CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Severe Growth Retardation, Delayed Bone Age, and Facial Dysmorphism in Two Patients with Microduplications in 2p16 → p22

Anya Blassnig-Ezeh,[1a] Claude Bandelier,[2a] Anne Frühmesser,[3] Nicole Revencu,[2] Birgit Krabichler,[3] Véronique Beauloye,[4] Marie Ravoet,[2] Christine Fauth,[3] Johannes Zschocke,[3] Burkhard Simma,[1] and Dieter Kotzot[3]*

[1]Department of Pediatrics, Landeskrankenhaus Feldkirch, Academic Teaching Hospital, Austria

[2]Center for Human Genetics, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

[3]Division of Human Genetics, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria

[4]Paediatric Endocrinology Unit, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

Manuscript Received: 3 January 2013; Manuscript Accepted: 16 July 2013

Interstitial duplications of the short arm of chromosome 2 have been rarely described. Here, we report on two unrelated patients with overlapping chromosome 2p16 → p22 de novo microduplications found by SNP-array analysis. The affected individuals were an 8-year-3-month-old boy with a direct duplication of approximately 14.6 Mb harboring 63 genes, and a 12-year-old girl with a direct duplication of around 9.6 Mb harboring 48 genes. Both patients have severe growth retardation, delayed bone age, prominent veins on trunk and extremities, total IGF1 level in the low range, mild developmental delay, and facial dysmorphism such as relative macrocephaly, a broad and prominent forehead, and a large anterior fontanelle. Comparison with patients previously reported in the literature and in the DECIPHER 5.1 and ECARUCA databases indicates a common region of interest of around 1.9 Mb responsible for most of the features. Two candidate genes (*EPAS* and *RHOQ*), may be particularly relevant for the marked growth retardation and developmental delay.

Key words: chromosomal rearrangements; delayed bone age; growth retardation; microduplication 2p

How to Cite this Article:
Blassnig-Ezeh A, Bandelier C, Frühmesser A, Revencu N, Krabichler B, Beauloye V, Ravoet M, Fauth C, Zschocke J, Simma B, Kotzot D. 2013. Severe growth retardation, delayed bone age, and facial dysmorphism in two patients with microduplications in 2p16 → p22.
Am J Med Genet Part A 161A:3176–3181.

INTRODUCTION

Pure duplications involving the short arm of chromosome 2 have been reported in more than 20 patients [Aviram-Goldring et al., 2000; Thangavelu et al., 2004]. The duplication sizes have been quite variable, and in most cases the chromosomal breakpoints were defined by conventional karyotyping only. No association of specific clinical features with certain duplicated segments has been reported except a possible association of neural tube defects with a locus on 2p24 [Lurie et al., 1995; Winsor et al., 1997; Thangavelu et al., 2004].

Growth retardation has been frequently observed in patients with 2p duplications but length and height below the 3rd centile are unspecific features of many chromosomal rearrangements and may be influenced by many different genes and exogeneous factors.

Here, we report on two patients with growth retardation, delayed bone age, mild developmental delay, and similar dysmorphic features caused by overlapping duplications on the short arm of chromosome 2.

Conflict of interest: none.

[a]These two authors contributed equally to this work.

*Correspondence to:
Dr. med. D. Kotzot, Division of Human Genetics, Department of Medical Genetics, Molecular and Clinical Pharmacology, Schoepfstr. 41, A-6020 Innsbruck, Austria.
E-mail: dieterkotzot@gmx.de

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 24 September 2013
DOI 10.1002/ajmg.a.36176

CLINICAL REPORT

The first patient, an 8-year-old boy, is the second child of healthy, nonconsanguineous, Caucasian parents of normal stature (mother´s height 166 cm, father´s height 184 cm). At birth, the mother was 30 years old and the father 32 years old. The family history is unremarkable. An older male sibling is healthy and of normal stature. Routine ultrasound during pregnancy showed decelerated growth from 32 weeks onward. Delivery at 42 weeks and 2 days by cephalic presentation was normal, the child´s Apgar score was 9/10/10. Birth weight was 2,880 g (−1 SD), length was 47 cm (−1.5 SD), and occipitofrontal head circumference (OFC) was 36 cm (0 SD).

