Review of Literature
2. REVIEW OF LITERATURE

Advances in medicine have led to decline in diseases like infection and malnutrition, hence the congenital malformations have emerged gaining great importance in perinatal mortality. An estimated, 3,90,000 with G6PD deficiency, 21,400 with Down syndrome, 9,000 with beta-thalassaemia, 5,200 with sickle cell disease, 9,760 with amino acid disorders and 4,95,000 infants with congenital malformations are born each year in India (Verma and Bijarnia, 2002).

In general, there are 1,07,488 articles (CHD+CDH+ACC) available in Pub Med as on date and therefore an attempt has been made to briefly review the relevant information in this chapter.

2.1 CONGENITAL MALFORMATION

Congenital malformation, birth defects and congenital anomalies are interchangeable terms used to describe developmental defects that are present at birth. According to WHO, only structural defects at birth should be termed as congenital malformation. A series of precisely timed genetic and environmental interactions determine the complex transition from a single fertilized ovum to a normally formed human being. The stage of development that these interactions affect during the process of embryogenesis influences the type of birth defect (Tanteles and Suri, 2007).
2.1.1 Prevalence

Patel and Adhia (2005) detected that congenital malformations affect 2.5% of infants and are responsible for 15% of perinatal mortality. Out of 17,653 consecutive births, 294 (1.6%) had a major malformation, 1,400 (7.92%) had minor malformation and 328 (1.85%) were still borns out of which 52 (15.8%) were malformed. The incidence of congenital anomalies was higher amongst still born than among live babies. The overall incidence of congenital malformations in the study was 1.63%.

Bhat and Babu (1998) studied congenital malformations prospectively from September 1989 to December 1992 covering 12,797 consecutive deliveries. The overall incidence of malformations was 3.7% and it was 3.2% among live births and 15.7% among still births. It was significantly higher among male babies (p< 0.001), stillbirths (p < 0.001), low birth weights (p< 0.001) and preterm babies (p< 0.001) and more common (p < 0.001) in babies born to consanguineous parents. Musculo-skeletal malformations were the commonest (9.69 per 1000) followed by cutaneous (6.33 per 1000), genitourinary (5.47 per 1000), gastrointestinal (5.47 per 1000), central nervous system (3.99 per 1000) and cardiac anomalies (2.03 per 1000).

In another study from Mumbai, India the incidence of congenital anomalies was found to be 3.61%. The incidence in live born was 3.51% and in stillborns was 8%. The most common system involved was the musculoskeletal system followed by gastrointestinal and cardiac anomalies. Prematurity, increased maternal age, increasing birth order and low birth weight were found to have a higher risk of congenital anomalies (Desai and Desai, 2006).
About 3% of children born in the United States have a major birth defect. The infant mortality rate was 7.0/1000 live births in 2002 and birth defects account for about 20% of all infant deaths (Martin et al., 2005). Lifetime costs have been estimated at $6 billion for those infants born in a single year with 1 or more of 17 major birth defects (Boyle and Cordero, 2005).

2.1.2 Etiology
These defects can occur for many reasons including inherited conditions, toxic exposure of the fetus (for example, to alcohol) and birth injury. In most of the cases, the cause is unknown. It has been estimated that genetic factors account for about 25% of all birth defects (Brent, 2004), and nearly 85% of all those with a known cause. Genetic causes of birth defects include chromosomal aberrations, mutations in genes (single gene defects) and the interaction of both environmental and genetic factors (multifactorial disorders). Environmental factors can be wholly or partially be responsible for some birth defects. These factors may be inherent in the maternal environment (e.g. acetylcholine receptor antibodies in a mother with myasthenia giving rise to congenital contractures in the fetus), or may be extraneous agents such as drugs taken by the mother or maternal infections in the early antenatal period (Clayton-Smith and Donnai, 2001). Environmental factors are responsible for approximately 10% of congenital anomalies (Brent, 2004).

2.1.3 Consanguinity
Higher incidence of congenital defects has been reported due to consanguinity (Rao, 1991; Jain et al., 1993; Badaruddozah et al., 1998; Hornby et al., 2001 and Muthukumaravel et al., 2005).
2.1.4 Maternal Age
Mothers with age more than 30 years stand at a higher risk of producing malformed babies (Murphy, 1947; Anand et al., 1998; Bhat and Babu, 1998; Desai and Desai, 2006).

2.1.5 Classification
Congenital malformation can be classified based on their severity (their medical or social consequences), their pathogenesis (their clinical presentation) or based on etiology.

2.1.5.1 Classification based on severity (Marden et al., 1964)
**Major malformations:** They are defined as those malformations which are either lethal or significantly affect the child’s function and/or appearance. About 2–3% of children are born with a major anomaly that is evident at birth, such as cleft lip and palate, anophthalmia and radial aplasia. A similar number of children are born with a major anomaly that only becomes evident later in life, such as atrial septal defect, polymicrogyria or hemivertebrae.

**Minor malformations:** They are defined as those which do not have functional but may be cosmetic importance such as up- or down slanting palpebral fissures, high-arched or narrow palate, single palmar crease, partial syndactyly of the second and third toes. Approximately, 15% of children are born with one or more minor anomalies. Sometimes multiple minor malformations may signify an underlying genetic disorder (Hurst et al., 2001).

2.1.5.2 Classification based on pathogenesis (Spranger et al., 1982)
Based on the pathogenesis, congenital malformation can be classified into four groups-
**Malformation:** Birth defects that result from failure or inadequate completion of normal developmental processes are called ‘malformations’

**Deformation:** Birth defect that results from an aberrant mechanical force distorting normally developing structures.

**Disruption:** Birth defects that result from destructive processes that alter a structure after it has formed normally.

**Dysplasia:** Abnormal cellular organisation or function within a specific tissue type throughout the body, resulting in apparent structural changes.

### 2.1.5.3 Classification based on etiology (Wellesley et al., 2005)

Classification based on etiology for epidemiological study would take into account information available at the time of registration, which may include family history, and diagnostic and laboratory test data in addition to the anomalies.

Categories for the classification of birth defects in hierarchical order-

**Chromosome (C):** For microscopically visible, unbalanced chromosome abnormalities includes trisomies (+ mosaics), triploidy, visible deletions such as 4p (Wolf-Hirschorn) etc.

**Microdeletion (MD):** For all submicroscopic chromosome abnormalities including microdeletions, uniparental disomy and imprinting mutations like Di-George, Prader willi, Sotos syndrome etc.

**Teratogen (T):** For known teratogens and prenatal infections like TORCH infection.
New dominant (ND): For new dominant mutations like Achondroplasia, Apert’s syndrome, Thanatophoric dysplasia, Campomelic dysplasia, Osteogenesis imperfecta types II

Familial (F): For familial disorders not included as a new dominant like Tuberous sclerosis, Meckel-Gruber syndrome, Autosomal recessive polycystic kidney disease, Fragile X syndrome.

Syndrome (S): For recognised non-familial, non-chromosomal syndromes not included in one of the previous categories such as Kabuki syndrome.

Isolated (I): For isolated anomalies like gastroschisis, talipes, cleft lip etc.

Multiple (M): For multiple anomalies and associations like VATER, CHARGE etc.

Congenital malformations can involve many different organs including the brain, heart, lungs, liver, bones, and intestinal tract. Children with congenital malformation have a wide array of problems including complex medical management issues, abnormalities in growth, special educational needs, behavioral and psychological problems, and cosmetic concerns. The pediatrician is faced with the challenge of making a diagnosis, pursuing therapeutic or prophylactic options, offering a prognosis and often, discussing recurrence risks with the family. Congenital malformation are being diagnosed in an increasing number in antenatal due to improved diagnostic technology especially USG.

2.2 OBSTETRIC SONOGRAPHY / FETAL MEDICINE
Fetal medicine is a branch of medicine that deals with the growth, development, care, and treatment of the fetus. Ultrasound allows examination of external and

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internal anatomy of the fetus and detection of major defects and subtle minor markers indicative of the chromosomal abnormality and genetic syndromes. It has become useful in the assessment of the cervix in women at risk for premature birth.

2.2.1 History
Scottish physician Ian Donald was one of the pioneers for medical use of ultrasound. His article "Investigation of Abdominal Masses by Pulsed Ultrasound" was published in The Lancet in 1958 (Donald et al., 1958). It was considered as the most important paper on Obstetrical and Gynaecological sonography that was ever written.

In 1962, after about two years of work, Joseph Holmes, William Wright, and Ralph Meyerdirk developed the first compound contact B-mode scanner. Wright and Meyerdirk launched the first commercial hand-held articulated arm compound contact B-mode scanner in 1963 (Woo, 2002). This was the start of the most popular design in the history of ultrasound scanners.

