(19)(p13.3) (RP11-330I7-)dn	4 844 383	6 043 505	6 859 584	6 881 792	816 079	2 037 409	*dn*	23	P	
48	M	MCA/MR		del	Xp11.3	arr cgh Xp11.3 (RP11-151G3→ RP11-48J14)x0	ish del(X)(p11.3) (RP11-203D16-)mat	44 403 077	44 433 162	46 795 584	46 795 588	2 362 422	2 392 511	*mat*	18	P	
49	M	MCA/MR		dup	3p26.3	arr cgh 3p26.3 (RP11-630I)x3	ish dup(3)(p26.3) (RP11-630I++)pat	2 377 366	2 443 357	2 619 407	2 628 216	176 050	250 850	*pat*	1	B	
50	M	MCA/MR		dup	5p14.3	arr cgh 5p14.3 (RP11-91A5)x3	ish dup(5)(p14.3) (RP11-91A5++)pat	19 046 234	19 485 530	19 656 108	20 798 445	170 578	1 752 211	*pat*	1	B	
51	M	MCA/MR		dup	5q13.3	arr cgh 5q13.1 (RP11-40N8→ RP11-91C10)x3	ish dup(5)(q13.1) (RP11-105A11++)mat	66 417 271	66 481 371	67 501 700	67 838 977	1 020 329	1 421 706	*mat*	3	B	

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a] Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)	Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
52	M	MCA/MR		dup	7p22.3	arr cgh 7p22.3 (RP11-23D23)x3	ish dup(7)(p22.3) (RP11-23D23++, RP11-1133D5+)mat	1	954 016	954 584	1 101 944	568	1 101 943	*mat*	12	B	
53	F	MCA/MR		dup	8p23.2	arr cgh 8p23.2 (RP11-79I19→ RP11-89I12)x3	ish dup(8)(p23.2) (RP11-89I19++, RP11-89I12++)pat	3 324 954	3 726 061	4 564 671	5 973 493	838 610	2 648 539	*pat*	1	B	
54	M	MCA/MR		dup	9q33.1	arr cgh 9q33.1 (RP11-150L1)x3	ish dup(9)(q33.1) (RP11-150L1++)pat	118 980 752	119 452 372	119 614 984	120 011 559	162 612	1 030 807	*pat*	2	B	
55	F	MCA/MR		dup	10q22.3	arr cgh 10q22.3 (RP11-79M9)x3	ish dup(10)(q22.3) (RP11-79M9++)mat	77 356 915	77 718 484	77 873 148	78 230 039	154 664	873 124	*mat*	1	B	
56	M	MCA/MR	ELBW, hepato-blastoma	dup	12q21.31	arr cgh 12q21.31 (RP11-91C4)x3	ish dup(12)(q21.31) (RP11-91C4++, RP11-142L2+)pat	80 924 954	82 678 148	82 830 190	85 768 388	152 042	4 843 434	*pat*	3	B	
57	M	GS		del	Xp11.23	arr cgh Xp11.23 (RP11-876B24) x0 mat	not performed (X-tiling array)	47 752 808	47 747 918	47 852 109	47 868 412	104 191	115 604	*mat*	3	B	
58	M	MCA/MR		dup	8q11.23	arr cgh 8q11.23 (RP11-221P7)x3	ish dup(8)(q11.23) (RP11-221P7++, RP11-26P22++)	53 665 974	53 717 675	54 235 229	54 576 654	517 554	910 680		3	VOUS	
59	F	MCA/MR	Micro-cephaly	dup	10q11.21	arr cgh 10q11.21 (RP11-178A10)x3	ish dup(10)(q11.21) (RP11-178A10++)	41 986 946	42 197 693	42 320 775	43 603 027	123 082	1 616 081		15	VOUS	
60	M	MCA/MR		dup	11p14.2p14.1	arr cgh 11p14.2p14.1 (RP11-1L12)x3	ish dup(11) (p14.2p14.1) (RP11-1L12++)	26 723 462	27 033 270	27 213 374	27 445 504	180 104	722 042		4	VOUS	
61	F	MCA/MR		dup	12p11.1	arr cgh 12p11.1 (RP11-88P4)x3	ish dup(12)(p11.1) (RP11-472A10++)	33 333 493	33 359 944	33 572 956	33 572 956	213 012	239 463		2	VOUS	
62	F	aRS		dup	12q21.31	arr cgh 12q21.31 (RP11-91I24→ RP11-91C4)x3	ish dup(12)(q21.31) (RP11-91C4++, RP11-142L2++)	79 949 648	82 172 368	83 968 319	85 768 388	1 795 951	5 818 740		12	VOUS	
63	F	MR	Congenital myopathy	dup	Xq12	arr cgh Xq12 (RP11-90P17→ RP11-383C12)x3	Not performed (X-tiling array)	66 212 661	66 216 353	66 921 699	66 948 538	705 346	735 877		1	VOUS	

Abbreviations: aRS, atyplical Rett syndrome; B, benign; CNV, copy-number variant; *dn*: *de novo* CNV observed in neither of the parents; ELBW, extremely low birth weight; FISH, fluorescence *in situ* hybridization; GS, Gillespie syndrome; *mat*: CNV identified also in mother; P, pathogenic; *pat*: CNV identified also in father; RTS, Rubinstein–Taybi syndrome; SMS, Smith–Magenis syndrome; VOUS, variant of uncertain clinical significance; ZLS, Zimmermann–Laband syndrome.
[a]The sizes were estimated by WGA-4500, X-array, FISH or Agilent Human Genome CGH microarray 244K.
[b]The notation systems is based on ISCN2005.[36]
[c]The number of protein-coding genes contained in the respective CNVs.
[d]The result of CNV assessment.