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Severe Developmental Delay in a Patient with 7p21.1–p14.3 Microdeletion Spanning the *TWIST* Gene and the *HOXA* Gene Cluster

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Key Words
array-CGH · Contiguous gene syndrome · Hand-foot-uterus syndrome · *HOXA* · Saethre-Chotzen syndrome · *TWIST*

Abstract
We describe a patient with a rare interstitial deletion of chromosome 7p21.1–p14.3 detected by array-CGH. The deletion encompassed 74 genes and caused haploinsufficiency (or loss of allele) of 6 genes known to be implicated in different autosomal dominant genetic disorders: *TWIST, DFNA5, CYCS, HOXA11, HOXA13,* and *GARS*. The patient had several morphological abnormalities similar to Saethre-Chotzen syndrome (caused by *TWIST* mutations) including craniosynostosis of the coronal suture and anomalies similar to those seen in hand-foot-uterus syndrome (caused by *HOXA13* mutations) including hypospadias. The combined phenotype of Saethre-Chotzen syndrome and hand-foot-uterus syndrome of our patient closely resembles a previously reported case with a cytogenetically visible small deletion spanning 7p21–p14.3. We therefore conclude that microdeletions of 7p spanning the *TWIST* gene and *HOXA* gene cluster lead to a clinically recognizable 'haploinsufficiency syndrome'.

The short arm of chromosome 7 harbors different developmental regulator genes, including the *TWIST* gene on 7p21 and the *HOXA* gene cluster on 7p15. Haploinsufficiency of these genes either by deletion or mutation leads to Saethre-Chotzen syndrome (SCS) (OMIM-#141400) and hand-foot-uterus syndrome (HFU) (OMIM-#140000), respectively.

SCS is characterized by craniosynostosis, maxillary hypoplasia, prominent ear crura, and cutaneous syndactyly [Howard et al., 1997; Johnson et al., 1998; Zackai and Stolle, 1998]. Mutations of *TWIST* are also found in cases of isolated single-suture craniosynostosis (coronal or sagittal) [Seto et al., 2007].

In the past, Robinow-Sorauf syndrome was used to describe patients that in fact had SCS with hallucal duplication and valgus deformity. Similarly, blepharophimosis ptosis epicanthus syndrome (BPES) was used to describe patients who really had SCS with ptosis of the eyelids [Robinow and Sorauf, 1975; Maw et al., 1996; Dollfus et al., 2001].

HFU (or hand-foot-genital syndrome) is characterized by small hands with hypoplastic proximally placed thumbs and feet with small halluces. Postaxial polydac-

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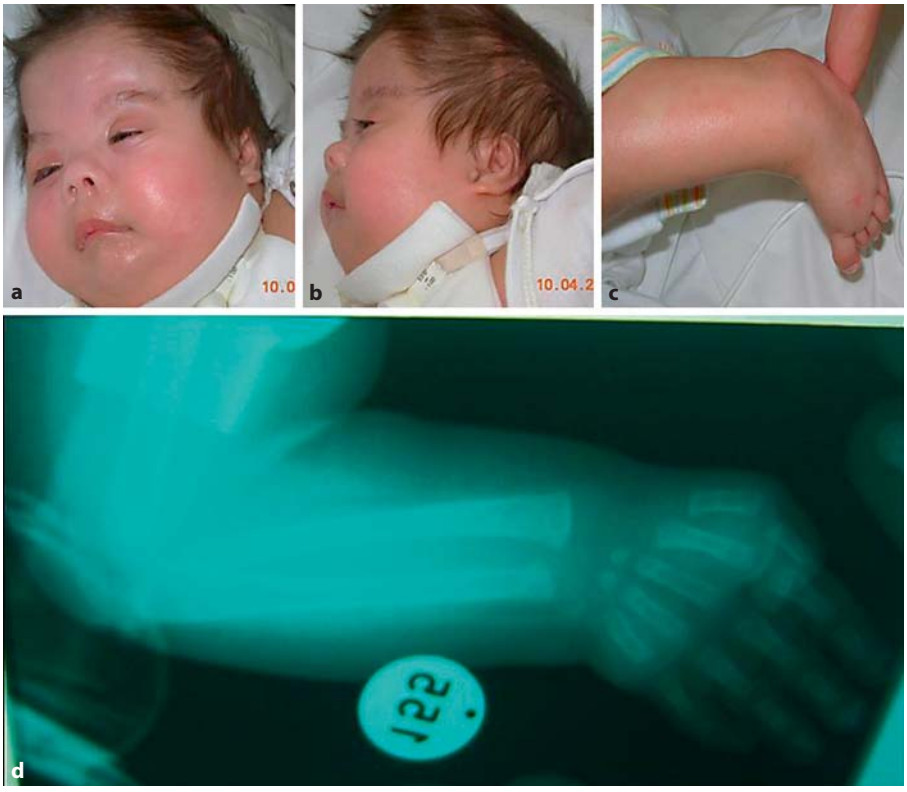