The boy displayed persistent inspiratory stridor since birth. At the age of 12 months he had an incarcerated inguinal hernia and hydrocele. At that time he had achieved sitting without support and standing with support; his weight was 6.47 kg (−3 SD), length was 70.5 cm (−2 SD), and OFC was 46.5 cm (−0.5 SD). Facial dysmorphism included relative macrocephaly with a broad and prominent forehead, a large anterior fontanelle, and sparse eyebrows. Prominent veins were noted on forehead, chest, and upper abdomen. Chest X-ray, abdominal and cranial ultrasound, and echocardiography were normal. Complete blood count and laboratory studies including thyroid function and screening for celiac disease revealed no cause for the poor weight gain. Cerebral MRI, ophthalmological evaluation, and BERA at a follow-up visit at the age of 17 months showed no pathological results. At that time the patients weight was 7.5 kg (−3 SD), length was 74 cm (−2 SD), and OFC was 48 cm (−0.1 SD).

Recently, at the age of 8 years and 3 months the boy was again referred for further evaluation. His weight was 16 kg (−3.2 SD), his length was 110.5 cm (−3 SD), and OFC was 53.5 cm (+0.5 SD). The past 7 years had been uneventful, and the child was described as generally healthy. The parents reported walking at 18 months. At present he attends the first year of primary school but requires a special-needs teacher. A nonverbal intelligence test showed an IQ of 79 in the lower normal range. The previously noted dysmorphic features such as a broad forehead, sparse eyebrows, and prominent veins were still present (Fig. 1A–C). Inner canthal distance (3 cm), interpupillary distance (5.5 cm), and outer canthal distance (8 cm) were around the 75th centile. Total hand length (12 cm) and middle finger length (5 cm) were below the 3rd centile. Also hyperopia of 4.5 diopters was present. Endocrinological assessment showed normal thyroid function and normal values for HGH, ACTH, Cortisol, LH, and FSH. IGF1 (85, 129, and 58.2 ng/ml (normal range 88–452 ng/ml)) and IGFBP3 (3.8 mg/L, and 1.7 mg/L (normal range 2.1–7.7 mg/L)) were repeatedly in the lower normal range or below. Furthermore, HGH stimulation test with arginine hydrochloride showed sufficient release of HGH. X-ray of the left hand revealed markedly delayed bone age of 3 yrs and 5 yrs in wrist and forehand, respectively (assessment according to Greulich and Pyle). It was decided to commence growth hormone therapy starting with a dose of 0.035 mg/kg/day somatropin. Before initiating GH therapy, insulin (15.7 μU/ml) and C-peptide (2.86 ng/ml) values were confirmed to be in the normal range (2.6–24.9 μU/ml and 0.9–7.1 ng/ml, respectively).