Stuart Campbell's landmark publication in 1969 "An improved method of fetal cephalometry by ultrasound" described the use of both the A- and B-mode scan to measure the fetal biparietal diameter (BPD) (Campbell, 1969). Campbell (1970) had developed a technique of measuring the BPD of the fetal head in which the midline echo of the brain was visualised on the two-dimensional display before the measurement was taken, making the technique very precise.

2.2.2 Types of Scan

2.2.2.1 Nuchal Translucency Scan
Nuchal translucency (NT) is the sonographic appearance of subcutaneous accumulation of fluid behind the fetal neck in the first trimester of pregnancy (Fig.1). The term translucency is used, irrespective of whether it is septated or not
and whether it is confined to the neck or envelopes the whole fetus. In 1866 Langdon Down noted that common characteristics of patients with Trisomy 21 are skin deficient in elasticity, giving the appearance of being too large for the body, and flat face with a small nose. In the 1990s, it was realized that the excess skin of individuals with Down’s syndrome can be visualized by USG as increased NT in the third month of intrauterine life. Fetal NT thickness at the 11–13+6 weeks scan has been combined with maternal age to provide an effective method of screening for Trisomy 21.

Every woman has a risk of having fetus with a chromosomal defect. At 11–13+6 weeks, all major chromosomal defects are associated with increased NT thickness (Snijders et al., 1998). The incidence of chromosomal and other abnormalities is related to the size, rather than the appearance of NT. For NT scan, the gestation should be 11–13+6 weeks and the fetal crown–rump length should be 45–84 mm. A mid-sagittal section of the fetus should be obtained and the NT should be measured with the fetus in the neutral position. During the second trimester, the translucency usually resolves and, in a few cases, it evolves into either nuchal edema or cystic hygromas with or without generalized hydrops. Other fetal abnormalities associated with increased NT are shown in Table 1.
Fig. 1-NT scan

Fig. 2- Nasal bone
**Nasal Bone:** Several studies have demonstrated a high association between absent nasal bone at 11–13\textsuperscript{+6} weeks and Trisomy 21, as well as other chromosomal abnormalities (Fig. 2) (Nicolaides, 2004).

**Ductus Venosus:** It is a unique shunt directing well-oxygenated blood from the umbilical vein to the coronary and cerebral circulations by preferential streaming through the foramen ovale into the left atrium. Blood flow in the ductus has a characteristic waveform with high velocity during ventricular systole (S-wave) and diastole (D-wave), and forward flow during atrial contraction (a-wave). In the second and third trimesters of pregnancy abnormal flow with absent or reverse a-wave is observed in impending or overt cardiac failure. At 10–13\textsuperscript{+6} weeks abnormal ductal flow (Fig. 3) is associated with chromosomal defects, cardiac abnormalities and adverse pregnancy outcome (Matias et al., 1998, Borrell et al., 2003).

**Tricuspid Flow:** Pulsed wave Doppler flow across the tricuspid valve is carried out to ascertain the presence or absence of Tricuspid regurgitation (TR). TR happens due to backward (leak) flow of the blood into the right atrium when the right ventricle contracts (Fig. 3). At 11 to 13\textsuperscript{+6} weeks, TR is a common finding in fetuses with trisomies 21 and 18 (Falcon et al., 2006).

**CDH** - Increased NT thickness is present in about 40% of fetuses with diaphragmatic hernia, including more than 80% of those that result in neonatal death due to pulmonary hypoplasia and in about 20% of the survivors (Sebire et al., 1997). It is possible that in fetuses with diaphragmatic hernia and increased NT the intrathoracic
Table 1. Fetal abnormalities associated with increased NT

<table>
<thead>
<tr>
<th>Central nervous system defect</th>
<th>Gastrointestinal defect</th>
<th>Fetal anemia</th>
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<tbody>
<tr>
<td>Acrania / anencephaly</td>
<td>Crohn’s disease</td>
<td>Blackfan Diamond</td>
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<td>Agenesis of the corpus callosum</td>
<td>Duodenal atresia</td>
<td>Congenital</td>
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<tr>
<td>Craniosynostosis</td>
<td>Esophageal atresia</td>
<td>Dyserythropoietic</td>
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<td>Dandy Walker malformation</td>
<td>Small bowel obstruction</td>
<td>Fanconi anemia</td>
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<td>Diastematomyelia</td>
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<td>Parvovirus B19</td>
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<td>Encephalocele</td>
<td>Genitourinary defect</td>
<td>Thalassaemia-a</td>
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<td>Fowler syndrome</td>
<td>Ambiguous genitalia</td>
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<td>Holoprosencephaly</td>
<td>Congenital adrenal</td>
<td>Neuromuscular defect</td>
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<td>Hydrolethalus syndrome</td>
<td>Congenital nephrotic</td>
<td>Fetal akinesia</td>
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<td>Iniencephaly</td>
<td>Hydronephrosis</td>
<td>Myotonic dystrophy</td>
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<td>Joubert syndrome</td>
<td>Hypospadia</td>
<td>Spinal muscular</td>
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<td>Macrocephaly</td>
<td>Infantile polycystic kidneys</td>
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<td>Microcephaly</td>
<td>Meckel-Gruber syndrome</td>
<td>Metabolic defect</td>
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<td>Spina bifida</td>
<td>Megacystis</td>
<td>Beckwith-</td>
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<td>Trigonocephaly C</td>
<td>Multicystic dysplastic GM1 gangliosidosis</td>
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<tr>
<td>Ventriculomegaly</td>
<td>Renal agenesis</td>
<td>Mucopolysaccharidos</td>
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<td></td>
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<td>Smith-Lemli-Opitz</td>
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<td>Facial defect</td>
<td>Skeletal defect</td>
<td>Vitamin D resistant</td>
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<td>Agnathia/micrognathia</td>
<td>Achondrogenesis</td>
<td>Zellweger syndrome</td>
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<tr>
<td>Facial cleft</td>
<td>Achondroplasia</td>
<td>Other defect</td>
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<td>Microphthalmia</td>
<td>Asphyxiating thoracic</td>
<td>Body stalk anomaly</td>
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<td>Treacher-Collins syndrome</td>
<td>Blomstrand osteochondrodysplasia</td>
<td>Campomelic dwarfism</td>
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<td>Brachmann-de Lange syndrome</td>
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<td>Nuchal defect</td>
<td>Cleidocranial dysplasia</td>
<td>Stickler syndrome</td>
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<td>Cystic hygroma</td>
<td>Hypochondroplasia</td>
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<td>Neck lipoma</td>
<td>Hypophosphatasia</td>
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<tr>
<td>Cardiac defect</td>
<td>Jarcho-Levin syndrome</td>
<td>Deficiency of the immune system</td>
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<td>Di George syndrome</td>
<td>Limb reduction defect</td>
<td>EEC syndrome</td>
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<td>Pulmonary defect</td>
<td>Nance-Sweeney syndrome</td>
<td>Neonatal neonatal myoclonic</td>
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<td>Cystic adenomatoid malformation</td>
<td>Osteogenesis imperfecta</td>
<td>Noonan syndrome</td>
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<td>Diaphragmatic hernia</td>
<td>Roberts syndrome</td>
<td>Perlman syndrome</td>
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<td>Fryn syndrome</td>
<td>Robinow syndrome</td>
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<td>Sirenomelia</td>
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<tr>
<td>Abdominal wall defect</td>
<td>Talipes equinovarus</td>
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<tr>
<td>Cloacal extrophy</td>
<td>Thalatophoric dwarfism</td>
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<tr>
<td>Exomphalos/Gastrichiasis</td>
<td>VACTER association</td>
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Ref.: Nicholaides, 2004, P.75
Fig. 3 Ductus Venosus and Tricuspid flow at 11-13+6 wks
herniation of the abdominal viscera occurs in the first trimester and prolonged compression of the lungs causes pulmonary hypoplasia. In the cases where diaphragmatic hernia is associated with a good prognosis, the intrathoracic herniation of viscera may be delayed until the second or third trimesters of pregnancy.

Other markers seen during NT scan are maxilla, ear, megacystis, exomphalos, heart rate and fetal biometry.

### 2.2.2.2 Anomaly Scan / Genetic Sonogram

A complete second trimester ultrasound provides information about the number of fetuses, the gestational age, the location of the placenta and fetal and maternal anatomy. It is used to survey the anatomy of the fetus to find out if everything is developing correctly. It is carried out between 18-23 wk of gestation and includes systematic examination of fetus for detection of major or minor defects. Some of the common defect picked up during anomaly scans are Ventriculomegaly, Holoprosencephaly, Choroid plexus cysts, Dandy Walker complex, Facial cleft, Micrognathia, Nasal hypoplasia, Nuchal edema, Cystic hygromas, Diaphragmatic hernia, Cardiac defect, Exomphalos, Duodenal atresia, Esophageal atresia, Renal defects, Short limbs, Clinodactyly, Overlapping fingers, Polydactyly, Syndactyly, Talipes, Echogenic bowel, Echogenic intra cardiac foci, hydrenephrosis, pelvicectasia, Fetal growth restriction etc.

