Prenatal Testing for SOX9 Gene Mutations: Campomelic Dysplasia

Also known as: CD; campomelic dwarfism; campomelic syndrome with autosomal sex reversal; CMPS.

Includes: acampomelic campomelic dysplasia; ACD

Mendelian Inheritance in Man Number: 608160 (SOX9 gene); 114290 (campomelic dysplasia)

Clinical Features in Newborns and Children:
Campomelic dysplasia (CD) is a rare, often lethal skeletal dysplasia characterized by angular bowing and shortening of the long bones, severe respiratory distress, and XY sex reversal. It is caused by chromosome abnormalities or mutations affecting expression of the SOX9 gene located on chromosome 17q24.3-q25.1. Approximately 75% of patients with CD that have a 46,XY karyotype exhibit partial or complete sex reversal, ranging from ambiguous genitalia to normal female external genitalia (Mansour et al. 1995). In addition to bowing of the long bones, skeletal features of CD include club feet, a bell-shaped and underdeveloped thorax, eleven pairs of ribs, and hypoplastic scapulae. CD is also associated with micrognathia and Pierre-Robin malformation. Many infants die shortly after birth from respiratory compromise. Children who survive the neonatal period often develop hearing loss, developmental delay, short stature and progressive kyphoscoliosis (Mansour et al., 2002). A small subgroup of patients with CD lack significant bowing of the limbs and are described as having acampomelic campomelic dysplasia (ACD). Patients with ACD have other characteristic features of campomelic dysplasia, and may exhibit mild campomelia on careful radiographic study.

Prenatal Ultrasound Findings:
A 46,XY karyotype in a fetus with female or ambiguous genitalia on ultrasound accompanied by limb shortening and/or bowing is strongly suggestive of CD; however, CD should be considered in any fetus with shortening of the long bones with or without limb bowing. In the first and second trimester, 46,XX and 46,XY fetuses with CD may exhibit an increased nuchal translucency and/or cystic hygroma accompanied by shortening of the long bones of the lower extremities, with or without accompanying limb bowing (Massardier et al., 2008). Scapular hypoplasia is extremely difficult to detect in utero, and other characteristic facial features such as micrognathia may be difficult to identify by ultrasound in the first or second trimester of pregnancy. Ultrasound examination may be normal in affected fetuses; therefore, pregnancies at risk to inherit a specific known familial mutation can be offered targeted molecular testing regardless of ultrasound findings, if desired.

Inheritance Pattern:
Autosomal dominant, typically de novo. Germline and somatic mosaicism have been reported (Wagner at al 1994; Cameron et al 1996; Smyk et al 2007)

Indications for Fetal Testing:
- Full sequencing and deletion/duplication testing for fetuses with ultrasound findings suggestive of CD
- Mutation-specific testing for fetuses with a family history of a known SOX9 mutation

Test Method:
Using genomic DNA, analysis is performed by bi-directional sequencing of the coding region (exons 1-3) and the flanking splice sites of the SOX9 gene. Concurrently, targeted array CGH analysis with exon-level resolution (ExonArrayDx) is performed to evaluate for a deletion or duplication of one or more exons of the SOX9 gene. For known familial mutations, the relevant portion of the SOX9 gene will be analyzed in duplicate.

Additionally, genotype analysis of maternal and fetal DNA for several polymorphic markers to test for maternal cell contamination is performed. Therefore, in all prenatal cases a maternal sample should accompany the fetal sample.

Test Sensitivity:
SOX9 is the only gene known to be associated with campomelic dysplasia. Of patients with a clinical diagnosis of CD or ACD that have a normal karyotype, an estimated 90-95% have mutations in the SOX9 gene that are identifiable by sequencing (Pop et al. 2004). Additionally, an estimated 5% have deletions of the SOX9 gene that would be missed by sequencing but would be detected by ExonArrayDx. Of note, SOX9 mutations have not been identified in patients with isolated XY sex reversal in the absence of skeletal malformations (Meyer et al, 1997). The sensitivity of SOX9 testing in pregnancies with ultrasound anomalies suggestive of campomelic dysplasia is not currently known.
Mutation Spectrum:
To date, approximately 30 missense, nonsense, frameshift and splice site mutations have been identified throughout the coding region of the SOX9 gene. Most mutations are private, although the Y440X mutation accounts for about 8% of all mutations in the SOX9 coding region (Pop et al., 2005). In addition, 15 patients with CD or ACD have been reported with genomic rearrangements, including inversions, translocations and deletions affecting the SOX9 region. Chromosome rearrangements and missense mutations in the DNA binding domain of SOX9 are typically associated with a less severe phenotype and a longer survival (Moog et al., 2001; Mansour et al., 2002). In fact, individuals with ACD and/or long-term survival are more likely to have a genomic rearrangement than to have a mutation within the SOX9 coding region (Pfeifer et al, 1999; Leipoldt et al., 2007). Of note, one patient with XX sex reversal was reported to harbor a duplication of 17q23.1-q24.3 that included the SOX9 gene (Huang et al., 1999).

Specimen Requirements and Shipping/Handling:
- **Prenatal Specimen – Based on Abnormal Ultrasound/Other Findings (test #2503):** 20mg villi preferred (minimum 15 mg) or 20 mL amniotic fluid or 2 T25 flasks of cultured CV or cultured amniocytes. Ship overnight at ambient temperature, using a cool pack in hot weather.
- **Prenatal Specimen- Based on Specific Known Mutation (test #902):** 20mg villi preferred (minimum 15mg) or 20 mL amniotic fluid or 2 T25 flasks of cultured CV or cultured amniocytes. Ship overnight at ambient temperature, using a cool pack in hot weather.
- **Maternal cell contamination studies (required for all prenatal testing):** 1-4 ml maternal blood in a lavender-top EDTA tube. Ship overnight at ambient temperature, using a cool pack in hot weather. Specimens may be refrigerated for 7 days prior to shipping. Alternatively, buccal brushes (GeneDx kit only) or DNA can be used. The maternal sample should accompany the prenatal specimen or be shipped to arrive prior to or concurrently with the prenatal sample.

*If more than one prenatal test is ordered, 30 mL amniotic fluid, 30mg villi or 3 T-25 flasks of cultured cells are requested*

**Required Forms:**
- Sample Submission (Requisition) Form – complete all pages
- Payment Options Form or Institutional Billing Instructions (last page of submission form)

**Prices and Turn-Around Time – Fees subject to change without notice:**
- Test #3383: Prenatal diagnosis based on ultrasound findings: $2,100 Approx. 2-3 weeks
- Test #902: Prenatal diagnosis for a specific known mutation: $2,000 Approx. 2 weeks

### CPT codes for Test # 3383 Prenatal Testing for campomelic dysplasia – All codes and units apply:
- 83891 x 12 units = $90
- 83898 x 23 units = $575
- 83894 x 23 units = $172
- 83904 x 24 units = $620
- 88386 x 1 unit = $488
- 83892 x 2 units = $40
- 83912 x 3 units = $115
- TOTAL $2,100

### CPT codes for Test #902 Prenatal Testing for Specific Known SOX9 mutation - All codes and units apply:
- 83891 x 5 units = $160
- 83898 x 10 units = $710
- 83894 x 5 units = $160
- 83904 x 10 units = $750
- 83892 x 2 units = $ 60
- 83912 x 5 units = $160
- TOTAL $ 2000

Possible ICD9 Codes: 655.83 – abnormal ultrasound findings; 655.23 – family history possibly affecting fetus