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Deletions of Xp Provide Evidence for the Role of Holocytochrome C-Type Synthase (*HCCS*) in Congenital Diaphragmatic Hernia

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TO THE EDITOR

Microphthalmia with linear skin defects (MLS) is a rare, congenital, X-linked dominant syndrome most commonly caused by terminal deletions of Xp (OMIM 30980). Inactivation of the holocytochrome c-type synthase gene (*HCCS*, Xp22.2) has been implicated as the cause of many of the characteristic findings in MLS including linear skin defects, microphthalmia and other ocular anomalies, cardiac anomalies, and mild to severe mental retardation [Wimplinger et al., 2006, 2007]. Inactivation of *HCCS* is usually lethal in 46,XY males but several males with MLS and Xp;Yp translocations involving the sex-determining region Y gene (*SRY*) have been reported [Morleo et al., 2005; Kapur et al., 2008]. Congenital diaphragmatic hernia (CDH) is sometimes listed as a feature of MLS syndrome and terminal Xp deletions have been documented in at least four patients with CDH [Allanson and Richter, 1991; Plaja et al., 1994; Nowaczyk et al., 1998; Pober et al., 2005]. In all of these cases, the Xp deletions were defined by cytogenetic analyses without detailed molecular characterization.

The patient reported here was a male child born to healthy, unrelated parents both of whom were of Caucasian/Hispanic descent. He has one healthy brother and three healthy maternal half -sisters. His mother also had one 12-week miscarriage with his father. At 20 weeks gestation, prenatal ultrasound showed a large, left-sided congenital diaphragmatic hernia with gastric herniation. Amniocentesis performed at 24 weeks gestation showed a 46,X,der-(X)t(X;Y)(p22.3;p11.3) chromosome complement. A FISH study for *SRY* (probe: Vysis LSI SRY, Abbott Molecular, Abbott Park, IL) confirmed its presence on the terminal short arm of the der(X) chromosome. Parental chromosome studies were normal. Bilateral cerebral ventriculomegaly was seen at 35 weeks gestation.

The pregnancy was complicated by maternal arrhythmia and pregnancy-induced hypertension. The patient was born via spontaneous vaginal delivery at 37 6/7 weeks with

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vacuum assisted extraction. Apgar scores were 3, 3, and 6 at 1, 5, and 10 min, respectively, after which he was intubated. His birth weight was 2,610 g (10–25th centile), length 46 cm (10–25th centile) and head circumference was 34 cm (50–75th centile). Postnatal evaluation showed bilateral microphthalmia with optic atrophy, a congenital cataract, linear vascular lesions on the cheeks, neck and nose, apparently small, cupped poorly formed ears, anteverted nares, small areolae, a prominent xiphoid, an apparently small phallus, and right cryptorchidism. Echocardiography showed a patent ductus arteriosus, a patent foramen ovale and severe pulmonary hypertension attributed to pulmonary hypoplasia. Head ultrasound showed bilateral ventriculomegaly and agenesis of the corpus callosum. He was also noted to have poor tone and diminished reflexes on neurological examination. These findings were consistent with a diagnosis of MLS syndrome. In light of his CDH, increasing respiratory distress, and the multiple anomalies listed above, his prognosis was deemed to be poor. Support was withdrawn and he died at 4 days of age.

Clinical array comparative genomic hybridization (aCGH) analysis of the patient's DNA was performed in the Medical Genetics Laboratories at Baylor College of Medicine (BCM) using an array which consists of ~105,000 60-mer oligonucleotide probes and provides coverage of the whole genome with an average resolution of ~30 kb and increased coverage at known disease loci (CMA OLIGO V7.4, Agilent Technologies, Santa Clara, CA). This analysis showed that the terminal deletion of Xp extended from Xpter to 11,619,963 bp (minimal) or 11,660,647 bp (maximum) and the additional material from Yp extended from Ypter to 10,387,718 bp (minimal) or 10,388,109 bp (maximal) based on human genome build 18 (hg18; see supporting information Fig. 1 which may be found in the online version of this article). The final karyotype designation was 46,X,der(X)t(X;Y)(p22.2;p11.2). Though additional chromosome material from chromosome Yp may have predisposed the patient to develop CDH, it is more likely that the major contribution was due to haploinsufficiency of a gene(s) on Xp, such as *HCCS*, since previously reported patients had isolated terminal deletions of Xp based on standard chromosome analyses [Allanson and Richter, 1991; Plaja et al., 1994].

