detail in only a few cases. Pangalos *et al.* reported three patients with mirror duplication who had the breakpoints at 21q22.3,[2] as in the present patient. More detailed analysis, however, indicated that chromosome breakpoints were variable among those including the present patient (Table 2; Fig. 2).

The present patient, as well as patient B described by Pangalos *et al.*, had TOF. Barlow *et al.* reported association of the region around the *PFKL* gene on 21q22.3 with TOF.[6] Although the detailed information is not available, similarity of chromosomal organization between the two patients (Table 2; Fig. 2) may confirm the report by Barlow *et al.*

In addition to the present patient, all three patients reported by Pangalos *et al.* were phenotypically DS, and monosomy of distal 21q22.3, ranging from the telomere to *PFKL*, apparently had no significant effect on the expression of DS phenotype. Based on analysis of genotype–phenotype correlation of the present case, the region from RP11-323F14 to RP11-135B17 does not appear to play an important role for the phenotype of DS. Monosomy in the present patient involved three genes: *ITGB2 (CD18)*, *COL6A1*, and *COL6A2* (Fig. 2). The mutations of *ITGB2* gene and *COL6A1*/*COL6A2* gene are responsible for leukocyte adhesion deficiency and Bethlem myopathy, respectively.[7,8] However, since both gene products work as a heterodimer, the monosomic state would not influence the protein structure. Therefore it is not surprising that the present patient lacked symptoms suggestive of infectious susceptibility or myopathy. Likewise, the present patient lacked any other phenotypic feature suggestive of monosomy 21q22.3, such as large ears, high nasal bridge, or retromicrognathia, which have been described in other reports.[9]

As discussed here, mirror duplication of chromosome 21 can provide an opportunity to precisely determine phenotype–genotype correlation. Further accumulation of these cases and detailed cytogenetic and molecular analysis are warranted.

References

1 Pfeiffer RA, Loidl J. Mirror image duplications of chromosome 21. Three new cases and discussion of the mechanisms of origin. *Hum. Genet.* 1982; **62**: 361–3.

2 Pangalos C, Theophile D, Sinet PM *et al.* No significant effect of monosomy for distal 21q22.3 on the Down syndrome phenotype in 'mirror' duplications of chromosome 21. *Am. J. Hum. Genet.* 1992; **51**: 1240–50.

3 Delabar JM, Theophile D, Rahmani Z *et al.* Molecular mapping of twenty-four features of Down syndrome on chromosome 21. *Eur. J. Hum. Genet.* 1993; **1**: 114–24.

4 Korenberg JR, Chen XN, Schipper R *et al.* Down syndrome phenotypes: The consequences of chromosomal imbalance. *Proc. Natl Acad. Sci. USA* 1994; **91**: 4997–5001.

5 Jackson JF, North ER III, Thomas JG. Clinical diagnosis of Down's syndrome. *Clin. Genet.* 1976; **9**: 483–7.

6 Barlow GM, Chen XN, Shi ZY *et al.* Down syndrome congenital heart disease: A narrowed region and a candidate gene. *Genet. Med.* 2001; **3**: 91–101.

7 McDowall A, Inwald D, Leitinger B *et al.* A novel form of integrin dysfunction involving β1, β2, and β3 integrins. *J. Clin. Invest.* 2003; **111**: 51–60.

8 Lucioli S, Giusti B, Mercuri E *et al.* Detection of common and private mutations in the COL6A1 gene of patients with Bethlem myopathy. *Neurology* 2005; **64**: 1931–7.

9 Cantu JM, Hernandez A, Plascencia L, Vaca G, Moller M, Rivera H. Partial trisomy and monosomy 21 in an infant with an unusual de novo 21/21 translocation. *Ann. Genet.* 1980; **23**: 183–6.

American Journal of Medical Genetics Part A

What is RSS?

