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A *de novo* 2q35-q36.1 deletion incorporating *IHH* in a Chinese boy (47,XYY) with syndactyly, type III Waardenburg syndrome, and congenital heart disease

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ABSTRACT. Reports of terminal and interstitial deletions of the long arm of chromosome 2 are rare in the literature. Here, we present a case report concerning a Chinese boy with a 47,XYY karyotype and a *de novo* deletion comprising approximately 5 Mb between 2q35 and q36.1, along with syndactyly, type III Waardenburg syndrome, and congenital heart disease. High-resolution chromosome analysis

to detect copy number variations was carried out using an Affymetrix microarray platform, and the genes affected by the patient's deletion, including *IHH*, were determined. However, no copy number changes were observed in his healthy parents. The present case exhibited novel syndactyly features, broadening the spectrum of clinical findings observed in individuals with 2q interstitial deletions. Our data, together with previous observations, suggest that *IHH* haploinsufficiency is the principal pathogenic factor in the syndactyly phenotype in this study, and that different types of variations at the *IHH* locus may cause divergent disease phenotypes. This is the first report of the involvement of *IHH* haploinsufficiency in syndactyly phenotype.

Key words: Deletion 2q35-q36.1; *IHH*; Syndactyly; Type III Waardenburg syndrome; Congenital heart disease

INTRODUCTION

Deletions in the long arm of chromosome 2 are rare, although interstitial (Warter et al., 1976; Marković et al., 1985; Narahara et al., 1985; Gorski et al., 1989; Pasteris et al., 1993; Wu et al., 1993; Nye et al., 1998; Kramer et al., 2000) and terminal deletions (Young et al., 1983; Sánchez and Pantano, 1984; Halal et al., 1989; Waters et al., 1993) in the 2q35-q36 region have been reported in previous papers. Cases of interstitial deletions primarily involve Waardenburg syndrome (WS), and of all the reports concerned, only three describe syndactyly. A *de novo* 2q35-q36.1 deletion was identified in our patient, who had syndactyly, type III WS, and congenital heart disease (CHD). However, it is unknown whether deletion in this region is associated with syndactyly.

Syndactyly is a digital malformation in which adjacent fingers and/or toes are webbed because they fail to separate during limb development. The current classification scheme defines nine types of syndactyly according to phenotype and genotype, principally based on isolated cases of syndactyly occurring within families and exhibiting the same pattern of phenotype, justifying categorization as a distinct type (Malik, 2012). However, the syndactyly phenotype in this study differs from each of the nine types previously described, and may be syndromic in nature. Our aim was to determine whether a gene potentially responsible for syndactyly was present within the deleted chromosomal region identified.

MATERIAL AND METHODS

Clinical report

The 7-year-old patient investigated here was admitted to our institute because of CHD. He is the third child of healthy, non-consanguineous Chinese parents and was born 1 month premature. His older brother was also born 1 month premature and had CHD, but died unexpectedly 15 days after birth. His older sister is healthy, as are his grandparents.

The patient was born with a congenital ventricular septal defect and bilateral sensorineural hearing loss. His eyesight is normal and the irises of his eyes are blue and exhibit heterochromia irides (Figure 1a), a key characteristic of type III WS. A W-index value of 2.46

(>1.95) indicated telecanthus. Flat nasal bridge, hypoplastic alar cartilage, and facial freckles are evident. The thumb of the left hand of the patient shows hypotonia and cannot bend fully, indicating hypogenesis of the flexor pollicis longus. These clinical features are consistent with symptoms of type III WS (MIM No. 148820). Syndactyly of toes 1-2 and 3-4 of both feet was observed (Figure 1b). X-ray examination showed no bone fusion (Figure 1c). In addition, an extra, shortened middle phalanx was identified in both of the patient's feet. These limb malformations have not been reported in previous studies.

