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SHORT COMMUNICATION

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Phenotype–genotype correlation in two patients with 12q proximal deletion

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Abstract Proximal 12q deletion is a very rare chromosomal abnormality. Only five cases have been reported. Among the five, an Argentinian patient (Case 1) with del(12)(q11q13) and a Japanese patient (Case 2) with del(12)(q12q13.12) were analyzed because they shared several clinical features: growth and psychomotor developmental delay; strabismus; broad and short nose with anteverted nostrils; high, arched palate; large, low-set ears; widely set nipples; short fingers and clinodactyly of fifth fingers; and abnormality of the second and third toes. To clarify the correlation between the deleted genes and their phenotypes, we delimited their deleted regions by fluorescence in situ hybridization (FISH). The overlapped region in the deletions spanned 6.2 Mb where at least 15 genes were predicted to localize on the current human genome database. Among them, *YAF2* and *AMIGO2* were the most plausible candidates to affect growth and psychomotor retardation, respectively, in both cases. Regarding unique symptoms in each case, congenital fibrosis of the extraocular muscles found only in Case 1 may be caused by *KIF21A* deletion and hearing loss and cleft palate in Case 2 by *COL2A1* defect.

Keywords 12q proximal deletion · Mental retardation · Congenital anomaly · FISH mapping · Genotype–phenotype correlation

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Introduction

Proximal 12q deletion is very rare, and to the best of our knowledge, only five cases have been reported; del(12)(q13.3q21.1), del(12)(q15q21.2), del(12)(q12q13.12), del(12)(q24.31q24.33), and del(12)(q11q13) (Meinecke and Meinecke 1987; Watson et al. 1989; Tonoki et al. 1998; Sathya et al. 1999; Gallego et al. 2000). Among them, an Argentinian patient (Case 1) with del(12)(q11q13) reported by Gallego et al. (2000) and a Japanese patient (Case 2) with del(12)(q12q13.12) reported by Tonoki et al. (1998) shared several clinical manifestations including psychomotor and growth retardation; strabismus; broad and short nose with

anteverted nostrils; high, arched palate; large, low-set ears; widely set nipples; short fingers and clinodactyly of fifth fingers; and abnormality of the second and third toes (Table 1). Myopia, congenital fibrosis of the extraocular muscles (CFEOM), and lower-limb hypertonia were observed only in Case 1, and severe intrauterine growth retardation (IUGR) and sensorineural hearing loss were specific to Case 2 (Table 1). We delimited their deletions, and the relationship between deleted genes and their possible consequent clinical features will be discussed.

Materials and methods

The samples of Case 1 and Case 2 were sent to us after receiving written informed consents. Their metaphase chromosomes were prepared for fluorescence in situ hybridization (FISH) from immortalized lymphoblastoid cell lines or peripheral blood lymphocytes according to standard protocols. Fifteen RPCI-11 human BAC clones (RP11-170L1, RP11-496H24, RP11-1009E3, RP11-91K15, RP11-157L7, RP11-297E10, RP11-490D11, RP11-91A7, RP11-105J16, RP11-579E15, RP11-152E14, RP11-1058B16, RP11-956H14, RP11-96K4 and RP11-480H15) that have been mapped around the deleted regions were selected for FISH analyses [UCSC genome browser, 2003 July version (http://genome.ucsc.edu/cgi-bin/hgGateway)]. BAC clone DNA was extracted using an automatic DNA extraction system (Kurabo, Osaka, Japan). Extracted BAC clone DNA were labeled with SpectrumGreen-11-dUTP or SpectrumOrange-11-dUTP (Vysis, Downers Grove, IL, USA) by nick translation and denatured at 70°C for 10 min. Probe-hybridization mixtures (15 μl) were applied on the chromosomes, incubated at 37°C for 16–72 h, then washed. Fluorescence photomicroscopy was performed under a Zeiss Axioskop microscope equipped with a quad filter set with single-band excitation filters (84000, Chroma Technology Corp., Brattleboro, VT, USA). Images were collected and merged using a cooled CCD camera (TEA/CCD-1317-G1, Princeton Instruments, Trenton, NJ, USA) and IPLab/MAC software (Scanalytics, Inc., Fairfax, VA, USA).

Results and discussions

Deletions in Cases 1 and 2 spanned a 36.3–46.3-Mb segment and a 40.1–46.8-Mb segment from the 12p terminal end, respectively. The overlapped region in the deletions spanned 6.2 Mb (from RP11-490D11 to RP11-579E15) and contained at least 15 genes (Fig. 1).