Our re-analysis of their data showed that these exons belong, instead, to AC073529.1, a processed transcript with no known protein product. The *HCCS* gene was deleted in the present patient, and in three of the four patients with CDH and Xp deletions previously reported [Allanson and Richter, 1991; Plaja et al., 1994; Nowaczyk et al., 1998; see supporting information Table I and supporting information Fig. 2 which may be found in the online version of this article]. Disruption of *Hccs* in mice causes fetal death before E10.5, a time point before diaphragm development, limiting the utility of null mice to understand the role of HCCS in diaphragm development [Prakash et al., 2002]. The strongest evidence implicating *HCCS* in the development of CDH was a report of a family with MLS in which one daughter had bilateral anophthalmia and died at 6 hr of age from complications of a left-sided CDH [Wimplinger et al., 2006]. This individual's two living affected siblings and their unaffected mother were subsequently shown to have a 8.6 kb deletion that included exons 1, 2, and the first 83 bp of exon 3 of *HCCS*. They also reported that two untranslated exons of midline 1 (*MID1*) were deleted. However, *HCCS* and *MID1* are separated by approximately 280 kb. Our re-analysis of their data showed that these exons belong, instead, to *AC073529.1*, a processed transcript with no known protein product. This suggests that disruption of *HCCS* is sufficient to cause CDH in some individuals.

It is unusual for CDH to be fully penetrant and variations in genetic background and/or environmental factors may explain why other individuals in the family described by Wimplinger et al. [2006] were not affected by CDH and why *HCCS* deletions do not always cause CDH. Incomplete penetrance may also explain why individuals bearing the three known point mutations in *HCCS* (c.589C>T (p.Arg197Ter), c.649C>T (p.Arg217Cys), c.

475G>A (p.Glu159Lys)) developed MLS syndrome or isolated ocular findings without CDH [Wimplinger et al., 2006, 2007].

Holocytochrome c-type synthase is expressed in many tissues including the diaphragm and catalyzes the covalent attachment of heme to apocytochrome c, forming the mature form of holocytochrome c, a highly conserved protein involved in mitochondrial electron transport [Bernard et al., 2003; Shyamsundar et al., 2005]. Mouse studies suggest that HCCS also plays an important role in cell proliferation in vivo [Drenckhahn et al., 2008]. Current hypotheses suggest that a decrease in the amount of tissue within the rostral and lateral aspects of the pleural peritoneal fold (which contains the precursor tissue of the diaphragm) causes posterolateral CDH as seen in patients with terminal Xp deletions [Ackerman and Greer, 2007]. It is possible that inactivation of *HCCS* results in respiratory chain deficiency and abnormal levels of cell proliferation which, in turn, results in decreased pleural peritoneal fold tissue and a higher risk for CDH.

The only reported case in which an Xp deletion not including *HCCS* was associated with CDH was a patient with a familial translocation 46,X,der(X)t(X;Y)(p22.3;q11.2) [Pober et al., 2005], see supporting information Fig. 2 which may be found in the online version of this article. Although the original manuscript did not contain a detailed clinical description, this patient presented with CDH, a VSD that closed spontaneously, mild developmental delay, exotropia, and short stature [height of 103.4 cm (~1st centile)] and weight of 17.4 kg (~15th centile) at 5 years 10 months of age [B. Pober, unpublished data]. A FISH analysis showed that the steroid sulfatase gene (*STS*, located distal to *HCCS* on Xp22.3) was present on the der(X) chromosome. This translocation was also found in her mother and maternal grandmother who did not have CDH but had short stature—adult heights of 147 cm (<1st centile) and 150 cm (<3rd centile), respectively. Although the CDH in this patient may have been associated with extra material from Yq, we know of no other cases of CDH involving Yq duplications. In addition, males with either 47,XXY or 47,XYY do not have an increased frequency of CDH [Holder et al., 2007]. It is more likely that deletions of one or more genes distal to *STS* on Xp contributed to the development of CDH in this patient and may also contribute to the overall risk of developing CDH in patients with terminal Xp deletions.

The *MID1* gene is located between *STS* and *HCCS* (outside the CDH-related interval defined above) but has been considered as a candidate gene for CDH (Supplemental Fig. 2) [Slavotinek, 2007]. Although it is possible that deletions of *MID1* contribute to the risk of developing CDH, it is unlikely that haploinsufficiency of *MID1* alone is sufficient to cause CDH since mutations in *MID1* cause X-linked Opitz G/BBB syndrome which is not typically associated with CDH. Indeed, the only published case report linking Opitz G/BBB syndrome and CDH is a male fetus prenatally diagnosed at 19 weeks gestation whose physical findings and family history were thought to be consistent with this diagnosis. However, no molecular confirmation was possible since this case was described before *MID1* was identified as the cause of this disorder [Hogdall et al., 1989; Quaderi et al., 1997].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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