**ACC:** ACC may be suspected by absence of cavum septum pellucidum and enlargement of the posterior horns. It is demonstrated in mid-saggital or mid-coronal view by trans-vaginal scan (Fig.4 and 5).
Fig. 4 ACC (tear drop)

No Cavum Septum Pellucidum
‘Teardrop’ Lateral Ventricle (Posterior Horn dilated, Anterior Horn pinched)

Fig. 5 ACC
CDH: It can be diagnosed by presence of stomach and intestine or liver in the thorax and the associated mediastinal shift to opposite side (Fig.6 and 7).

2.2.2.3 Fetal Echocardiography
Fetal echocardiography provides a valuable means to better understand intrauterine growth and development of the heart and great vessels. The prenatal diagnosis of structural heart disease and the physiologic evaluation of fetal arrhythmias are perhaps the most important insights provided by this technique. Cardiac examinations are easiest to perform between 20 and 24 wk. The heart is usually examined by real-time and, in some circumstances, with M-mode or Doppler. The fetal circulation differs from the adult circulation in many respects. The heart can be observed in infinity of planes, but a few sections are the basis on which most of the diagnoses are made. These planes include:

- the four-chamber view,
- short axis (or axial),
- left and right chambers and
- great vessels views

Some of the tachyarrhythmias can be treated in utero. When the anomaly is treatable and the fetal condition is worsening, premature delivery and/or referral of the infant to a center familiar with the treatment of the disease should be encouraged. When a lethal anomaly is discovered, interruption of the pregnancy can be offered or, when after the legal limit of termination, non-interventional obstetrical care given. Cardiac anomalies are associated with extracardiac anomalies in 5-10% (Greenwood et al., 1975;
Fig. 6 CDH

Fig. 7 CDH (herniation)
Wallgren et al., 1978; Gallo et al., 1976). Conversely, non-cardiac anomalies occur in 7-17% of fetuses with a cardiac anomaly (Jeanty et al., 2001).

2.2.3 Prenatal diagnosis

In the literature, transabdominal amniocentesis in the third trimester has been reported by Prochownick, Von Schatz and Lambl in 1877 and Schatz in the 1890s. The first use of amniotic fluid examination in the diagnosis of genetic disease was reported by Fuchs and Riis in 1956 in their seminal article in "Nature". They determined fetal sex from cells found in amniotic fluid, basing on the presence or absence of the Barr body (Fuchs and Riis, 1956). The determination of fetal sex led to the prenatal management of patients with Haemophilia A in 1960 and Duchenne muscular dystrophy in 1964. Steele and Breg very importantly demonstrated in their seminal paper in the Lancet in 1966 that cultured amniotic fluid cells were suitable for karyotyping (Woo, 2002).

The year 1966 is an important milestone in prenatal diagnosis, as this year saw the introduction of amniotic fluid cell culture. Geneticists could advise on the degree of risk and the avoidance of pregnancy, but it was evident that genetic counselling had little impact without fetal diagnosis. The emergence of amniocentesis and amniotic cell culture for fetal chromosomal and metabolic disorders in 1966 changed this practice forever (Ferguson-Smith and Bianchi, 2010). In 1974, Hobbins and Mahoney reported a technique for obtaining fetal erythrocytes for prenatal diagnosis of haemoglobinopathies (Hobbins and Mahoney, 1974). Important trends in practice are revealed by the survey, such as the gradual change from transcervical to transabdominal Chorionic villi sampling from 1982 to 1986.
2.2.3.1 Chorionic villi sampling

Chorionic villi sampling (CVS) is the removal of a small part of placenta tissue (chorionic villi) from the uterus. CVS can be done through the cervix (transcervical) or through the abdomen (transabdominal) (Fig. 8). The techniques are equally safe when done by an experienced fetal medicine specialist, although miscarriage rates are slightly higher when done through the cervix. Prior to procedure, an abdominal ultrasound is performed to determine the position of the uterus, the size of the gestational sac, and the position of the placenta within the uterus. Under aseptic precaution, the transabdominal procedure is performed by inserting a needle through the abdomen and uterus and into the placenta. Ultrasound is used to help guide the needle, and a small amount of tissue is drawn into the syringe.

Apart from the risk of miscarriage, there is a risk of infection and amniotic fluid leakage. Random studies have demonstrated that the rate of fetal loss following first-trimester transabdominal CVS is the same as with second-trimester amniocentesis. There is an association between chorionic villus sampling before 10 weeks and fetal transverse limb abnormalities, micrognathia and microglossia. It is therefore imperative that chorionic villus sampling is performed only after 11 weeks and before 15 weeks by appropriately trained operators (Alfirevic et al., 2010).
Fig. 8 Transabdominal Chorionic Villus Sampling
2.2.3.2 Amniocentesis

Amniocentesis is a prenatal procedure in which small amount of amniotic fluid, which contains fetal tissues, is extracted from the amnion or amniotic sac surrounding a developing fetus. Amniocentesis is performed between the 15th - 20th wks of pregnancy. Under aseptic precaution, with the aid of ultrasound-guidance, a fetal medicine specialist punctures the sac in an area away from the fetus and extracts approximately 20 ml of amniotic fluid. Apart from a risk of miscarriage, there is a risk of infection, injury to the fetus and amniotic fluid leakage. It is also possible at 10–14 wk of gestation. However, randomized studies have demonstrated that after early amniocentesis the rate of fetal loss is about 2% higher and the incidence of talipes equinovarus is 1.6% higher than after first-trimester CVS or second-trimester amniocentesis. Amniocentesis should not be performed before 15 wks (Alfirevic et al., 2010).

2.2.3.3 Cordocentesis

Cordocentesis, also sometimes called Percutaneous Umbilical Cord Blood Sampling (PUBS), is a highly specialized prenatal test that examines blood from the fetal umbilical cord. An advanced imaging ultrasound determines the location for needle insertion into the placenta, and the needle is guided through the mother's abdomen and uterine wall into the fetal vein of the umbilical cord, where a fetal blood sample is removed. It can be done at 18 wk of pregnancy or later. This test carries a significant risk of complication and includes blood loss at the puncture site, infection, premature rupture of membranes and the rate of fetal loss is higher than amniocentesis (Percutaneous umbilical cord blood sampling, 2010).
2.3 Cytogenetics

Cytogenetics is the study of chromosomes which are carriers of the gene. A normal human karyotype contains 22 pairs of autosomes and one pair of sex chromosomes. The year 1956 is considered as beginning of modern human cytogenetics. Before this the human chromosomes numbers were believed to be 48 and XX-XY mechanism of sex determination was assumed to work in same way as it does in Drosophila. Due to improvement of technique, Tijo and Levan (1956) discovered that human chromosome number is 46. Historians have divided the discipline of human cytogenetics into five “eras”: the “Dark Ages”, the “Hypotonic Period”, the “Trisomy Period”, the “Banding Era”, and the “Molecular Era” (Jung et al., 2009; Wolstenholme and Rooney, 2010).

During the “Dark Ages”: (prior to 1952) mammalian tissue culture techniques were used for arresting cells during division.

The “Hypotonic Era”: (started in 1952 by TC Hsu) denotes the use of a solution with a lower salt concentration than the cells, it contains. This causes the cells to absorb water through their membranes and swell (but not burst). The swollen cells allow the chromosomes to readily separate, making them easier to count.

During the “Trisomy Period”: Cytogeneticists discovered patients with an additional copy of a small chromosome, eg. Trisomy 21 (Down syndrome), Trisomy 13 (Patau syndrome) and Trisomy 18 (Edward syndrome). Numerical abnormalities involving sex chromosomes (the X and Y chromosomes) were also described for the first time and such as Turner syndrome and Klinefelter syndrome.

Further advances in technology led to banding techniques (hence the “Banding Era”), which brought out horizontal bands of differential staining intensity.
The most recent developments in cytogenetics have led to the “Molecular Era”. Advances in the use of DNA probes have allowed cytogeneticists to hybridize these probes to chromosomes and determine if a specific DNA sequence is present on the target chromosome. This has been useful in detecting abnormalities beyond the resolution level of studying banded chromosomes at the microscope, and also in determining the location of specific genes on chromosomes. Recent advances in cytogenetic techniques made a valuable contribution toward the practice of modern medicine (Jung et al., 2009; Wolstenholme and Rooney, 2010).