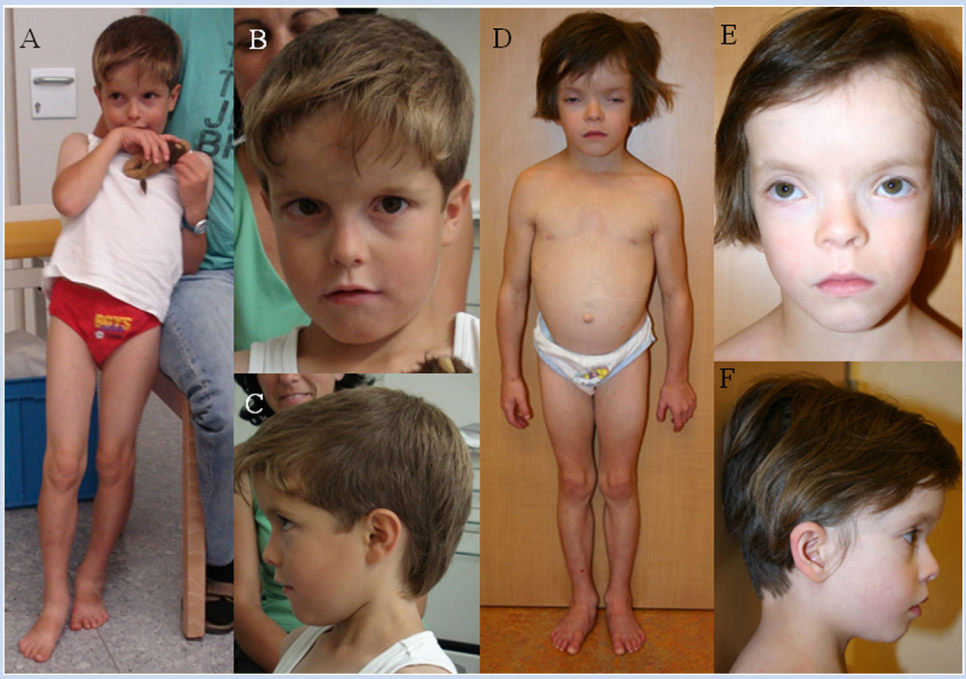

FIG. 1. A–C: Patient 1 at the age of 8 years and 3 months. Dysmorphic features included short stature, relative macrocephaly, a broad forehead, and sparse eyebrows. D–F: Patient 2 at the age of 7 years: relative macrocephaly, large and prominent forehead, hypertelorism, short stature, hypotrophy, hyperlordosis, and thin skin with prominent veins.

Patient two, a 12-year-old girl, is the second child of healthy, nonconsanguineous, Belgian parents with short stature: mother's height is 154 cm (−2.1 SD) and father's height is 156.5 cm (−3.6 SD). Otherwise, the familial history was unremarkable. At birth, the mother was 30 years old and the father 36 years old. The patient was born at 38 weeks by caesarean after a pregnancy complicated by intrauterine growth retardation and oligohydramnios detected in the third trimester. Birth weight was 2,300 g (−2.2 SD), length was 43 cm (−3.1 SD), and OFC was 33.5 cm (−1 SD). Because of a weak suck the patient required tube feeding during the first days.

On examination at 9 months the girl was noted to have severe growth retardation, was unable to sit without support and showed moderate cranio-facial dysmorphism including a prominent forehead, relative macrocephaly, marked hypertelorism, narrow palpebral fissures, thin lips and large anterior fontanelle. Brain MRI was normal. She started to walk at 2 years of age.

At the age of 3 years the patient was referred to us for further evaluation. Her growth parameters were: weight 7.65 kg (−4.5 SD), height 73 cm (−5.5 SD), and OFC 46.5 cm (−2 SD). On physical examination the previously observed dysmorphism as well as thin skin, prominent veins on trunk, forehead and extremities, clinodactyly of the fifth fingers, palmar and plantar erythema, and lumbar hyperlordosis were noted. The anterior fontanelle was still open. She had feeding difficulties and recurrent respiratory tract infections. At the age of 4 years, a left diaphragmatic hernia was diagnosed and surgically repaired. She went to regular school and was described as "choleric" with a difficulty to concentrate but socially integrated. She required speech therapy. At the age of 5 years, endocrinologic assessment revealed normal thyroid function and total IGF1 in the low range (64 ng/ml; normal range: 78–405 ng/ml). The growth velocity was 5.8 cm/year (−0.8 SD) and the bone age was 2 years (assessment according to Greulich and Pyle). At that time, height was 86 cm (−5.3 SD) and weight was 10.65 kg (−5.1 SD). Taking into account the fact that she was small for gestational age without catch-up growth, growth hormone (GH) therapy was started (somatropine 0.035 mg/kg/day). Treatment had little effect and was stopped after 19 months. The IGF1 level (86 ng/ml) did not increase during the GH therapy. At the age of 8 years inner canthal distance was 3.5 cm and outer canthal distance was 9.7 cm; both above the 97th centile. At the age of 10 years, gastroesophageal reflux was treated by omeprazole and a hypercaloric diet was started. At the age of 10 years and 6 months, bone age was 6 years and 10 months.