Among the 15 genes in the common deleted region, the YY1-associated factor-2 gene (*YAF2*) and the amphoterin-induced gene 2 (*AMIGO2*) are relevant to some common manifestations in the two cases. YAF2 is one of upregulating factors during in vitro differentiation of both skeletal and cardiac muscle cells and potentiates proteolytic cleavage of a zinc finger protein YY1, which

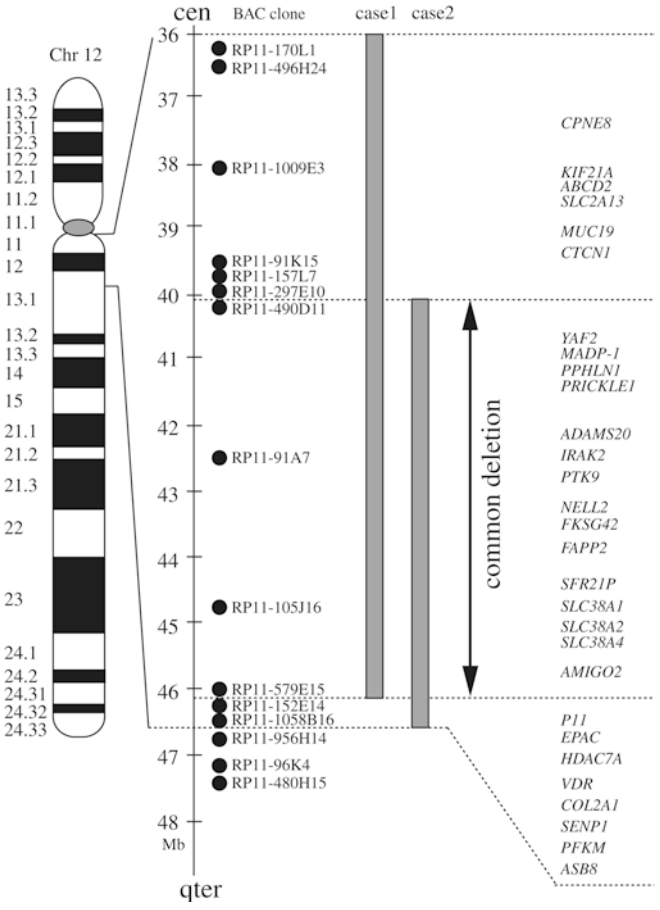

Fig. 1 Schematic presentation of deleted regions, BAC clones used for this study, and genes. Two longitudinal *gray bars* denote the deleted regions in Cases 1 and 2

is downregulated during myogenic differentiation (Kalenik et al. 1997). Thus, a *YAF2* deletion may have caused the growth retardation and/or hypotonicity in the two patients. As *AMIGO2* is highly expressed in the nervous system and plays a role in growth of axonal connection and myelination (Kuja-Panula et al. 2003); its deletion can cause psychomotor developmental delay.

Among the six genes deleted in Case 1 only, *KIF21A* is known to be mutated in patients with CFEOM type 1 (CFEOM1, OMIM #135700) (Yamada et al. 2003). Thus, CFEOM observed in Case 1 may be accounted for by the lack of *KIF21A* due to the deletion. Case 2 did show strabismus and blepharoptosis but did not have CFEOM. Eight deleted genes were specific to Case 2. *COL2A1* encodes a type-II collagen, a main component of hyaline cartilage essential for skeletal development. *COL2A1* mutations can cause various types of autosomal-dominant connective-tissue disorders such as achondrogenesis II (OMIM #200610), spondyloepiphyseal dysplasia congenita (OMIM #183900), Kniest dysplasia (OMIM #156550), and Stickler syndrome type I (OMIM #108300) (Kuivaniemi et al. 1997; Wilkin et al. 1999). The common features of the type-II collagen disorders by *COL2A1* mutations are myopia, sensorineural hearing loss, cleft palate, and short-trunked

Table 1 Clinical manifestations of two cases of interstitial 12q deletions

	Case 1	Case 2
Deletion	12q11–q13	12q12–q13.12
Age at estimation (months)	20	24
Psychomotor retardation	+	++
Intrauterine growth retardation	–	++
Growth retardation	+(−3SD)	+(<−3SD)
Blepharoptosis	–	+(bilateral)
Strabismus	+	+
Palpebral fissure	Upward	Downward
Cleft or high-arched palate	+	+
Low-set ear	+	+
Sensorineural hearing loss	–	+
Widely set nipples	+	+
Small hand	+	+
Clinodactyly of 5th finger	+	+
2nd and 3rd toe anomaly	Syndactily	Overlapping toes
Muscle tonus	Hypertonia (lower limbs)	Hypotonia

+, positive
++, severe

dwarfism (Wilkin et al. 1999). Thus, sensorineural hearing loss and cleft palate in Case 2 may be explained by *COL2A1* deletion.

In conclusion, *YAF2* and *AMIGO2* may have contributed to growth and psychomotor retardation in our two patients with proximal 12q deletion. CFEOM in Case 1 and hearing loss and cleft palate in Case 2 may be explained by deletion of *KIF21A* and *COL2A1*, respectively. Establishment of genotype–phenotype correlation as described here will indicate possible functions of uncharacterized genes in humans.

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