2.4 Fluorescence in situ hybridization (FISH)

FISH is a molecular cytogenetics technique that allows identification and detection of the gene of interest within its natural environment of chromosomes, cells or tissues. The basic principle involved in this technique is natural affinity of base pairing of nucleotide sequences with the complementary sequences. Pardue and Gall (1969) reported the hybridization of radioactive DNA probes for repetitive sequences to mouse and drosophila chromosomes. In 1981 Harper and Saundres reported an improved technique for in situ hybridization allowing detection of unique DNA sequence along human metaphase chromosome spread (Harper and Saunders, 1981). In 1987 novel non-isotopic in situ hybridization technique was described to overcome the serious disadvantages of radioactive probes like scattering of emitted radiation, prolonged auto radiographic exposure times (several weeks) and limited special resolution (Garson et al., 1987).

2.4.1 Types of FISH probes

- Centromeric α-satellite
- Locus specific
Telomeric and Subtelomeric

Whole chromosome painting probes

2.4.2 Application of FISH

1. FISH allows rapid analysis of chromosome copy number in interphase cells of Amniotic fluid, Chorionic villi, Cord blood and peripheral blood samples.

2. FISH has been extensively applied in cancer cytogenetics to confirm various translocations like t(9;22) in Chronic Myelogenous Leukemia (Philadelphia chromosome), t(15;17) Acute promyelocytic Leukemia, t(12;21) Acute Lymphoblastic Leukemia etc. and prognostication of Hemato-Oncologic malignancies.

3. It is used for post sex-mismatched bone marrow transplant.

4. Detection of microdeletion syndromes like DiGeorge Syndrome, Prader-Willi Syndrome.

5. It is also used in Biodosimetry, Genotoxic study, Genetic Mapping and Evolutionary Studies.

FISH technique was used for microdeletion study in patient with Congenital heart defect, Congenital diaphragmatic hernia and Agenesis of corpus callosum.

2.5 Congenital Heart Defect

The first reference in history to the presence of CHD comes from a Babylonian tablet which dates back to around 4000 BC. The description mentions:“When a woman gives birth to an infant that has the heart open and has no skin, the country will suffer from calamities”, which might refer to ectopia
cordis. Leonardo da Vinci then was the first to describe a congenital heart defect in humans in his *Quaderni de Anatomia* (Rashkind, 1979).

CHD is the leading cause of infant morbidity in the Western world, but only in the past ten years has its etiology been understood. Recent studies have uncovered the genetic basis for some common forms of the disease and provide new insight into how the heart develops and how dysregulation of heart development leads to disease (Bruneau, 2008). CHD are the malformation of heart and large blood vessels associated with the heart which is present at birth, affecting various parts or function. Since many lives born children with congenital heart defects nowadays survive into adulthood, congenital heart defects are more frequently seen in adults who require specialized care (reproductive issues, heart failure, arrhythmias etc).

2.5.1 Prevalence

CHD is the most common form of human birth defects accounting for about 30% of the total anomalies, which is found to affect nearly 1% of newborns. This is certainly an underestimation of the total incidence of congenital heart disease, as the incidence of congenital heart disease is tenfold higher in fetuses that die prenatally (Gowda *et al.*, 2010). Population based studies on the prevalence of CHD worldwide is found to range between 1.0 – 150 per 1,000 live births whereas, in India it is found to range from 2.2 to 50.89 per 1,000 livebirths (Tank *et al.*, 2004; Smitha *et al.*, 2006).

2.5.2 Classification

Various classification systems have been devised for CHD. It can be classified into two broad categories: Cyanotic heart disease and Acyanotic heart disease which is
Atrial Septal Defect (ASD): It is an opening in the interatrial septum, causing a left-to-right shunt and volume overload of the right atrium and right ventricle. ASDs account for about 6 to 10% of cases of CHDs.

ASDs can be further classified by location:

Ostium secundum (defect in the fossa ovalis—in the center)

Sinus venosus (defect in the posterior aspect of the septum)

Ostium primum (defect in the anteroinferior aspect of the septum)

Ventricular septal defect (VSD): It is an opening in the interventricular septum, producing a shunt between ventricles. It is the 2nd most common congenital heart anomaly after bicuspid aortic valve, accounting for 20% of all defects.

Tetralogy of fallots (TOF): It consists of 4 features: a large ventricular septal defect, right ventricular outflow tract and pulmonary valve obstruction, right ventricular hypertrophy, and over-riding of the aorta. It accounts for 7 to 10% of congenital heart anomalies.

Patent ductus arteriousus (PDA): It is a persistence of the fetal connection, ductus arteriosus between the aorta and pulmonary artery after birth, resulting in a left-to-right shunt. It accounts for 5 to 10% of CHDs.

Pulmonary stenosis (PS): It is narrowing of the pulmonary valve causing obstruction of blood flow from the right ventricle to the pulmonary artery during systole and accounts for 8 to 12% of CHDs.
Aortic stenosis (AS): It is narrowing of the aortic valve obstructing blood flow from the left ventricle to the ascending aorta during systole and accounts for 3 to 6% of CHDs.

Coartation of aorta (COA): It is a localized narrowing of the aortic lumen that results in upper-extremity hypertension, left ventricular hypertrophy, and malperfusion of the abdominal organs and lower extremities. It accounts for 6 to 8% of CHDs.

Interrupted aortic arch (IAA): In this the aorta is not completely developed and there is a gap between the ascending and descending thoracic aorta. There are three types of IAA: Type A is distal to the left subclavian artery. Type B, which is the most common form, is between the left common carotid and the left subclavian arteries. Type C, is between the innominate and left common carotid arteries. It accounts for 1% of CHDs.

Transposition of the great arteries (TGA): It occurs when the aorta arises directly from the right ventricle and the pulmonary artery arises from the left ventricle, resulting in independent, parallel pulmonary and systemic circulations; oxygenated blood cannot reach the body except through patent foramen ovale and VSD. It accounts for 5 to 7% of CHDs.

All above defects accounts for 85% of all CHDs. The remaining 15% of rare and complex CHDs are Atrioventricular Septal Defect (AVSD), Persistent Trucus Arteriosus (PTA), Tricuspid Atresia, Total Anomalous Pulmonary Venous Connection (TAPVC), Pulmonary Artesia (PA), Hypoplsatic Left Heart Syndrome
(HLHS), Double Outlet Right Ventricle (DORV), Single Ventricle (SV), Ebstein Anomaly (EA) and Dextrocardia (Smitha et al., 2006).

2.5.3 Embryology
Congenital heart diseases arise from abnormal heart development during embryogenesis, so understanding how the heart forms normally is important. The heart is the first organ to form in an embryo and must function to support the rapidly growing embryo before it has the opportunity to shape itself into the four chambered organ (Fig.9). The combination of complex morphogenetic events necessary for cardiogenesis and the superimposed hemodynamic influences may contribute to exquisite sensitivity of the heart to perturbation. The fraction of congenital heart malformations those are hemodynamically compatible with the intrauterine circulation form the spectrum of CHD that is observed clinically (Deepak, 2001).

The earliest cardiac progenitors arise from lateral plate mesoderm, controlled by a cascade of interacting transcription factors. Discovery of a ‘second’ heart field (SHF) led to a rethinking of the origin and patterning of the embryonic heart. The SHF is medial and dorsal to the early differentiating cardiomyocytes that comprise the ‘cardiac crescent’, and gives rise to a large portion of the heart, including the outflow tract, right ventricle and most of the atria. The SHF is further subdivided into a number of lineage pools, which contribute either to anterior structures (such as the outflow tract) or posterior components (such as the atria) (Buckingham et al., 2005).
Fig. 9 Heart development

Ref.: Bruneau, 2008, P946
Human genetic studies have identified numerous genes that are responsible for inherited and sporadic congenital heart diseases. Most of these genes encode transcription factors that regulate specific events in heart development, such as ventricular septation or outflow tract morphogenesis. A core set of evolutionarily conserved transcription factors (NK2, MEF2, GATA, TBX and Hand) control cardiac cell fates, the expression of contractile protein-encoding genes and cardiac morphogenesis (Olson, 2006). In turn these transcription factors regulate one another, and many other transcription factors are involved. Of these, MEF2 is the key myogenic transcription factor, involved in the differentiation of all types of myocyte. In turn it is under regulation by NK2 homeobox genes, particularly tinman in Drosophila and its orthologues in mammals. The homeodomain factor NKX2-5 is a key transcription factor in cardiac development (Dunwoodie, 2007). It is expressed in cardiac progenitor cells of both the first and second heart fields. Expression continues in the primary heart tube and in the looping heart, in the outflow tract, ventricles, common atrium and the proximal horns of the sinus venosus. Expression continues in muscular layers of the heart throughout the remainder of embryogenesis and into postnatal and adult life (Prall et al., 2002). The absence of Nkx2-5 is catastrophic to heart development in the mouse embryo, resulting in complete failure of cardiac morphogenesis, chamber formation and outflow tract development.