Currently, at the age of 12 years, she has a height of 114.0 cm (−5.6 SD), weight of 18.5 kg (−5.8 SD), OFC of 49.4 cm (−2.2 SD), and a body mass index of 14.2 (−2.3 SD). She is still prepubertal. She attends normal school, but requires extra help. The previously described craniofacial dysmorphism such as a prominent forehead, relative macrocephaly, marked hypertelorism, narrow palpebral fissures, and thin lips are still present (Fig 1D–F).

METHODS AND RESULTS

Investigation of Patient 1 and his parents with the Illumina CytoChip-12v2.1 SNP-array according to the manufacturer´s instructions and FISH with locus specific probes (RP11-17L5 and RP11-347F1) revealed a de novo direct duplication of approximately 14.6 Mb harboring 63 genes, breakpoints between rs2540240 (39,791,569 bp) and rs2540229 (39,797,559 bp) in 2p22.1 and between rs843622 (54,401,986 bp) and rs1682139 (54,410,054 bp) in 2p16.2, respectively in the son (Fig. 2A,B).

Investigation of Patient 2 with the 250K NspI Affymetrix SNP-array according to the manufacturer´s instructions and FISH with locus specific probes (RP11-391M15 and RP11-82P15) revealed a direct duplication of approximately 9.6 Mb harboring 48 protein-coding Ensembl genes. Breakpoints were between SNP_A-2157520 = (rs11895590) (42,259,573 bp) and SNP_A-2272083 = (rs10200000) (42,295,716 bp) in 2p21 and between SNP_A-2189059 = (rs12622885) (51,981,249 bp) and SNP_A-4214575 = (rs6759448) (52,036,133 bp) in 2p16.3, respectively (Fig. 2C,D). Karyotypes of both parents were normal.

In both patients endocrinological investigations were performed according to standard protocols in routine laboratories.

DISCUSSION

Submicroscopic deletions and duplications of the short arm of chromosome 2 have been rarely published. Mégarbané et al. [1997] reviewed the literature for patients with duplication in 2p and found inconsistent phenotypes. However, breakpoints in only few patients were defined by molecular array techniques [Guilherme et al., 2009; Kasnauskiene et al., 2013]. Several authors describe growth retardation, cardiopulmonary malformations, and depending on the latter, poor life expectancy. Delayed bone age was reported in two cases only [Parruti et al., 1989; Sawyer et al., 1994]. Diaphragmatic hernia has been described as a possible complication in duplication 2p syndrome; a minimal overlapping segment common for the respective patients was delineated on 2p23–p25 [Lurie et al., 1995]. Although left-sided diaphragmatic hernia was identified in Patient 2, the duplication does not encompass the previously suggested critical region which is more telomeric. Our Patient 2 and the fetus reported by Guilherme et al. [2009] indicate a region of interest for diaphragmatic hernia in 2p16.3 between 49.5 Mb and 52 Mb. Termination of pregnancy at 23 weeks of gestation in the patient reported by Guilherme et al. [2009] makes a more specific phenotypic comparison difficult.

The major clinical hallmark in our patients is severe growth retardation which was non-responsive to regular doses of GH in Patient 2. A search in the ECARUCA database revealed two additional patients with prenatal onset of short stature, one with duplication 2p21 → p24 and a second with duplication 2p12 → 21 [Mégarbané et al., 1997; Magee et al., 1998]. The respective breakpoints were determined by conventional karyotyping and are therefore imprecise but in conjunction with our observations indicate one or more candidate genes for growth in 2p21. The patient reported by Kubo et al. [1999] also showed a large fontanelle, prominent forehead, and hypertelorism similar to our patients. Kasnauskiene et al. [2013] described a 17-year-old girl with growth retardation, macrocephaly, developmental delay, and a de novo duplication of 2p16.1–p22.1 (position 40,059,584–57,546,352; NCBI build 36). Although the duplication is telomerically larger than in our patient (approximately 5 Mb and 7 Mb, respectively), the phenotype is very similar (Table I). A more precise phenotypic comparison is hampered by limited clinical data.

