Prenatal 46,XY Disorders of Sex Development Panel

This panel includes 10 genes; some variants in these genes may manifest with genital ambiguity or external genitalia that are discordant with gender based on genetic testing findings. Pathogenic variants in these genes may be suspected when the fetal karyotype is 46,XY or non-invasive prenatal screening is consistent with the presence of a Y chromosome but ultrasound reveals apparently female or ambiguous external genitalia. Ultrasound detection of abnormalities of the genitalia can be detected as early as the 2nd trimester of pregnancy. Depending on the underlying disorder, renal malformations, congenital heart defects, limb/skeletal malformations, intrauterine growth restriction, cleft lip/cleft palate, and congenital brain defects may also be observed by ultrasound. Note that some ultrasound findings may not be detectable until the 3rd trimester of pregnancy. In addition, some disorders may also present with abnormal findings on second trimester maternal serum screening, such as abnormal maternal serum estriol (MS-uE3) levels.

As disorders of sex development (DSD) may also be caused by genomic copy number variations, it is recommended that this panel be performed following or concurrent with whole genome chromosomal microarray analysis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>OMIM References</th>
<th>Inheritance</th>
<th>Prenatal ultrasound/maternal serum findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRY</td>
<td>400044 (46, XY Sex Reversal 1/46,XY Complete or Partial Gonadal Dysgenesis)</td>
<td>Y-linked</td>
<td>Male karyotype and female or ambiguous genitalia [1]</td>
</tr>
<tr>
<td>NR5A1</td>
<td>612965 (46,XY Sex Reversal 3)</td>
<td>Autosomal dominant/Autosomal recessive</td>
<td>Male karyotype and female or ambiguous genitalia [1]</td>
</tr>
<tr>
<td>AR</td>
<td>300068 (Androgen Insensitivity Syndrome, AIS) 300633 (Hypospadias 1, X-Linked)</td>
<td>X-linked recessive</td>
<td>Complete (CAIS): Male karyotype and female genitalia Partial (PAIS): male karyotype and female or ambiguous genitalia [2]</td>
</tr>
<tr>
<td>SRD5A2</td>
<td>264600 (5-α Reductase Deficiency/Pseudovaginal Perineoscrotal Hypospadias)</td>
<td>Autosomal recessive</td>
<td>Male karyotype and female or ambiguous genitalia [3]</td>
</tr>
<tr>
<td>SOX9</td>
<td>114290 (Campomelic Dysplasia)</td>
<td>Autosomal dominant</td>
<td>Male karyotype and female or ambiguous genitalia and limb shortening and/or bowing [4]</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Mode of Inheritance</td>
<td>Clinical Features</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>DYNC2H1</td>
<td>613091 (Short-Rib Thoracic Dysplasia 3 with or without Polydactyly)</td>
<td>Autosomal recessive / digenic recessive (NEK1)</td>
<td>Narrow thorax, bilateral short limbs, polydactyly, bilateral enlarged kidneys, and male karyotype and female or ambiguous genitalia [5]</td>
</tr>
<tr>
<td>NEK1</td>
<td>263520 (Short-Rib Thoracic Dysplasia 6 with or without Polydactyly)</td>
<td>Autosomal recessive/digenic recessive (DYNC2H1)</td>
<td>Polydactyly, choroid plexus cysts, short ribs and narrow chest, short fibula, tibial aplasia, and genital abnormalities [6]</td>
</tr>
<tr>
<td>DHCR7</td>
<td>270400 (Smith-Lemli-Opitz Syndrome)</td>
<td>Autosomal recessive</td>
<td>Male karyotype and female or ambiguous genitalia and IUGR, brain, cardiac, renal, or limb malformation and/or low MS-uE3 levels on maternal serum screening [7]</td>
</tr>
<tr>
<td>CHD7</td>
<td>214800 (Charge Syndrome) 612370 (Hypogonadotropic Hypogonadism 5 with or without Anosmia)</td>
<td>Autosomal dominant</td>
<td>Heart defects, cleft lip/palate, CNS malformations, renal, gastrointestinal anomalies, and hypogonadism (micropenis) [9]</td>
</tr>
<tr>
<td>ARX</td>
<td>300215 (Lissencephaly, X-Linked, 2) 300004 (Agenesis of Corpus Callosum with Abnormal Genitalia)</td>
<td>X - linked</td>
<td>Structural brain anomalies, agenesis of the corpus callosum, ambiguous genitalia, hypospadias [9]</td>
</tr>
</tbody>
</table>