NKX2-5 acts as part of a pathway in which it physically interacts with a set of other transcription factors to activate target genes. For example, the zinc finger transcription factor GATA4 (one of a group of genes named because their protein products bind to the nucleotide sequence GATA) physically interacts with NKX2-5. When co-expressed, their effect on the transcription of some cardiac genes is
synergistically augmented (Prall et al., 2002). GATA4 protein is regulated by other co-transcription factors including the Friend of GATA (Fog) proteins. Gata4 null mouse embryos have severely disrupted cardiac development, with failure to form the primitive heart tube among other severe developmental abnormalities (Molkentin et al., 1997).

The T-box genes are a group of transcription factors which share a highly conserved 180-amino acid DNA binding domain called the T-box (Stennard and Harvey, 2005). Of the seven or more T-box genes expressed in the developing human heart, TBX1, TBX5 and TBX20 have been implicated in human congenital heart disease. TBX1 is important in the secondary heart field and subsequently the outflow tract, consistent with its role as the major determinant of the cardiac phenotype in velocardiofacial syndrome (characterized by conotruncal malformations) (Yagi et al., 2003).

There is evidence that TBX5 functions as part of the NKX2-5 pathway (Prall et al., 2002; Dunwoodie, 2007). Mouse Tbx5 associates directly with Nkx2-5 and Gata4, synergistically stimulating chamber-specific genes in later stages of cardiac development. Tbx5 is specifically expressed in the first heart field, at the cardiac crescent stage and later in the primary heart tube. Tbx5 null mouse embryos are severely dysmorphic and fail to undergo cardiac looping. Interestingly, mice heterozygous for a Tbx5 null allele have similar cardiac abnormalities to those seen in Holt-Oram syndrome, with septal defects and AV conduction block (Stennard and Harvey, 2005; Prall et al., 2002).

Mouse Tbx20 is expressed in the cardiac crescent, and in some cells of the secondary heart field. In the heart tube, it is expressed in myocardium and in endothelial cells associated with the endocardial cushions; this latter expression
persists with further development, as myocardial expression weakens. Tbx20 interacts directly with Tbx5, Nkx2-5, and Gata4 (Stennard and Harvey, 2005).

Tbx20 null mouse embryos have hypoplastic, unlooped hearts. Expression of Tbx20 is required for normal levels of Nkx2-5 expression (Dunwoodie, 2007).

Notch signalling pathway, NOTCH1 is expressed in the endocardium of the great vessels of the heart, where it is thought to be important for epithelial-to-mesenchymal transition and valve formation. Individuals with NOTCH1 mutations have a spectrum of defects, including AS, VSD, TOF and, in one patient, mitral atresia, DORV and hypoplastic left ventricle. NOTCH1 also represses a bone-related pathway, which might explain calcifications in the cardiac valves of patients with NOTCH1 mutations (Bruneau, 2008).

2.5.4 Maternal Age

Long and group (2010) showed that there was evidence of a significant linear increase in the risk of CHDs with advancing maternal age (Reller et al., 2008).

2.5.5 Consanguinity

Ramegowda and Ramchandra (2006) revealed that first-cousin marriages (44.68%) and uncle-niece marriages (46.81%) increases the risk of birth of a child with CHDs. The types of CHDs associated with consanguinity were found to be ASD and PDA (Becker et al., 2001; Yunis et al., 2006; Al-Ani, 2010).
2.5.6 Etiology

Most of the congenital heart defects are sporadic. The major genetic cause for congenital heart defects includes the following: (a) chromosomal disorders and single gene disorders constituting 8%, (b) 2% of environmental teratogens and (c) 90% multifactorial disorders (Payne et al., 1995). A multifactorial means both genetic and environmental factors interact, to interfere with the development of the heart. Increased incidence of CHDs has been noted with intrauterine viral infections, maternal drug and alcohol consumption during first trimester of pregnancy and pregnancy-induced systemic maternal disease (Ramegowda and Ramachandra, 2005).

2.5.6.1 Chromosomal aberration

The association of CHDs with chromosomal anomalies varies between 4-12% (Chaoui et al., 1999). The following are common chromosomal abnormalities associated with CHD (Burn and Goodship, 2002; Ramegowda and Ramachandra, 2005).

Trisomy 21 (Down syndrome) - At least 40% of Trisomy 21 children will have heart disease; furthermore, 50% of those children with heart abnormalities will specifically be affected with AVSD (Ramegowda and Ramachandra, 2005).

Trisomy 18 (Edward syndrome): This is the second most common autosomal aneuploidy after Down syndrome. Common CHDs include VSD, AVSD, double outlet right ventricle, and hypoplastic left heart.
Trisomy 13 (Patau syndrome): Common CHDs include ASD, VSD, PDA and cardiac malpositions especially Dextrocardia (6%).

45, X (Turner syndrome): Common CHDs include VSD, COA, bicuspid aortic valve, hypoplastic left heart, mitral valve prolapse, and idiopathic aortic root dilatation.

Tetrasomy 22q (Cat eye syndrome): The CHDs association is found to be 30% of the patients with total anomalous pulmonary venous drainage as major problem.

Tetrasomy 12q (Pallister-Killian syndrome): The CHDs association is found to be 25% of the patients that includes VSD, COA, PDA, ASD, and AS.

Chromosome Deletion syndrome-

Deletion 22q11.2 syndrome (OMIM: 611867): It comprises of 3 major syndromes: DiGeorge syndrome (DGS), Velo cardio facial syndrome (VCFS) and Conotruncal anomaly face syndrome (CTAFS). The incidence of this syndrome is estimated to be at least 1 in 4,000-6,000 live births, but this might be an underestimation as many cases with mild features may remain undiagnosed (Tézenas et al., 1996; Devriendt et al., 1998; Botto, et al., 2003).

In 1965, DiGeorge described a patient with hypoparathrodism and cellular immune deficiency secondary to thymic hypoplasia which was expanded later with inclusion of dysmorphic feature, 3rd and 4th branchial arch defect. In 1978, Shprintzen and colleague described Velo cardio facial syndrome or Shprintzen
syndrome with cleft palate, cardiac defects, velopharyngeal incompetence and prominent nose. In 1980 Tako and colleague reported Conotruncal anomaly face syndrome or Takao syndrome with outflow tract defect. It was subsequently determined that all of them have a deletion of chromosome 22q11.2. Burn and Goodship (2002) suggested that a useful acronym would be CATCH: cardiac defect, abnormal facies, T-cell deficit due to thymic hypoplasia, cleft palate, hypocalcemia. Various diagnostic terms have been assigned to the constellation of features of DiGeorge syndrome including Velo cardio facial syndrome (VCF), 22q11.2 deletion syndrome, Takao syndrome and CATCH22. All of these terms are now acknowledged to represent variant manifestations of the same entity, as all of these syndromes are caused by the same 22q11.2 microdeletion and demonstrate an extensive overlap of phenotypes.

Dysmorphic facial feature were small mouth, retrognathia, elongated face, narrow palpebral fissure, facial palsy with squared nasal root and pinched nares. Other abnormalities include slender hyperextensible fingers, renal anomaly, hypothyroidism, hearing loss with mild to severe learning problem. Skeletal differences are possible, including mild short stature and, less frequently, abnormalities of the spinal bones.

Mortality and morbidity after corrective surgery for congenital heart defects is higher in these patients than in those with isolated congenital heart defects. Mortality of 8% of cases due to cardiac defect before 6 months of life has been reported. Hypotonia in infancy is frequent. Speech development is often delayed and impaired with almost always hypernasal. Additionally, affected children are more likely to have attention deficit hyperactivity disorder (ADHD) and
developmental disorders such as autism that affect communication and social interaction. Hypocalcemia may present with seizure activity but responds promptly to replacement therapy and becomes less apparent with age. The immune deficit also resolves with time and is often less evident than in the original case. Later in life, they are at an increased risk of developing mental illnesses such as schizophrenia, depression, anxiety, and bipolar disorder. A variety of cardiac malformations are seen in particular affecting the outflow tract. These include TOF, IAA, VSD, TA, right aortic arch and aberrant right subclavian artery.

This disorder has an Autosomal dominant inheritance pattern. Familial cases of DGS have been described in 6 to 28%, but usually DGS occurs sporadically and results from a de novo deletion which occurs most often as a random event during the formation of reproductive cells (eggs or sperm) or in early fetal development 22q11.2 microdeletion (Carelle-Calmels et al., 2009). Although the penetrance of a 22q11 deletion is nearly 100%, the severity of the disorders is variable.