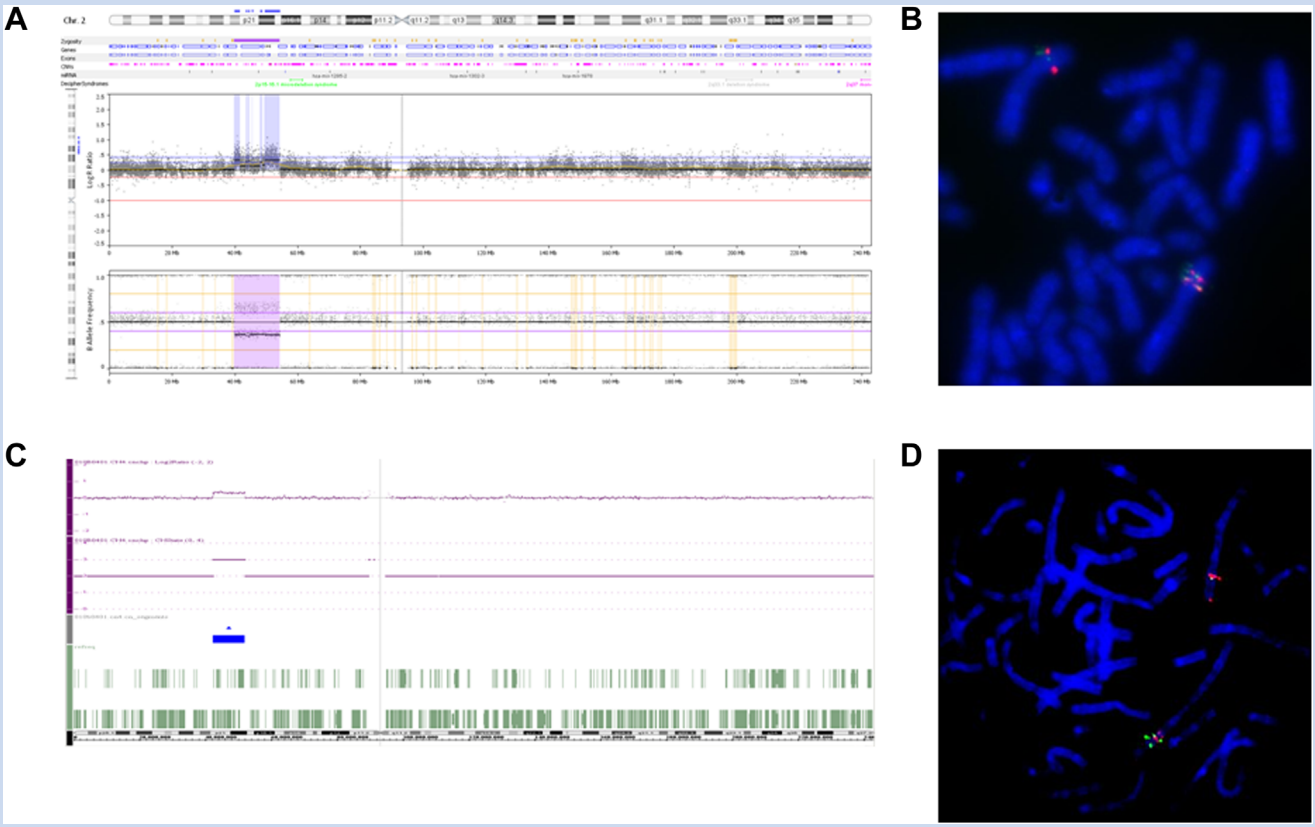

FIG. 2. A: Result of the SNP array analysis with the Illumina® CytoChip-12v2.1 (>300,000 SNPs) of chromosome 2 showing the duplication 2p16.2 → p22.1. The data were evaluated with the software Nexus Copy Number Version 6. Copy number information is given as log R ratio (LRR), the logged ratio of observed probe intensity to expected intensity (Norm = 0) (upper panel). Genotype information is given as B allele frequency (BAF), with homozygosity for the arbitrary "B" allele indicated by BAF = 1, homozygosity for the arbitrary "A" allele indicated by BAF = 0, and heterozygosity for A/B indicated by BAF = 0.5 (lower panel). The upper diagram shows a clear gain of signal intensity in the duplicated region, the lower diagram shows a fork of the B allele values in the same region. B: FISH analysis with the clones RP11-17L5 (2p21; 43,429,488 bp–43,597,601 bp; signal: red) and RP11-347F1 (2p16.3; 51,195,545 bp–51,361,176 bp; signal: green) (BlueGnome, Cambridge, UK) revealed a direct duplication. Shown is only a part of a metaphase including both chromosomes 2. C: Molecular results in patient 2: Affymetrix GeneChip Human Mapping 250K SNP-array analysis of chromosome 2 showing p16.3 → p21 duplication. Copy number and log2 ratio were obtained using Affymetrix Genotyping Console v4.0. Data were analyzed with Cartagenia software. D: FISH analysis with the RP11-391M15 clone (2p21; 42,992,855 bp—43,173,584 bp; signal: green) (selected from the Ensembl database and obtained from the BACPAC Resources Center at the Children's Hospital Oakland Institute, Oakland, CA) and RP11-82P15 clone (2p16.3; 51,136,218 bp—51,219,186 bp; signal: red) (BlueGnome) revealed direct duplication.

Evaluation of the phenotypical traits listed in the DECIPHER 5.1 database in patients with similar duplications in 2p16 → p22 for growth retardation allowed us to narrow the region of interest from 9.6 Mb in Patient 2 of this report to around 1.9 Mb (45.4–47.3 Mb) harboring 19 genes (Fig. 3). Two of these, *endothelian PAS domain protein 1* (*EPAS1* or *HIF-2α*) and *ras homolog family member Q* (*RHOQ* or *TC10*) are interesting candidate genes for growth retardation.