Fig. 1. Photographs of the patient. **A** Asymmetry of the face (particularly on the left side), hypertelorism, epicanthic folds, low nasal bridge, small nose with upturned nostrils, fish-shaped mouth, long and simple philtrum. **B** Low-set ears, interrupted ear tragus, hyperplastic anti-helix and anti-tragus and tracheostomy. **C** Brachydactyly of toes II–IV with a long and broad hallux. **D** X-ray of the lower arm and hand showed brachyphalangy, cone-shaped epiphyses, hypoplastic middle-phalanx of the 5th finger with clinodactyly. No radio-ulnar synostosis was noted.

tyly of the hands and short or uniphalangeal second toes with absent nails have also been reported. Males have hypospadias, while females have duplication of the uterus and sometimes the cervix, and they may have a septate vagina. Ureteral malformations are common in both sexes. HFU is caused by mutations of the *HOXA13* gene on 7p15.2 [Devriendt et al., 1999; Goodman et al., 2000; Goodman, 2002; Frisen et al., 2003]. Guttmacher syndrome is allelic to HFU syndrome with phenotypic overlap (with the exception of postaxial polydactyly of the hands and short or uniphalangeal second toes with absent nails) [Innis et al., 2002]. Larger deletions of the *HOXA* gene cluster *(HOXA1–HOXA13)* result in HFU in combination with other malformations such as velopharyngeal insufficiency and a persistent patent ductus botali [Devriendt et al., 1999].

We report a patient with clinical features of both SCS and HFU due to a microdeletion on chromosome 7p21.1–p14.3 detected by array-CGH and encompassing the *TWIST* and *HOXA* gene clusters. This suggests that microdeletions spanning *TWIST* and *HOXA* represent a recognizable 'haploinsufficiency syndrome'.

Patient and Methods

Case Report

A Greek boy was born at 38 weeks' gestation by caesarian section due to a previous one. The patient was the second child of unrelated parents, and there was no relevant family history. The pregnancy was uncomplicated and prenatal tests (serologic, ultrasonographic) were normal. Birth weight was 2,225 g (2nd centile), length 45 cm (3rd centile), and head circumference 34.5 cm (50th centile). At delivery the neonate was cyanotic, hypotonic, and bradycardic, requiring resuscitation. APGAR scores were 1 and 5 at 1 and 9 min, respectively, and the child was admitted to the intensive care unit where he stayed for 2 months. Brain magnetic resonance imaging on the sixth day was within normal limits.

The infant was initially bottle fed, but feeding by nasogastric tube became necessary because of choking and cyanotic episodes. He had laryngomalacia and severe gastroesophageal reflux and failed to thrive.