**Clinical Features in Newborns and Children:**

The 46,XY DSDs are a highly variable group of disorders, which arise from abnormalities in the complex process of sex determination and differentiation. Approximately 1 in 20,000 live male births are affected with a 46,XY DSD, with the severity ranging from mild hypogonadism (such as micropenis) to complete gonadal dysgenesis [1,10]. The genes included in the prenatal 46,XY DSD panel include disorders associated with non-syndromic DSD, steroid abnormalities, skeletal dysplasia syndromes, and multiple malformation syndromes where the presenting fetal ultrasound finding is discrepant/ambiguous gender.

A brief overview of the more common conditions associated with the genes included in this panel is given below:

**Gene variants associated with non-syndromic DSD / sex determination abnormalities (SRY and NR5A1):**

Pathogenic variants in SRY are associated with 46, XY Complete or Partial Gonadal Dysgenesis. 46,XY complete gonadal dysgenesis (CGD) is marked by a lack of testicular development, streak gonads, the presence of well-developed Mullerian structures (a uterus
and fallopian tubes), underdeveloped breasts, and female external genitalia. Individuals often are not diagnosed until puberty when they present with amenorrhea and the absence of secondary sexual characteristics. In rare cases, SRY mutations have been associated with 46,XY partial gonadal dysgenesis (also called SRY-related 46,XY disorder of sex development or SRY-related 46,XY DSD). 46,XY DSD is characterized by the presence of ambiguous genitalia, dysgenetic testes, and absent to fully developed Mullerian structures. Both 46,XY CGD and 46,XY DSD are associated with an increased incidence of gonadoblastoma and germinoma [1].

Pathogenic variants in NR5A1 result in a 46,XY disorder of sex development (DSD) with or without adrenal insufficiency. At the severe end of the spectrum, individuals with NR5A1 mutations have presented with primary adrenal failure and 46,XY complete gonadal dysgenesis characterized by female external genitalia, severe testicular dysgenesis, and the presence of Mullerian structures or in patients presenting at puberty with 46,XY primary amenorrhea. At the milder end of the spectrum, NR5A1 mutations have been reported in individuals with normal adrenal function and 46,XY partial gonadal dysgenesis resulting in ambiguous genitalia, bilateral testes, and no evidence of Mullerian structures. Mutations in the NR5A1 gene have also been identified in several patients with severe (penoscrotal) hypospadias and undescended testes, and in males with idiopathic infertility [1].

Gene variants associated with abnormalities of androgen synthesis or action / sex differentiation abnormalities (AR and SRD5A2): These disorders are associated with abnormalities in the steroid pathway and can lead to abnormal levels of glucocorticoids and mineralcorticoids, which can potentially lead to salt-wasting, hypertension, and/or hypokalemia. The steroid abnormalities also affect the production of enzymes need to make hormones involved in sex development. 46,XY individuals can present with a variable phenotype ranging from female external genitalia, blind vaginal pouch, absence of Mullerian structures, to ambiguous genitalia, hypospadias, and cryptorchidism.

Androgen Insensitivity Syndrome (AIS) is caused by pathogenic variants in the AR gene and may be complete or partial. Patients with AIS may come to attention in utero or at birth because of inconsistency between prenatal karyotype (male) and ultrasound findings of a female fetus, or at birth because of ambiguous genitalia. Alternatively, patients may present during the pubertal years with a presumed inguinal hernia (abdominal or inguinal testes), absence of pubic/auxiliary hair, or lack of onset of menses. The mature phenotype is often distinctly feminine with very well-developed breasts and abundant scalp hair. In the partial form, patients may exhibit hypospadias, micropenis, or fusion of the labial folds, associated with the occurrence of virilization at puberty [2].

Individuals with pathogenic variants in SRD5A2 are affected with 5-α reductase deficiency. Most patients have ambiguous genitalia noted in infancy, individuals with female external genitalia may not present until puberty with primary amenorrhea, a lack of breast development, and virilization of the external genitalia. If the diagnosis of 5-α reductase deficiency is not made, the majority of infants are assigned a female gender based on the appearance of the external genitalia; however, significant virilization of the external genitalia occurs at puberty unless a gonadectomy is performed [3,11].
Gene variants associated with a skeletal dysplasia (SOX9, DYNC2H1, and NEK1): These disorders may present with additional ultrasound anomalies, although the additional skeletal anomalies may not be detectable until the third trimester of pregnancy, and can be associated with lethality in the neonatal period.