EPAS1 encodes a transcription factor involved in oxygen mediated induction of gene activity [Hu et al., 2003]. Expression is reported in various organs from fetal to adult development including bone and connective tissues [Patel and Simon, 2008]. Of particular interest is the regulation of enchondral ossification during skeletal growth by activating type X collagen (*COL10A1*), matrix metalloproteinase 13 (*MMP13*), and vascular endothelial growth factor (*VEGFA*) [Saito et al., 2010]. Patients with osteartrhitis show cartilage destruction due to chondrocyte hypertrophy and apoptosis and increased levels of *EPAS1* [Ryu et al., 2012]. It is tempting to speculate that increased levels of *EPAS1* due to three copies of the gene might also cause increased chondrocyte apoptosis which might affect growth and final height.

RHOQ or *TC10* is a member of the RAS superfamily of small GTP-binding proteins, which are molecular switches in signal transduction. Expression has been described in bone and connective tissues (NCBI—Unigene). *RHOQ* is involved in insulin-stimulated glucose uptake [Baumann et al., 2000]. Moreover, *IGF1* activates *RHOQ*, which is important in axon elongation in developing neurons indicating relevance for psychomotor

TABLE I. Clinical Features of our Patients and the Patient Reported by Kasnauskiene et al. [2013]

	Kasnauskiene et al. [2013]	Patient 1	Patient 2
Short stature at birth	+ (?)	+	+
Short stature at the last evaluation	+ (?)	+	+
Macrocephaly	+	+	+
Developmental delay	+	+	+
Muscular hypotonia	+		+
Abnormal behaviour	+		
Delayed bone age	n.a.	+	+
Large anterior fontanelle		+	+
High and prominent forehead	+	+	+
Prominent veins		+	+
Hypertelorism		+	+
Tapering fingers	+	+	
Feeding difficulties	+	+	+
Others			Diaphragmatic hernia

?, not clearly mentioned in the paper; n.a., not available.

development [Dupraz et al., 2009]. Overexpression of *RHOQ* due to the duplication and a negative feed-back might also explain the low-normal values for total IGF1 in both patients and IGFBP3 in Patient 1. Thus, as IGF1 and IGFBP3 are important for pre- and postnatal growth, low-normal levels might reflect the pathophysiological basis for growth retardation in our patients.