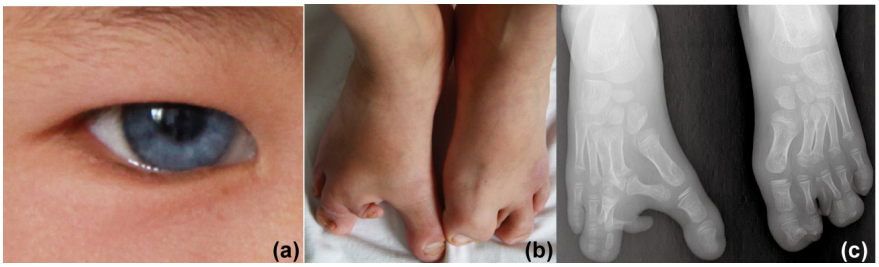

Figure 1. Photographs and radiograph of the patient. **a.** Heterochromia irides. **b.** Syndactyly of toes 1-2 and 3-4 of both feet. **c.** Radiograph of the patient's feet, showing five metatarsals and one extra, shortened middle phalanx in each foot, with no bone fusion.

Cytogenetic and G-banding analyses

Cytogenetic analysis was performed according to standard procedures using cultures of peripheral blood lymphocytes from the patient and his parents. Twenty metaphases were analyzed for each subject using the GTG banding technique. Data were analyzed according to the criteria of the International System for Human Cytogenetic Nomenclature.

High-resolution chromosome analysis of copy number variations (CNVs)

A CytoScan HD Array (Affymetrix, Santa Clara, CA, USA) was used for high-resolution detection of CNVs across the genome of the patient and his parents, following the manufacturer's protocol. Scanned images were analyzed with the Affymetrix GeneChip Command Console 3.2 software, and data analysis was performed using Affymetrix Chromosome Analysis Suite 1.2.2.

Ethics Committee

Written informed consent was obtained from the patient's father to carry out this study, which was approved by the ethics committee of Qilu Children's Hospital of Shandong University.

RESULTS

G-banding analysis of peripheral blood lymphocyte cultures revealed that the patient has a 47,XYY male karyotype with a small, non-mosaic interstitial deletion of chromosome 2q35-q36 (**Figure S1**). His parents have normal karyotypes.

High-resolution chromosome analysis confirmed the patient's 47,XYY karyotype, and identified segmental monosomy of approximately 5.04 Mb in the 2q35-q36.1 region (218,271,744-223,313,592 according to the University of California Santa Cruz Genome Browser hg19 assembly), but detected no chromosomal structural aberrations in his parents (**Figure S2**). Figure 2 shows the patient's chromosome microarray profile with an overview of the deleted region and the 78 genes that it covers (as per the hg19 assembly), including *DIRC3*, *TNS1*, *MIR6809*, *RUFY4*, *CXCR2P1*, *CXCR1*, *ARPC2*, *GPBAR1*, *AAMP*, *PNKD*, *TMBIM1*, *MIR6513*, *MIR6810*, *CATIP*, *SLC11A1*, *CTDSP1*, *MIR26B*, *VIL1*, *USP37*, *RQCD1*, *PLCD4*, *ZNF142*, *BCS1L*, *RNF25*, *STK36*, *TTLL4*, *CYP27A1*, *PRKAG3*, *WNT6*, *WNT10A*, *LOC101928537*, *CDK5R2*, *LINC00608*, *FEV*, *CRYBA2*, *MIR375*, *LOC100129175*, *CCDC108*, *IHH*, *MIR3131*, *NHEJ1*, *SLC23A3*, *CNPPD1*, *FAM134A*, *ZFAND2B*, *ABCB6*, *ATG9A*, *ANKZF1*, *GLB1L*, *STK16*, *TUBA4A*, *TUBA4B*, *DNAJB2*, *PTPRN*, *MIR153-1*, *RESP18*, *DNPEP*, *DES*, *SPEG*, *LOC100996693*, *GMPPA*, *ASIC4*, *CHPF*, *TMEM198*, *MIR3132*, *OBSL1*, *INHA*, *STK11IP*, *SLC4A3*, *MIR4268*, *EPHA4*, *AX747413*, *Mir_649*, *PAX3*, *CCDC140*, *DD413687*, *LOC440934*, and *SGPP2*.