**Williams syndrome (Williams-Beuren syndrome)(OMIM: 194050):** It was first described in 1961 by Williams and is characterized by learning disability, malar flattening, periorbital fullness, heavy sagging cheeks, short nose with hypercalcemia and supravalvular aortic stenosis. It is caused by the deletion of the elastin (ELN) gene from a specific region of chromosome 7q11.23.

**Wolf-Hirschhorn syndrome (OMIM: 194190):** This syndrome is due to deletion of terminal segment of chromosome 4p. There is increased incidence of cleft lip, palate, seizures and heart disease (30%).
Alagille syndrome (OMIM: 118450): It’s characterized by prominent forehead, deep set eyes, thin nose, butterfly vertebrae, arcus juvenilis with pulmonary artery stenosis. It is caused by the deletion of the JAGGED1 gene from a specific region of chromosome 20p11.2.

2.5.6.2 Single Gene disorder

Noonan syndrome (OMIM: 163950): It was first described in 1962. Children with this syndrome have specific features such as valvar pulmonary stenosis, short stature, mild learning difficulties, and dysmorphic appearance. The cardiac disease seen in this syndrome includes PS, ASD, PDA, VSD and asymmetric septal hypertrophy. It’s caused by Mutation of PTPN11 gene which is linked to chromosome 12q24.1.

Holt-Oram syndrome (OMIM 142900): It was first describe by Holt and Oram in 1960. It’s characterized by upper limb defect, narrow shoulders and cardiac anomaly. Cardiac anomaly includes majority as secundum ASD with occasional reports of VSD, AVSD and TA. It’s caused by Mutation of TBX5 gene which is linked to chromosome 12q24.1.

Ellis- van Creveld syndrome (OMIM: 225500): It shows skeletal dysplasia characterized by short limbs, short ribs, postaxial polydactyly, dysplastic nails and teeth. CHDs occur in 60% of affected individuals that are disease in primary atrial septation; single atrium and Hypoplastic left heart syndrome. It’s caused by Mutation of EVC gene which is linked to chromosome 4p16.
**Kabuki syndrome (OMIM: 147920):** It is characterized by distinct facial anomalies, variable degrees of mental retardation, CHDs and skeletal malformation. CHDs occur in 50% of affected individuals which include ASD, VSD, TOF, PDA, TGA, aortic Coarctation, single ventricle with common atrium and right bundle branch block.

**Metabolic Disorder**

**Pompe's Disease (OMIM 232300):** It is an inborn error of metabolism and is caused by an accumulation of glycogen in the lysosome due to deficiency of the lysosomal acid alpha-glucosidase enzyme. There is accumulation of glycogen in certain organs and tissues. It is manifested as hypotonia, generalized muscle weakness, feeding difficulties, failure to thrive, cardiomegaly and hypertrophic cardiomyopathy.

**Zellweger syndrome (OMIM: 214100):** It is characterized by hypotonia, high forehead, flat facies, hepatomegaly and CHDs like PDA and septal defect. It is caused by defects in number of PEX genes.

**Smith-Lemli-Opitz syndrome (OMIM: 270400):** It is characterized by severe learning disability, failure to thrive, cleft palate, bitemporal narrowing, anteverted nares and syndactyly. 50% will have CHDs like AVSD or ASD. It is due to severe defect in cholesterol biosynthesis resulting in deficiency of 7-dehydrocholesterol reductase (DHCR7) gene which is mapped to chromosome 11q12-13.
2.5.7 Genes associated with CHD

Most of the isolated congenital heart defects do not show a typical Mendelian inheritance pattern, but as mentioned earlier a genetic component is very likely to contribute (Table 2.).
Table 2. Genes causing different types of CHD’s with their chromosomal region in humans.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome Locus</th>
<th>Type of defects</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSX/ NKX2-5</td>
<td>5q35</td>
<td>ASD, VSD, AV block, TOF, Ebstein malformations and Tricuspid valve abnormalities</td>
</tr>
<tr>
<td>GATA4</td>
<td>8p22-23</td>
<td>ASD, VSD, AVSD, Pulmonary valve thickenings</td>
</tr>
<tr>
<td>TBX5</td>
<td>12q24.1</td>
<td>ASD, VSD, AVSD, TOF, HLHS, AS</td>
</tr>
<tr>
<td>dHAND/eHAND</td>
<td>4q33 and 5q33 respectively</td>
<td>AS</td>
</tr>
<tr>
<td>IRX4</td>
<td>5p15.3</td>
<td>SV</td>
</tr>
<tr>
<td>JAGGED-1</td>
<td>20p12</td>
<td>TOF</td>
</tr>
<tr>
<td>Elastin</td>
<td>7q11</td>
<td>AS</td>
</tr>
<tr>
<td>TFAP2B</td>
<td>6p12</td>
<td>PDA</td>
</tr>
<tr>
<td>Fibrillin</td>
<td>15q21</td>
<td>AA</td>
</tr>
</tbody>
</table>

Ref.: Ramegowda and Ramchandra, 2005, P19
2.5.7.1 NKX2-5 (OMIM: 600584) NK2 HOMEOBOX 5 / CSX

Homeobox genes have been found to play a crucial role in regulating tissue specific gene expression. The cardiac homeobox protein NKX2-5 is essential in cardiac development and mutations in CSX (Cardiac-Specific Homeobox) (which encodes NKX2-5) cause various congenital heart malformations. The earliest molecule marker of the cardiac lineage is NKX2-5 in vertebrates. It is one of the members of NK2 family of homeobox genes and a homolog of the Drosophila tinman (Shiojima et al., 1995). It has highly conserved regions of DNA binding, protein-protein interactions, nuclear translocation, and regulation of other transcription factors. Their homeodomains have a tyrosine at position 54, making it the most unambiguous feature of this class and is a useful classification tool (Harvey, 1996). Mutations in this gene have been reported to cause ASD, VSD with atrial ventricular block, TOF and Tricuspid valve abnormalities. Mutations in this gene can also cause congenital hypothyroidism non-goitrous type 5, a non-autoimmune condition (Dentice et al., 2006).

Turbay and group (1996) mapped the CSX gene to chromosome 5q35, close to the junction with band 5q34.

Pauli and group (1999) described a distal 5q deletion, with karyotype del(5)(q35.1q35.3), in a 7½-year-old girl who, in addition to ASD and PDA, had ventricular myocardial noncompaction. FISH analysis showed that this deletion included the locus for CSX. Thus, they suggested that some instances of ventricular myocardial noncompaction may be caused by haploinsufficiency of CSX. They reviewed 4 other cases with deletions in the same region of 5q and pointed out that 2 of them had atrial septal defects and 1 had a cardiomyopathy.

Gibbons and associates (1999) presented infant girl with an interstitial deletion of
chromosome bands 5q33 to 5q35 inherited from a maternal interchromosomal insertion ins(8;5)(p23; q33q35) which was confirmed by FISH. She had increased tone, microcephaly, short neck, apparently low-set ears, micrognathia, camptodactyly, mild rocker bottom feet, and hammer toe. Cardiac anomalies included a large ventricular septal defect, patent ductus arteriosus, pulmonary hypertension and hypoplastic right ventricle. Schafer and group (2001) described two male sibs with partial monosomy of chromosome 5 [46,XY,der(5)inv ins(1;5)(p32;q35.4q34)]; maternally derived from a balanced insertion of 1 and 5 [inv ins (1;5)(p.32;q35.4q34)]. One sibling had microcephaly, cleft lip and palate, facial anomalies, ASD and VSD, camptodactyly 4th and 5th fingers, and developmental delay. The other sibling showed microcephaly, facial anomalies, ASD, hypotonia, primary optic nerve hypoplasia, and developmental delay. Both had only one copy of the cardiac specific homeobox CSX gene.

Schiffer and group (2003) described a boy with complex heart defect, club feet, adducted thumbs, and facial dysmorphic features. Karyotype identified an abnormal chromosome 5q suspected to be an interstitial deletion (5)(q33q35). Breakpoints of the deleted segment were confirmed as del(5)(q33.3q35.2) by multicolor FISH using two sets of combinatorially labeled band specific YAC clones. Baekvad-Hansen and group (2006) described a 15-year-old boy with EA, ASD, AV conduction defect, and microcephaly. He had an apparently balanced paracentric inversion of chromosome 5, with the karyotype 46,XY,inv(5)(q13q35) de novo. Further mapping of the chromosome breakpoints using FISH revealed a 2.2 Mb microdeletion at the 5q35 breakpoint, which spans 16 genes, including the cardiac homeobox transcription factor gene NKX2-5. They also suggested
presence of a new microcephaly locus within a 2.2 Mb region at 5q35.1-q35.2. Rauch and Dorr (2007) described a larger terminal deletions including chromosomal bands 5q35.1 and 5q35.2 cause a more severe phenotype significant CHD, microcephaly profound developmental retardation or early death due to respiratory failure. A heart defect is explained by haploinsufficiency of the NKX2-5 gene at 5q35.1.