Furthermore, particularly in muscle and adipose tissue *RHOQ* is relevant for translocation of the GLUT4 glucose transporter from intracellular storage sites to the cell surface [Chiang et al., 2001]. In some tissues increased *GLUT4* at the cell surface might reduce glucose level, which by a negative feed-back decreases insulin production resulting subsequently in relatively reduced expression of *RHOQ* and *GLUT4*.

A UCSC Genome Browser database analyses revealed repeats of identical short interspersed nuclear elements (mammalian interspersed repetitive (MIR3)) which belongs to the family of short interspersed nuclear elements (SINE) in both breakpoint regions of patient 1, which according to the array data have a size of around 6 kb and around 8 kb, respectively. This indicates that the duplication might have been formed by nonallelic homologous recombination which is thought to be the most common mechanism of formation for microdeletions and microduplications. In Patient 2

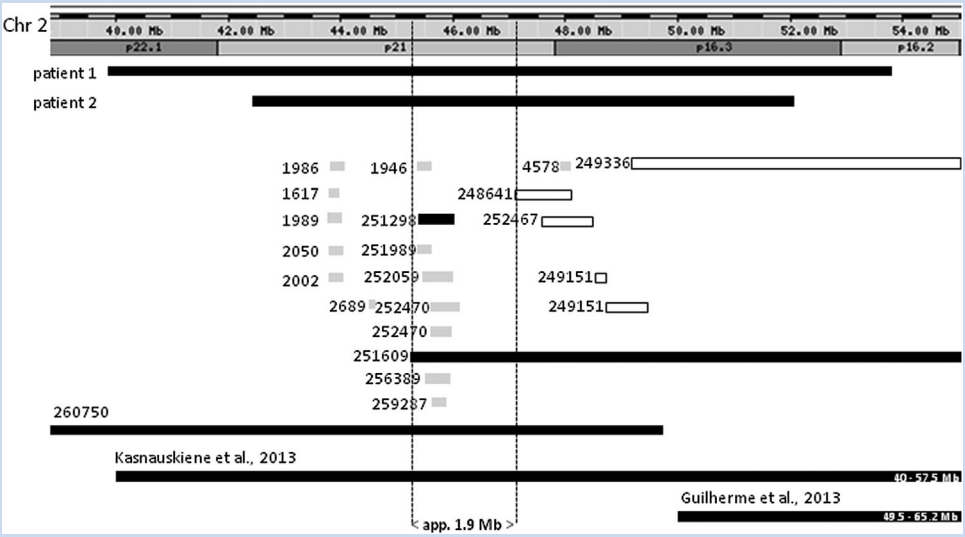

FIG. 3. Cut-out from DECIPHER v5.1 showing only cases with duplications overlapping the duplications in our patients and in the patients reported by Kasnauskiene et al. [2013] and Guilherme et al. [2009] (▬ = short stature, ▭ = no short stature, ▭ = no information about growth).

the breakpoint regions are much larger in size (around 36 kb and 55 kb) and contain a large number of repetitive elements, also compatible with formation by nonallelic homologous recombination.

In conclusion, the combination of pre- and post-natal growth retardation, delayed bone age, and specific craniofacial dysmorphic features in our patients point toward a clinically recognizable 2p16 → p22 duplication syndrome. Molecular characterization of new cases with overlapping duplications will contribute to a more precise assessment of genotype–phenotype correlations.

ACKNOWLEDGMENTS

We thank the patients and their families for their support of this publication.

REFERENCES

Aviram-Goldring A, Fritz B, Bartsch C, Steuber E, Daniely M, Lev D, Chaki R, Barkai G, Frydman M, Rehder H. 2000. Molecular cytogenetic studies in three patients with partial trisomy 2p, including CGH from paraffin-embedded tissue. Am J Med Genet 91:74–82.

Baumann CA, Ribon V, Kanzaki M, Thurmond DC, Mora S, Shigematsu S, Bickel PE, Pessin JE, Saltiel AR. 2000. CAP defines a second signalling pathway required for insulin-stimulated glucose transport. Nature 407:202–207.