Aberrations involving the SOX9 gene are associated with campomelic dysplasia (CD), a rare, often lethal skeletal dysplasia characterized by angular bowing and shortening of the long bones, severe respiratory distress, and XY sex reversal. 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 [4]. 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 scalpulae. CD is also associated with micronathia 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 [12].

Short-rib thoracic dysplasia (SRTD) is a group of autosomal recessive ciliopathies; some forms are lethal in the neonatal period. SRTD3 and SRTD6 are caused by homozygous, compound heterozygous, or digenic biallelic pathogenic variants in DYNC2H1 and NEK1, respectively, and are characterized by a constricted thoracic cage, short ribs, shortened tubular bones, and a ‘trident’ appearance of the acetabular roof. Polydactyly may or may not be present. The severity of these disorders varies, even within families. The thoracic abnormalities in SRTD3 tend to improve with age. SRTD6 is characterized by short ribs and limbs, medial cleft lip, pre- and postaxial polydactyly, genital abnormalities, polycystic kidneys, and anomalies of the epiglottis and viscera [6,13].

Gene variants associated with a multiple malformation syndrome (DHCR7, CHD7, and ARX): These disorders may present with additional ultrasound anomalies and/or abnormal maternal serum findings.

DHCR7 is associated with Smith-Lemli-Opitz Syndrome (SLOS), a severe developmental disorder. The clinical spectrum is wide and includes both pre- and post-natal growth retardation, mild to severe mental retardation, multiple congenital malformations (both major and minor), and characteristic facies. Frequent additionally observed findings include: microcephaly, micrognathia, cleft palate, cardiac defects, abnormal external genitalia, postaxial polydactyly, and 2-3 toe syndactyly. Infants are often hypotonic with poor suck, and fail to thrive. Older children commonly have behavioral concerns including autism, hyperactivity, aggression, and self-injurious behavior [7].

Individuals with pathogenic variants in CHD7 present with CHARGE syndrome. CHARGE syndrome is characterized by coloboma of the eye, heart defects, choanal atresia, intellectual disability, growth retardation and ear anomalies or hearing loss. Affected patients may also have genital abnormalities (hypogonadotrophic hypogonadism), pre- and post-natal growth deficiency, hypotonia, and characteristic hands (broad palms with “hockey-stick” palmar crease, short fingers and small/unusual thumbs). The characteristic facial appearance includes square face with broad prominent forehead, arched eyebrows, large eyes with or without ptosis, prominent nasal bridge and columella, flat midface, small mouth and facial asymmetry.
CHARGE syndrome encompasses additional nonspecific features such as intellectual disability, skeletal abnormalities, hypodontia, orofacial clefting, tracheoesophageal fistula, and urinary tract and renal anomalies [14].

Variants in ARX are associated with various clinical phenotypes causing intellectual disability, seizures, movement disorders, brain malformations, and/or abnormal genitalia [9].

**Test Methods:**
Using genomic DNA obtained from prenatal specimens, the coding exons and flanking splice junctions of 10 genes are enriched using a proprietary targeted capture method developed by GeneDx. The products are sequenced on an Illumina instrument using paired-end reads. The sequence data is aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Sanger sequencing is used to compensate for low coverage and refractory amplifications. The presence of any potentially disease associated sequence variant(s) is confirmed by dideoxy DNA sequence analysis or by other methods as appropriate.

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

**Test Sensitivity:**
The 46,XY DSDs are a genetically heterogeneous group of conditions with a wide variant spectrum. The clinical sensitivity of sequence analysis of the 10 genes included in this panel in prenatal cases ascertained based on fetal ultrasound gender discrepancy/ambiguity is currently unknown, and the clinical sensitivity of analysis of the genes included in the Prenatal 46,XY Disorders of Sex Development depends on the specific gene and the clinical phenotype of the patient.

The technical sensitivity of gene sequencing in prenatal cases ascertained based on fetal ultrasound abnormalities is estimated to be greater than 99%. It will not detect deletions, insertions, or rearrangements greater than or equal to ten base pairs.

Most pathogenic variants identified in these genes include frameshift, nonsense, missense, and splice site variants resulting in protein truncation or loss of expression of the allele with the variation. Pathogenic deletions and/or duplications have also been observed in majority of analyzed genes [15].

**References:**