The patient had many dysmorphic features such as asymmetry of the face, overlapping cranial sutures, hypertrichosis of the forehead, and a low nasal bridge with anteverted nostrils. There were small palpebral fissures, hypertelorism with epicanthic folds and ptosis of the eyelids (fig. 1a, b). The ears were low-set with underdevelopment of the helix, hyperplastic anti-helix and anti-tragus, and a prominent intertragic notch. The philtrum was long and smooth. The patient had a high palate and clefting of the soft palate. Additional features included a short neck and widely spaced nipples. There were several limb anomalies: short upper limbs (particularly the forearms and hands), bilateral hypoplastic fifth

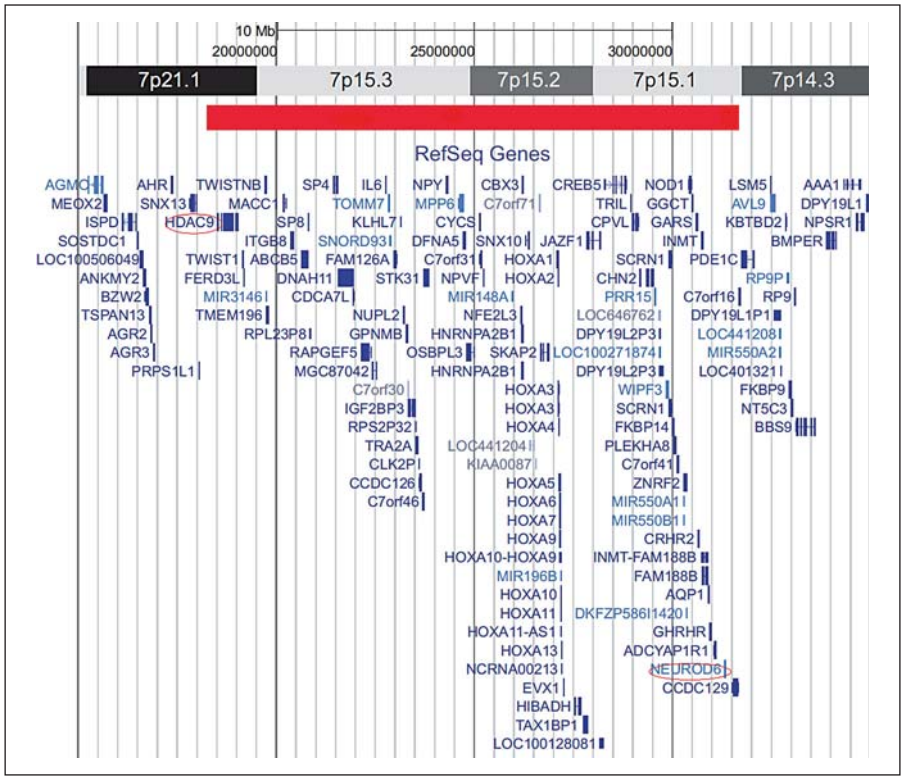

Fig. 2. The results of the array-CGH, revealing a deletion of 13 Mb on chromosome 7p21.1–p14.3 (red bar) and showing the 2 breakpoints (circled) and the genes located within the deleted region.

fingers with clinodactyly, digital webbing between the second, third, and fourth fingers, and abnormal hand creases. Toes II–IV were short, whereas the halluces were relatively long, broad and medially deviated (fig. 1c). There was no radioulnar synostosis (fig. 1d). The scrotum was hypoplastic with left cryptorchidism and first-degree hypospadias.

Cardiac ultrasound showed an open foramen ovale and a mild aortic insufficiency. He had multiple episodes of prolonged apnea, and the sleep EEG was abnormal with predominance of delta waves on the left side. The hematological and biochemical tests were normal, and he never developed thrombocytopenia.