Early View (Articles online in advance of print)

Published Online: 18 Jul 2008

Research Letter

Prenatal diagnosis of Costello syndrome using 3D ultrasonography amniocentesis confirmation of the rare HRAS mutation G12D†

Hideo Kuniba [1 2 *], Ritsuko K. Pooh [3], Kensaku Sasaki [4], Osamu Shimokawa [4], Naoki Harada [4], Tatsuro Kondoh [2 5], Masanori Egashira [2], Hiroyuki Moriuchi [2], Koh-ichiro Yoshiura [1], Norio Niikawa [1 6]

[1]Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[2]Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[3]CRIFM Clinical Research Institute of Fetal Medicine PMC, Osaka, Japan

[4]Kyushu Medical Science Nagasaki Laboratory (KMS), Nagasaki, Japan

[5]Department of Clinical Genetics, Misakae-no-sono Mutsumi, Institute for Severe Intellectual/Motor Disabled Persons, Isahaya, Japan

[6]Research Institute of Personalized Health Sciences, Health Sciences University of Hokkaido, Tobetsu, Japan

email: Hideo Kuniba (kuniba03@nifty.com)

*Correspondence to Hideo Kuniba, Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Sakamoto 1-12-4, Nagasaki 852-8523, Japan.

† How to cite this article: Kuniba H, Pooh RK, Sasaki K, Shimokawa O, Harada N, Kondoh T, Egashira M, Moriuchi H, Yoshiura K, Niikawa N. 2008. Prenatal diagnosis of Costello syndrome using 3D ultrasonography amniocentesis confirmation of the rare HRAS mutation G12D. Am J Med Genet Part A.

ABSTRACT

No Abstract.

Received: 8 February 2008; Accepted: 12 March 2008

DIGITAL OBJECT IDENTIFIER (DOI)

10.1002/ajmg.a.32335 About DOI

ARTICLE TEXT

To the Editor:

Costello syndrome (CS, OMIM #218040) is a rare disorder with a distinctive facial appearance, prenatal overgrowth, poor postnatal growth, loose skin of the hands and feet, characteristic hand position, developmental delay, papillomata, cardiac abnormalities, and tumor predisposition. *HRAS* is the only gene currently known to be causative for CS [Aoki et al., [2005]; Nava et al., [2007]; Rauen, [2007]]. Almost all of the mutations of the *HRAS* gene in CS patients which have been reported subsequently have been diagnosed after infancy [Estep et al., [2006]; Gripp et al., [2006]; Kerr et al., [2006]; Schulz et al., [2008]] except for patients presenting with severe neonatal manifestation of CS [Lo et al., [2008]]. We report on the first patient with prenatally diagnosed CS dues to the rare c.35G > A, p.G12D *HRAS* mutation.

A 31-year-old G2P1 woman was referred at 23 weeks of gestation for ultrasonography which showed polyhydramios, good fetal movement, and overgrowth with estimated body weight 1,300 g (+5.3 SD using a Japanese fetal growth curve). There was no pleural effusion, ascites or subcutaneous edema. Craniofacial features included large head

(+3.0 SD), pointed chin, full cheeks, wide nasal bridge, and low-set ears (Fig. 1A), but no macroglossia, omphalocele, hydrocephalus, or brain anomalies. The size of the abdomen was equivalent to that of a fetus at 28-31 weeks gestation. The fetal stomach could not be identified. Hepatomegaly was detected, but the other visceral organs were normal. The extremities were normal in length without deformity, although the wrists were deviated laterally.

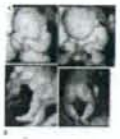

Figure 1. A: Three-dimensional ultrasound images of the fetus at 24 weeks of gestation with overgrowth and so-called "coarse face". Note his left hand presenting ulnar deviation and flexion of the wrist. B: Electropherogram of *HRAS* showing missense mutation at codon 12, c.35G > A, p.G12D. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.] [Normal View 47K | Magnified View 75K]

Cytogenetic and molecular analyses were performed after obtaining informed consent from parents. Standard chromosomes analysis by amniocentesis showed normal karyotype: 46,XY. The deletion of *NSD1* responsible for Sotos syndrome was not detected by fluorescence in situ hybridization (data not shown). By the time CS was suspected, the volume of amniotic fluid was only 1.8 ml which was frozen and stored in the clinic. To perform molecular diagnosis for CS, DNA was extracted with QIAvac vacuum manifold (Qiagen, Chatsworth, CA) from the specimen, and whole genome amplification was carried out with GenomePlex Whole Genome Amplification Kit (Sigma-Aldrich, St. Louis, MO), according to the manufacture's instructions. In this procedure, 3 ㎍ of DNA was obtained. All *HRAS* coding exons and their flanking intronic sequences were analyzed by direct sequencing on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA), and a rare missense mutation was found, that is, c.35G > A, p.G12D (Fig. 1B).