Bjørnstad and Leren (2009) have identified a mutation in the NKX2-5 gene on chromosome 5q35 responsible for autosomal dominantly inherited ASD in the oval fossa combined with disturbances of atrioventricular conduction in 7 patients spanning 4 generations. None of the 21 family members, who did not possess the mutation, had deficient atrial septation.

### 2.5.7.2 GATA4 (OMIM: 600576)

GATA4 is a transcription factor which is characterized by a highly conserved binding domain of two zinc fingers. It is expressed in the heart and is essential for mammalian cardiac development, localized to chromosome region 8p23.1. In mice, germline ablation of the gene encoding GATA4 results in abnormal ventral folding of the embryo, failure to form a single ventral tube, and lethality. Besides heart development, Gata4 is involved in the formation of multiple organs, such as intestine, liver, pancreas and swim bladder in zebrafish as well as gastric epithelial development in mouse through interaction with Fog cofactors (Reamon-Buettner et al., 2007). The mutation in GATA4 gene diminishes DNA-binding affinity and transcriptional activity. GATA4 is capable of synergizing with other transcription
factors such as NKX2-5, dHAND and TBX5 to activate cardiac-specific gene expression.

There are published deletions involving chromosome 8p23.1 range from large terminal deletions that are easily detectable by routine chromosome analysis and small interstitial deletions that are best identified using FISH or molecular techniques such as array comparative genomic hybridization (aCGH). Lubs and Lubs described (1973) the first case of an individual with a partial deletion of distal chromosome arm 8p and congenital heart disease. Bröcker-Vriends and group (1986) described two patients with partial monosomy of the short arm of chromosome 8. The chromosomal abnormality of partial monosomy of 8p was initially not considered. They stressed upon the importance of cytogenetic investigations in all infants with major congenital heart defect and facial dysmorphism or microcephaly or both.

Devriendt and group (1998) reported the prenatal diagnosis at 30 weeks of gestation of a del(8)(p21.3→pter) in a growth-retarded fetus with an unbalanced atrioventricular septal defect (AVSD) and a hypoplastic right ventricle. Pehlivan and group (1999) provided evidence that GATA4 may be involved in the etiology of some congenital heart defects. They performed FISH analysis using a GATA4 probe on 5 patients with interstitial deletions of 8p23.1. Hemizygosity for GATA4 was seen in the 4 patients with congenital heart disease but not in the patient without known cardiac anomalies. The authors proposed that haploinsufficiency of GATA4 may contribute to the congenital heart disease observed in some patients with del(8)(p23.1).
Reddy (1999) described a case with del(8)(p23.1) in amnionocyte culture with normal cardia. Similar deletion was revealed in father’s karyotype. The karyotype from the prenatal case was compared with the previous four cases of 8p23.1 deletions in his laboratory to see if there was a discernible difference in the size of the deletion. The deletion in the proband seemed to involve a more distal 8p23.1 breakpoint. In the father's high resolution chromosomes (550-850 band level), the breakpoint appeared to be 8p23.1 approximately 23.2 and FISH studies using an 8p telomeric probe confirmed a terminal deletion. Interstitial deletion of sub-band 8p23.1 was associated with phenotypic abnormalities and distal 8p23.2pter deletion was found in apparently normal individuals, therefore, 8p23.1 appears to be the critical region for clinical abnormalities.

Bhatia and group (1999) reported the prenatal diagnosis, at 18 wk gestational age of a del(8)(p23.1→pter) in a fetus with an AV canal, persistent left superior vena cava and hypoplastic right ventricle detected by sonographic imaging. Some of the common features reported with partial monosomy of 8p include growth and mental retardation, impulsive and aggressive behaviour, congenital cardiac defects, diaphragmatic hernia and in males, genital abnormalities. Devriendt and group (1999) have performed genotype-phenotype correlations in nine unrelated patients with a de novo del8p. In five patients, a uniform interstitial deletion of ~6 Mb in 8p23.1 was detected. One patient carried a large terminal deletion encompassing this commonly deleted region. All these patients have a similar phenotype, with a CHD, microcephaly, mild developmental delay, intrauterine growth retardation, and a characteristic behavioral phenotype. Features that have been recognized more recently are a characteristic behavioral phenotype (Claeys et al., 1997),
hypospadias, and seizures (Digilio et al., 1998). The del8p phenotype often is relatively mild, without associated facial dysmorphism or other major internal malformations (Fryns et al., 1989; Hutchinson et al., 1992; Wu et al., 1996). They defined an 8p heart defect– critical region spanning a 10-cM segment defined distally by D8S1706 and proximally by D8S1759, and they suggested the transcription factor GATA4 as a candidate gene.

Giglio and group (2000) narrowed this region by studying twelve del(8p) patients, including 6 new cases, 7 of whom had CHDs. Patients with 8p deletions distal to D8S1706, at ~10 cM from the 8p telomere, did not have CHD, whereas patients with a deletion that included the more proximal region suffered from the spectrum of heart defects reported in patients with 8p distal deletions. The 5-cM critical region is flanked distally by D8S1706 and WI-8327, both at ~10 cM, and proximally by D8S1825, at 15 cM.

2.5.7.3 Second DiGeorge syndrome locus (DGSII) (OMIM: 601362)

The DiGeorge syndrome and velocardiofacial syndrome may present many clinical problems, including cardiac defects, hypoparathyroidism, T-cell immunodeficiency, and facial dysmorphism. They are frequently associated with deletions within 22q11.2, but a number of cases have no detectable molecular defect of this region. Bourrouillou and group (1981) described a case of monosomy 10p with microcephaly, antimongoloid slant of the palpebral fissures, low-set ears, prominent anthelix, congenital heart disease, abnormalities of the limbs. Schuffenhauer and group (1995) described a 20 months old girl with DGS and a monosomy 10p13-pter and a trisomy 10q26-qter due to a meiotic recombination of
a maternal inversion (10)(p13q26). The proposita's phenotype demonstrates typical features of the del(10p) syndrome which include mental retardation, abnormally shaped skull, hypertelorism, low nasal bridge, micrognathia, dysmorphic low set ears, short neck, foot abnormalities, and cardiac defect. Daw and group (1996) stated that a number of single case reports with deletions of 10p suggested genetic heterogeneity of DGS. They compared the regions of hemizygosity in 4 patients with terminal deletions of 10p (1 patient with hypoparathyroidism and 3 with DGS) and 1 patient with VCFS and a large interstitial deletion. FISH analysis demonstrated that these patients had overlapping deletions at the 10p13/10p14 boundary. They concluded that the results strongly support the hypothesis that haploinsufficiency of a gene or genes within 10p (DGSII locus) can cause the DGS/VCFS spectrum of malformations.

Schuffenhauer and group (1998) performed FISH and PCR analyses in 12 patients with 10p deletions, 9 of them with features of DGS, and in a familial translocation 10p;14q associated with midline defects. The critical DGS2 region was defined by 2 DGS patients and mapped within a 1-cM interval including D10S547 and D10S585. The other 7 DGS patients were hemizygous for both loci. The breakpoint of the reciprocal translocation 10p;14q mapped at a distance of at least 12 cM distal to the critical DGS2 region. Interstitial and terminal deletions described in these patients were in the range of 10 to 50 cM and enabled the tentative mapping of loci for ptosis and hearing loss, features that are not part of the DGS clinical spectrum.

Lichtner and group (2000) reported a new case with the HDR phenotype: hypoparathyroidism, deafness, and renal dysplasia. They were found to have partial monosomy for 10p due to terminal deletions with breakpoints between
D10S585 and D10S1720. By comparison with data previously published on patients with DGS/VCFS associated with 10p monosomy, they concluded that this is a contiguous gene syndrome. Hemizygosity for a proximal region can cause cardiac defects and T cell deficiency; hemizygosity for a more distal region can cause hypoparathyroidism, sensorineural deafness, and renal dysplasia.

Berend and group (2000) tested 412 patients, 54 were found to be deleted for the DGSI locus on chromosome 22 (13%), and a single patient was found deleted for the DGSII locus on chromosome 10 (0.24%). The patient with the 10p deletion had facial features consistent with VCFS, plus sensorineural hearing loss, and renal anomalies. Cytogenetic analysis showed a large deletion of 10p [46, XX,del(10)(p12.2p14)] and FISH using a 10p telomere region-specific probe confirmed the interstitial nature of the deletion.