At 5 months, surgery was performed for severe unilateral craniostenosis of the left coronal suture (left metopic plagiocephaly). The post-surgical period was complicated by cardiorespiratory arrest, and neurological damage was suspected. He also developed moderate renal insufficiency. He required a tracheostomy and g-tube placement. At 18 months, he had severe neurological deficits with spastic quadriplegia and persistent opisthotonus responding only to noxious stimuli; it was therefore difficult to correctly estimate his hearing. His height, weight and head circumference were below the third centile. At the age of 2, the patient died after developing a severe respiratory infection. The parents refused an autopsy.

Molecular Analysis

Array comparative genomic hybridization analysis (array-CGH) consisted of hybridization of the patient and control DNA to the Agilent Human Genome CGH 4×44k microarrays (G4426B, AMADID#014950) (Agilent Technologies, Santa Clara, Calif., USA) according to the manufacturer's protocol with slight modifications. In brief, 150 ng of genomic DNA was labeled with Cy5 (patient) or Cy3 (control). After clean up, labeled fragments were pooled and 5 μl Cot-1 DNA (1 mg/ml), 10× blocking agent, and 2× hybridization buffer were added. This mixture was hybridized on the microarrays for 24 h at 65°C. After washing, the slides were scanned using an Agilent Microarray Scanner, quantified with Feature Extraction software 9.1 (Agilent Technologies), and analyzed with array-CGH base (http://medgen.ugent.be/arraycghbase/) [Menten et al., 2005].

Array-CGH analysis (Agilent 44 K, resolution of ~400 kb) revealed a microdeletion of ~13 Mb on chromosome 7p21.1–p14.3 (fig. 2). Within the deleted region 74 genes are located, with 6 of them responsible for a variety of autosomal dominant diseases: *TWIST, DFNA5, CYCS, HOXA11, HOXA13,* and *GARS*. Array-CGH analysis of the parents and siblings of the patient were normal, indicating that the microdeletion was a de novo event in this patient and most likely caused his abnormalities. Because of the size of the microdeletion, confirmation by FISH analysis was unnecessary.

Discussion

We describe a patient with a ~13 Mb deletion on chromosome 7p21.1–p14.3 that was detected by array-CGH. Loss-of-function mutations in the *TWIST* gene, which

encodes a basic helix-loop-helix transcription factor, are responsible for the SCS [Howard et al., 1997]. Our patient had the typical phenotypic features of SCS (craniostenosis, clefting of the soft palate, and limb anomalies), and his ears resembled those seen in SCS. He had an interrupted ear tragi and hyperplastic antihelices and antitragi but no prominent helical crura. He also had ptosis of the eyelids and short palpebral fissures (compatible with BPES syndrome) and the additional features of broad halluces medialy deviated which meet the clinical criteria for Robinow-Sorauf syndrome [Robinow and Sorauf, 1975; Chotai et al., 1994; Maw et al., 1996; Dollfus et al., 2001; Cox et al., 2002].

There are 4 *HOX* gene clusters *(HOXA, HOXB, HOXC,* and *HOXD)* which are the basic developmental regulators that define positional information for the embryo along the anterior-posterior axis of the body [Davis et al., 1995; Kmita et al., 2005; Zakany et al., 2007]. *HOXA13* (OMIM #142959) mutations lead to the dominantly inherited hand-foot-uterus (OMIM #140000) and Guttmacher syndromes (OMIM #176305) [Davis et al., 1995; Goodman et al., 2000; Thompson et al., 2001; Kosaki et al., 2005]. Typical limb defects in these disorders include shortening of the carpals and tarsals and fifth finger clinodactyly which were observed in our case. However, the first-digit hypoplasia (typical of HFU) was absent which might have been due to the concurrent *TWIST* gene haploinsufficiency that is typically associated with broadening of the first digits. Our male proband had the typical genital anomalies found in patients with a *HOXA13* mutation [Goodman et al., 2000].