The mother had been transported to a pediatric hospital after the fetal evaluation, and the fetus subsequently developed pleural effusion and deteriorated. He was born at 31 weeks gestation via cesarean. He weighed 2,926 g (+4.2 SD) and developed respiratory failure, severe hypoglycemia, cardiac hypertrophy and renal failure. Although he was treated in neonatal intensive care unit, he died soon after birth due to multiple organ failures. Permission for autopsy was not granted. We were unable to study the parental origin [Sol-Church et al., [2006]; Zampino et al., [2007]] because DNA samples from the mother and his 33-year-old father had not been obtained.

Prenatal overgrowth and polyhydramios were prominent in this case. Dysmorphic facial features and flexion of the wrist, imaged with striking clarity by three-dimensional (3D) ultrasonography led us to a clinical diagnosis of CS. Prenatal overgrowth syndromes include relatively few conditions, that is, Sotos syndrome, Simpson-Golabi-Behmel syndrome, Beckwith-Wiedemann syndrome, and CS. The presence of polyhydramios which occurs in over 90% of pregnancies with CS, supported by the 3D ultrasonographic imaging of facial features (broad nose, puffy cheeks, so-called "coarse" face, pointed chin, and flexion of the wrist) made the diagnosis likely. Three-dimensional ultrasonography is clearly more beneficial than two-dimensional (2D) ultrasonography in a diagnosis of genetic syndromes, since we can see overall fetal image of malformation which we hardly get with conventional 2D ultrasonography [Lee and Simpson, [2007]]. The phenotype of Noonan syndrome often overlaps with that of CS, and the prenatal findings of Noonan syndrome, polyhydramnios and so-called "coarse face" in a fetus with the T854C mutation in the *PTPN11* gene, have been reported [Levaillant et al., [2006]], although that fetus with Noonan syndrome did not develop overgrowth.

CS was diagnosed clinically in the prenatal period in monozygotic twins who died 57 days of life after birth at 30 weeks of gestation due to respiratory failure [Van den Bosch et al., [2002]]. Molecular diagnosis was not available. Neonatal deaths in two patients with CS confirmed by molecular diagnosis of G12D *HRAS* mutation were reported by Lo et al. [2008]. One patient was born at 36 weeks gestation weighing 2,950 g developed hypoglycemia, persistent and severe jaundice, persistent respiratory distress with tracheomalacia, bronchomalacia and chylothorax. The baby also had clenched hands, atrial septal defect, paroxysmal multifocal atrial tachycardia, pulmonary lymphangiectasia, and renal failure. She died at age 3 months due to respiratory failure. The other patient was a girl born at 37 weeks gestation weighing 3,115 g had hypoglycemia, rhizomelic limb shortening and flexion contractures at the wrist, hypertrophic cardiomyopathy, dysplasic pulmonary valve, atrial fibrillation, cardiac failure and persistent hyponatremia due to renal sodium leakage. She became ventilator dependent and died at 3 months of age from sepsis and renal failure. Both had pregnancies complicated by polyhydramios. Lo et al. [2008] suggested that differences in activating potential of G12D mutations in *HRAS* gene may result in severe manifestations, such as hypoglycemia, renal abnormalities, severe early cardiomyopathy, and congenital respiratory abnormalities, which result in multiple organ failure.

We believe this is the first case of prenatally diagnosed CS confirmed with molecular genetic analysis with a G12D mutation in *HRAS* gene. The mutation was not observed in previous natural history studies of CS, perhaps because of the rarity of the mutation and the fact that the patients die in early infancy. Our findings contribute to the natural history of this mutation which includes a severe clinical course. If prenatal ultrasonographic findings show both polyhydramnios and overgrowth, CS should be considered despite its rarity. Molecular diagnosis should be offered in the perinatal period without hesitation.

Acknowledgements

We are grateful to the family for their participation in this research. We thank Dr. Shoko Miura for her helpful comments.