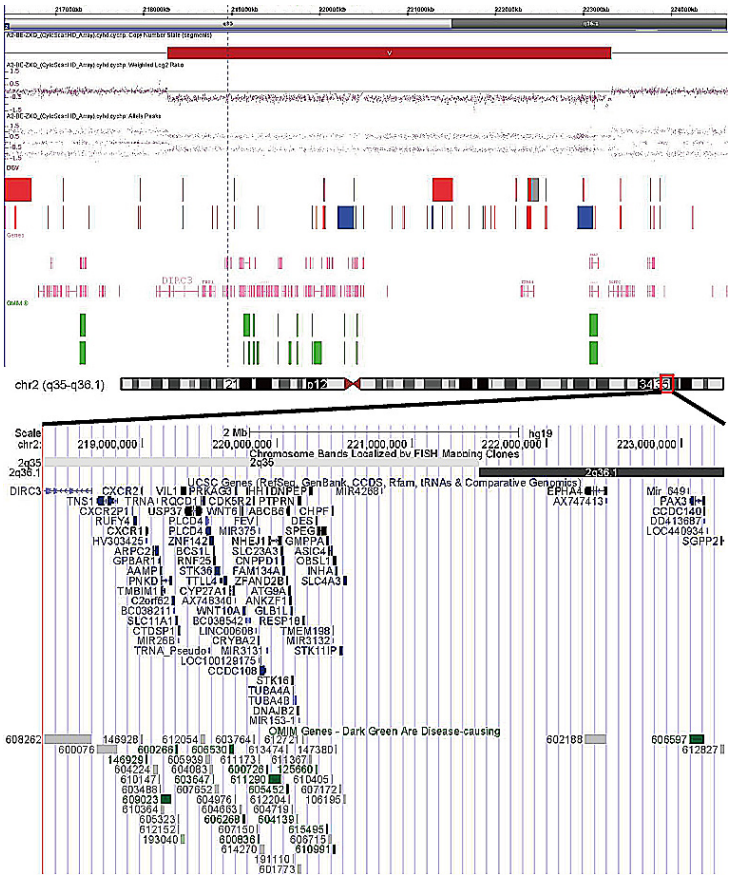

Figure 2. Deletion of 2q35-q36.1 in the patient described in this study. The chromosome microarray profile shows the deletion with a schematic overview of the region (University of California Santa Cruz Genome Browser view) and all Online Mendelian Inheritance in Man (OMIM) genes affected.

The DNA of the index patient was screened for point mutations in the *PAX3* and *IHH* genes using direct exon sequencing. However, analysis of all exons of these genes provided no evidence of such mutations.

DISCUSSION

Deletions in chromosome 2q35-q36 are extremely rare. We reviewed the literature for previous reports of deletions in this region overlapping that identified in this study. The clinical features of the current case have been documented in other patients carrying such deletions (Table 1).

Table 1. Clinical features of our patient shared with other reported cases involving a deletion in 2q35-q36.

Feature	Warter et al. (1976)	Young et al. (1983)	Sanchez and Pantano (1984)	Marković et al. (1985)	Pasteris et al. (1993)	Nye et al. (1998)		Our patient
						case 1	case 2	
Deletion	q34-q36	q36-qter	q35-qter	q31-q35	q35-q36	q35-q36.2	q35-q36.2	q35-q36.1
Sex	Female	Female	Female	Male	Female	Male	Female	Male
Age	6 years	8 months	NB	NB	5 years	4 years	4 years	7 years
Heterochromia irides	-	-	-	-	+	+	-	+
Congenital heart disease	+	-	-	+	-	+	+	+
Finger flexion contractures	-	-	-	-	+	-	-	+
Syndactyly of the toes	-	+	+	-	+	-	-	+
Sensorineural hearing loss	-	-	-	-	+	-	-	+
Telecanthus	-	-	-	-	+	+	+	+
Hypoplastic alar cartilage	-	-	-	-	+	+	+	+
Facial freckles	-	-	-	-	-	-	-	+

"+" = Present; "-" = absent; NB = newborn.