The *DFNA5* gene (OMIM #608798) is implicated in one of the autosomal dominant types of non-syndromic deafness (OMIM #600994). All 4 identified mutations result in the skipping of exon 8 of the *DFNA5* gene, raising the hypothesis that there is a specific gain-of-function effect [Van Laer et al., 1998, 2004; Cheng et al., 2007]. Two patients (cases 1 and 3) described by Duno et al. [2004] had deletions of the complete *DFNA5* gene without hearing loss. Unfortunately, we could not make a positive evaluation of our patient.

A single *HOXA11* (OMIM #142958) truncating mutation has been reported to cause a specific syndrome with skeletal defects (radioulnar synostosis) and amegakaryocytic thrombocytopenia (OMIM #605432) [Thompson and Nguyen, 2000; Thompson et al., 2001]. Double mice mutants for *Hoxa11* and *Hoxd11* have a very small radius and ulna and severe kidney defects [Davis et al., 1995]. Our patient did not have thrombocytopenia (220–550 $\times$ $10^3/\mu l$) or radioulnar synostosis.

A single missense mutation in the *CYCS* gene (OMIM #123970) encoding cytochrome c has been shown to cause autosomal dominant thrombocytopenia type 4 (OMIM #612004) [Morison et al., 2008], a syndrome not yet linked with haploinsufficiency which suggests it is a gain-of-function mutation.

Mutations of the *GARS* gene (OMIM #600287) are associated with Charcot-Marie-Tooth neuropathy type 2D (OMIM #601472) and distal spinal muscular atrophy V (OMIM #600794). Until now, only *GARS* missense mutations have been found [Antonellis et al., 2003], and it is unknown whether *GARS* haploinsufficiency leads to neurological deficits. Our patient did not have any neurological signs of Charcot-Marie-Tooth neuropathy or distal spinal muscular atrophy.

Several patients with deletions of chromosome 7p have been described in the literature [Chotai et al., 1994; Johnson et al., 1998; Cai et al., 1999; Cox et al., 2002; Hoover-Fong et al., 2003]. The boundaries of the deletions were not delineated in great detail because of the limited resolution of conventional cytogenetics, but the deletion breakpoints appeared distinct. The distal breakpoint of the deletion in the *HDAC9* gene of our patient was very close to the distal inversion breakpoint of a patient described by Duno et al. [2004] (case 2) and a translocation breakpoint reported by David et al. [2003]. Also, the distal breakpoint of a deletion described by Johnson et al. [1998] was located close to the *HDAC9* gene, which suggests that this region is a hot spot for recombination.

Only a single microdeletion encompassing both the *TWIST* and *HOXA* clusters has been described in the literature. Kosaki et al. [2005] described a patient with left coronal craniosynostosis, maxillary hypoplasia, prominent ear crura, rectoperineal fistula, anal atresia, patent ductus arteriosus, hypoplastic fifth finger, and psychomotor delay. Although no array-CGH was performed, fluorescent in situ hybridization analysis showed a deletion of the *TWIST* gene close to the *HOXA* gene cluster. The facial anomalies could be mainly attributed to haploinsufficiency of the *TWIST* gene, whereas the other anomalies were more difficult to assign to specific gene deletions. Chotai et al. [1994] reported a panel of 6 7p-deletion cases, 3 with craniosynostosis. There were 5 de novo deletions and 1 resulting from the unbalanced product of a paternal balanced insertion. The putative proximal locus at 7p13–p14 does not appear to be allelic with Greig cephalopolysyndactyly syndrome [Chotai et al., 1994].

In conclusion, microdeletion of 7p21 spanning the *TWIST* and the *HOXA* clusters leads to combined phenotype of Saethre-Chotzen syndrome and hand-foot-uterus syndrome. The phenotype of the microdeletion 7p21.1–p14.3 could not be explained only by variable expressivity of haploinsufficiency of other genes, but incomplete penetrance may play a critical role. Therefore, array-CGH testing is necessary to exclude a 7p21–p14 microdeletion in patients with craniosynostosis, limb anomalies, and genital hypoplasia.

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