REFERENCES

Aoki Y, Niihori T, Kawame H, Kurosawa K, Ohashi H, Tanaka Y, Filocamo M, Kato K, Suzuki Y, Kure S, Matsubara Y. 2005. Germline mutations in HRAS proto-oncogene cause Costello syndrome. *Nat Genet* **37**: 1038-1040. Links

Estep AL, Tidyman WE, Teitell MA, Cotter PD, Rauen KA. 2006. HRAS mutations in Costello syndrome: Detection of constitutional activating mutations in codon 12 and 13 and loss of wild-type allele in malignancy. *Am J Med Genet Part A* **140A**: 8-16. Links

Gripp KW, Lin AE, Stabley DL, Nicholson L, Scott CI Jr, Doyle D, Aoki Y, Matsubara Y, Zackai EH, Lapunzina P, Gonzalez-Meneses A, Holbrook J, Agresta CA, Gonzalez IL, Sol-Church K. 2006. HRAS mutation analysis in Costello syndrome: Genotype and phenotype correlation. *Am J Med Genet Part A* **140A**: 1-7. Links

Kerr B, Delrue MA, Sigaudy S, Perveen R, Marche M, Burgelin I, Stef M, Tang B, Eden OB, O'Sullivan J, De Sandre-Giovannoli A, Reardon W, Brewer C, Bennett C, Quarell O, M'Cann E, Donnai D, Stewart F, Hennekam R, Cave H, Verloes A, Philip N, Lacombe D, Levy N, Arveiler B, Black G. 2006. Genotype-phenotype correlation in Costello syndrome: HRAS mutation analysis in 43 cases. *J Med Genet* **43**: 401-405. Links

Lee YM, Simpson LL. 2007. Major fetal structural malformations: The role of new imaging modalities. *Am J Med Genet Part C Semin Med Genet* **145C**: 33-44. Links

Levaillant JM, Gerard-Blanluet M, Holder-Espinasse M, Valat-Rigot AS, Devisme L, Cave H, Manouvrier-Hanu S. 2006. Prenatal phenotypic overlap of Costello syndrome and severe Noonan syndrome by tri-dimensional ultrasonography. *Prenat Diagn* **26**: 340-344. Links

Lo IF, Brewer C, Shannon N, Shorto J, Tang B, Black G, Soo MT, Ng D, Lam ST, Kerr B. 2008. Severe neonatal manifestations of Costello syndrome. *J Med Genet* **45**: 167-171. Links

Nava C, Hanna N, Michot C, Pereira S, Pouvreau N, Niihori T, Aoki Y, Matsubara Y, Arveiler B, Lacombe D, Pasmant E, Parfait B, Baumann C, Héron D, Sigaudy S, Toutain A, Rio M, Goldenberg A, Leheup B, Verloes A, Cavé H. 2007. Cardio-facio-cutaneous and Noonan syndromes due to mutations in the RAS/MAPK signalling pathway: Genotype-phenotype relationships and overlap with Costello syndrome. *J Med Genet* **44**: 763-771. Links

Rauen KA. 2007. HRAS and the Costello syndrome. *Clin Genet* **71**: 101-108. Links

Schulz AL, Albrecht B, Arici C, van der Burgt I, Buske A, Gillessen-Kaesbach G, Heller R, Horn D, Hübner CA, Korenke GC, König R, Kress W, Krüger G, Meinecke P, Mücke J, Plecko B, Rossier E, Schinzel A, Schulze A, Seemanova E, Seidel H, Spranger S, Tuysuz B, Uhrig S, Wieczorek D, Kutsche K, Zenker M. 2008. Mutation and phenotypic spectrum in patients with cardio-facio-cutaneous and Costello syndrome. *Clin Genet* **73**: 62-70. Links

Sol-Church K, Stabley DL, Nicholson L, Gonzalez IL, Gripp KW. 2006. Paternal bias in parental origin of HRAS mutations in Costello syndrome. *Hum Mutat* **27**: 736-741. Links

Van den Bosch T, Van Schoubroeck D, Fryns JP, Naulaers G, Inion AM, Devriendt K. 2002. Prenatal findings in a monozygotic twin pregnancy with Costello syndrome. *Prenat Diagn* **22**: 415-417. Links

Zampino G, Pantaleoni F, Carta C, Cobellis G, Vasta I, Neri C, Pogna EA, De Feo E, Delogu A, Sarkozy A, Atzeri F, Selicorni A, Rauen KA, Cytrynbaum CS, Weksberg R, Dallapiccola B, Ballabio A, Gelb BD, Neri G, Tartaglia M. 2007. Diversity, parental germline origin, and phenotypic spectrum of de novo HRAS missense changes in Costello syndrome. *Hum Mutat* **28**: 265-272. Links