In this study, we identified a *de novo* deletion of approximately 5 Mb in 2q35-q36.1 and a 47,XYY karyotype in a boy with CHD, type III WS, and syndactyly, yet observed no copy number changes in his healthy parents. This deleted region contains 78 genes, including *PAX3*, which is known to be implicated in WS. To date, more than 70 *PAX3* mutations have been detected in patients with different types of this disease (Pingault et al., 2010), and deletions incorporating this gene have been described in other cases (Pasteris et al., 1993; Wu et al., 1993; Nye et al., 1998). Analysis of all *PAX3* exons in the present case provided no evidence of point mutations. This suggests that *PAX3* haploinsufficiency is the actual cause of our patient's type III WS. Syndactyly, as observed in our case, has also been documented in several other patients with WS (Young et al., 1983; Sánchez and Pantano, 1984; Pasteris et al., 1993). A review of previous reports of syndactyly in the literature revealed that the phenotype observed in this study is novel, differing from any described in the current classification scheme, and possibly being syndromic in nature. Further analysis was conducted to determine whether a candidate gene for syndactyly was present within the deleted region identified in this study. Using online databases (GeneCards Version 3, NCBI gene records, and PubMed literature searches), each of the 78 genes located within this region was investigated. Since particular emphasis was placed on involvement in skeletal development and endochondral bone growth, *IHH* was identified as the most likely candidate. *IHH* signaling is known to promote endochondral bone growth through positive regulation of chondrocyte proliferation and osteoblast differentiation (Karp et al., 2000; Long et al., 2004), and has been shown to regulate elongation of digit primordia (Zhou et al., 2007). Although mutations in human *IHH* cause brachydactyly type A1 (MIM No. 185900), small tandem duplications involving this

gene have been described in patients with mild syndactyly and craniosynostosis (Klopocki et al., 2011), and a duplication of approximately 900 kb at this locus has also been reported in two siblings with extensive polysyndactyly of the hands and feet (Yuksel-Apak et al., 2012). As discussed above, different variations in the *IHH* gene may cause divergent disease phenotypes (Table 2). In addition, Gofflot et al. (2003) developed an *in vivo* rat model showing that an imbalance in *IHH* expression in forming cartilage leads to reduced interdigital apoptosis and syndactyly. Therefore, we infer that CNVs at this locus result in aberrant *IHH* expression, ultimately causing limb malformations. Moreover, deletion and duplication of *IHH* may result in down- and up-regulation of its expression, respectively.

Table 2. Clinical phenotypes in reported cases of limb malformation involving different genetic alterations of the *IHH* locus.

Reported case	Genetic alteration	Location	Clinical phenotype
Gao et al. (2001)	Missense mutations	*IHH* exon	Brachydactyly type A1
Klopocki et al. (2011)	Small tandem duplications	Upstream of *IHH* or upstream of *IHH* plus entire *IHH* coding sequence	Mild syndactyly
Yuksel-Apak et al. (2012)	Large (~900-kb) duplication	Includes entire *IHH* locus	Extensive polysyndactyly of the hands and feet
Our patient	*De novo* ~5-Mb deletion	Chromosome 2q35 to q36.1 including entire *IHH* locus	Novel syndactyly phenotype

CONCLUSIONS

In summary, our patient demonstrated a novel syndactyly phenotype in which the gene *IHH* may be implicated. Specifically, *IHH* haploinsufficiency appears to be the principal pathogenic factor responsible. The present case broadens the spectrum of clinical findings observed in individuals with interstitial deletions in chromosome 2q.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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Supplementary material

Figure S1. Cytogenetic analysis of chromosome 2. The G-banded partial karyotype shows the normal chromosome on the left of the pair and its deletion-carrying homolog on the right. del = deletion.

Figure S2. Microarray profiles of the 2q35-q36.1 showing deletion in our patient and the corresponding regions of the normal chromosomes of his parents.