Chiang SH, Baumann CA, Kanzaki M, Thurmond DC, Watson RT, Neudauer CL, Macara IG, Pessin JE, Saltiel AR. 2001. Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. Nature 410:944–948.

Dupraz S, Grassi D, Bernis ME, Sosa L, Bisbal M, Gastaldi L, Jausoro I, Cáceres A, Pfenninger KH, Quiroga S. 2009. The TC10-Exo70 complex is essential for membrane expansion and axonal specification in developing neurons. J Neurosci 29:13292–13301.

Guilherme R, Guimiot F, Tabet AC, Khung-Savatovsky S, Gauthier E, Nouchy M, Benzacken B, Verloes A, Oury JF, Delezoide AL, Aboura A. 2009. Abnormal muscle development of the diaphragm in a fetus with 2p14–p16 duplication. Am J Med Genet A 149A:2892–2897.

Hu CJ, Wang LY, Chodosh LA, Keith B, Simon MC. 2003. Differential roles of hypoxia-inducible factor 1a (HIF-1a) and HIF-2a in hypoxic gene regulation. Mol Cell Biol 23:9361–9374.

Kasnauskiene J, Cimbalistiene L, Utkus A, Ciuladaite Z, Preiksaitiene E, Pečiulytė A, Kučinskas V. 2013. Two new de novo interstitial duplications covering 2p14-p22.1: Clinical and molecular analysis. Cytogenet Genome Res 139:52–58.

Kubo T, Kakinuma H, Nakamura T, Kitatani M, Ozaki M, Takahashi H. 1999. Infantile spasms in a patient with partial duplication of chromosome 2p. Clin Genet 56:93–94.

Lurie IW, Ilyina HG, Gurevich DB, Rumyantseva NV, Naumchik IV, Castellan C, Hoeller A, Schinzel A. 1995. Trisomy 2p: Analysis of unusual phenotypic findings. Am J Med Genet 55:229–236.

Magee AC, Humphreys MW, McKee S, Stewart M, Nevin NC. 1998. De novo direct duplication 2 (p12-->p21) with paternally inherited pericentric inversion 2p11.2 2q12.2. Clin Genet 54:65–69.

Mégarbané A, Souraty N, Prieur M, Theophile D, Chédid P, Augé J, Vekemans M. 1997. Interstitial duplication of the short arm of chromosome 2: Report of a new case and review. J Med Genet 34:783–786.

Parruti G, Di Ilio C, Calabrese G, Stuppia L, Guanciali Franchi P, Aceto A, Palka G. 1989. A new case of partial 2p trisomy due to de novo interstitial duplication 2 p21–22. Ann Genet 32:55–58.

Patel SA, Simon MC. 2008. Biology of hypoxia-inducible factor-2alpha in development and disease. Cell Death Differ 15:628–634.

Ryu JH, Shin Y, Huh YH, Yang S, Chun CH, Chun JS. 2012. Hypoxia-inducible factor-2α regulates Fas-mediated chondrocyte apoptosis during osteoarthritic cartilage destruction. Cell Death Differ 19:440–450.

Saito T, Fukai A, Mabuchi A, Ikeda T, Yano F, Ohba S, Nishida N, Akune T, Yoshimura N, Nakagawa T, Nakamura K, Tokunaga K, Chung UI, Kawaguchi H. 2010. Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. Nat Med 16:678–686.

Sawyer JR, Jones E, Hawks FF, Quirk JG, Jr. Cunniff C. 1994. Duplication and deletion of chromosome band 2(p21p22) resulting from a familial interstitial insertion (2;11)(p21;p15). Am J Med Genet 49:422–427.

Thangavelu M, Frolich G, Rogers D. 2004. Partial duplication 2p as the sole abnormality in two cases with anencephaly. Am J Med Genet A 124A:170–172.

Winsor SH, McGrath MJ, Khalifa M, Duncan AM. 1997. A report of recurrent anencephaly with trisomy 2p23-2pter: Additional evidence for the involvement of 2p24 in neural tube development and evaluation of the role for cytogenetic analysis. Prenat Diagn 17:665–669.