Lichtner and group (2002) constructed a deletion map of partial monosomy 10p patients and narrowed the critical region DGCRII to about 300 kb. The genomic draft sequence of this region contains only one known gene, BRUNOL3 (NAPOR, CUGBP2, ETR3). In situ hybridization of human embryos and fetuses revealed as well as in other tissues a strong expression of BRUNOL3 in thymus during different developmental stages. BRUNOL3 appears to be an important factor for thymus development and is therefore a candidate gene for the thymus hypoplasia or aplasia seen in partial monosomy 10p patients.

2.5.7.4 DiGeorge syndrome locus (OMIM: 188400; 600237)

Most cases of DGS result from a deletion of chromosome 22q11.2 (the DiGeorge syndrome chromosome region, or DGCR). Several genes are lost including the putative transcription factor TUPLE1 (TUP-like enhancer of split gene-1) which is
expressed in the appropriate distribution. Molecular biology studies revealed that approximately 90% of patients have a typically deleted region of 3Mb, which encompasses an estimated 30 genes, whereas about 8% of patients have a smaller nested deletion of 1.5Mb, which encompasses 24 genes (Shaikh et al., 2000).

Halford and group (1993) reported that TUPLE1 gene is an attractive candidate for the central features of the syndrome. This putative transcription factor shows homology to the yeast transcription factor TUP, and to Drosophila enhancer of split. It contains 4 WD40 domains and shows evidence of expression at the critical period of development in the outflow tract of the heart and the neural crest derived aspects of the face and upper thorax. Lamour and group (1995) isolated a cDNA that encodes a protein of 1,017 amino acids, designated HIRA (histone cell cycle regulation defective, S. cerevisiae, homolog of, A) on the basis of its homology to the HIR1 and HIR2 transcriptional repressors of S. cerevisiae. HIRA encompasses the entire TUPLE1 protein with an additional 207 internal amino acid residues and an extra 44 N-terminal residues, a result of an alternative start codon. Thus, TUPLE1 cDNA appears to represent a truncated version of the HIRA cDNA. Demczuk and group (1995) reported the isolation and cloning of a gene encoding a potential adhesion receptor protein in the DGCR. They designated the gene DGCR2 and suggested DGCR1 as a symbol for the TUPLE1 gene. Haploinsufficiency of the TBX1 gene is also responsible for most of the physical malformations. There is evidence that point mutations in the TBX1 gene can also cause the disorder.
De la Chapelle and group (1981) suggested that DiGeorge syndrome may be due to a deletion within chromosome 22 or partial duplication of 20p, based on finding the syndrome in members of a family with a 20;22 translocation. Specifically, they observed DGS in 4 members of one family and demonstrated monosomy of 22pter-q11 and 20p duplication. Their interpretation that DGS might result from monosomy for 22q11 was confirmed by Kelley and group (1982) in 3 patients with translocation of 22q11-qter to other chromosomes.

Greenberg and group (1984) observed partial monosomy due to an unbalanced 4;22 translocation in a 2-month-old male with type 1 TA and features of DGS. The asymptomatic mother showed partial T-cell deficiency and the same unbalanced translocation with deletion of proximal 22q11. The recognition of the importance of 22q11 deletion grew with improving techniques. Greenberg and group (1988) found chromosome abnormalities in 5 of 27 cases of DGS, 3 with 22q11 deletion though only one of these was an interstitial deletion. Wilson and group (1992) reported high resolution banding (more than 850 bands per haploid set) in 30 of 36 cases of DGS and demonstrated 9 cases of interstitial deletion. All other cases were apparently normal. Use of molecular dosage analysis and fluorescence in situ hybridization with probes isolated from within the deleted area revealed deletion in 21 of the 22 cases with normal karyotypes (Carey et al., 1992) giving pooled results of 33 deleted among the consecutive series of 35 cases.

Gowde and Patel (2007) screened families with congenital heart disease for chromosome 22 micro deletion; of the 105 patients screened 6 had microdeletion.
VCFS has an extremely expansive phenotypic spectrum. More than 180 clinical features, both physical and behavioral, have been described. No single clinical feature occurs in 100% of cases and there is no reported case of the syndrome that has all or even most of the clinical findings. The phenotype therefore shows markedly variable expression. The diagnosis is therefore defined by the deletion of DNA from chromosome 22 at the q11.2 band spanning the region that is regarded as the critical region. Other molecular genetics tests will clearly become widely available in the near future, such as microarray analysis and MLPA, but at the current time, FISH is widely available, relatively cost effective, and highly accurate (Shprintnzen, 2008).

2.5.8 Signs and symptoms

It is related to the type and severity of the heart defect. Symptoms frequently present early in life, but it's possible for some CHDs to go undetected throughout life. Some children have no signs while others may exhibit shortness of breath, cyanosis, syncope, murmur, under-developing of limbs and muscles, fatigue, poor feeding or growth, or respiratory infections and heart failure.

2.5.9 Management

2.5.9.1 Diagnosis

Physical Exam: Examination for signs of a heart defect, such as cyanosis, shortness of breath, rapid breathing, delayed growth, or signs of heart failure and auscultation of chest.

Echocardiography: It creates two-dimensional pictures of the cardiovascular system and can also produce accurate assessment of the velocity of blood and
cardiac tissue at any arbitrary point using pulsed or continuous wave Doppler ultrasound. This allows assessment of cardiac valve areas and function, any abnormal communications between the left and right side of the heart, any leaking of blood through the valves (valvular regurgitation), and calculation of the cardiac output as well as the ejection fraction. Other parameters measured include cardiac dimensions (luminal diameters and septal thicknesses).

**Electrocardiogram:** The test shows how fast the heart is beating and its rhythm. It also records the strength and timing of electrical signals as they pass through each part of the heart.

**X-ray:** This test can show whether the heart is enlarged or whether the lungs have extra blood flow or extra fluid, a sign of heart failure.

**Pulse oximetry:** It is a test that measures how much oxygen is present in your child’s blood.

**Cardiac catheterisation:** It is a useful way of finding out more information about exactly how the blood is pumping through the child’s heart.

**Cardiac MRI:** It's used to diagnose and evaluate a number of diseases and conditions, including: coronary artery disease, cardiac failure, cardiac valve problems, pericarditis, cardiac tumor and damaged caused by myocardial infarction.

### 2.5.9.2. Treatment

Sometimes CHD improves without treatment. Other defects are so small that they do not require any treatment. Most of the time, CHD is serious and requires surgery and medications. Medications include diuretics, which aid the baby in
eliminating water, salts, and digoxin for strengthening the contraction of the heart. This slows the heartbeat and removes some fluid from tissues. Some defects require surgical procedures to restore circulation back to normal and in some cases, multiple surgeries are needed. Interventional cardiology now offers patients minimally invasive alternatives to surgery. Device closures can now be performed with a standard trans-catheter procedure using a closure device mounted on a balloon catheter. Most patients require life-long specialized cardiac care, first with a pediatric cardiologist and later with an adult cardiologist.

2.6 Congenital Diaphragmatic Hernia (OMIM: 142340)

Congenital diaphragmatic hernia (CDH) is a malformation of the developing diaphragm, a mesodermally derived structure, separating the thoracic and abdominal cavities. Most often, the malformation is an actual ‘hole’ or discontinuity in the diaphragm. Less often, the defect is a thinning or under muscularization, which is generally referred to as eventration or sac-type CDH (Pober, 2008). CDH was first described in the medical literature in the early 18th century. Gross (1946) reported the first successful repair of a neonatal diaphragmatic hernia in the first 24 hours of life. Areechon and Reid (1963) observed that the high mortality rate of congenital diaphragmatic hernia was related to the degree of pulmonary hypoplasia at birth.

2.6.1 Prevalence

Prevalence in newborns ranges from 1 in 2,500 to 4,000, and there is a 30 to 60% mortality rate (Langham et al., 1996; Harrison et al., 1994; Nobuhara et al., 1996). A retrospective analysis of 5 year period at Gangaram Hospital, New Delhi,
India, showed 31 neonates with CDH at incident rate of 0.69% (Dhir et al., 2002). A retrospective study of all infant and neonatal autopsies done during last 30 years (1960 to 1989) was conducted. Ten cases of congenital diaphragmatic hernia were encountered among 588 autopsies and its incidence was 1.7% of all infant and neonatal autopsies (Bajaj et al., 1991). Babies born with CDH since 01 January 2000 is 6, 38,936 according to CHERUBS, an association of CDH research, awareness and support.

2.6.2 Classification: There are two major types of CDH (Fig.10)

2.6.2.1 Posteriolateral hernia or Bochdalek hernia

These hernias make up the majority of the cases comprising approximately 80-90%. It is often accompanied by herniation of the stomach, intestines, liver, and/or spleen into the chest cavity. An extremely large defect, or apparent absence of the hemidiaphragm, is called agenesis of the diaphragm; this defect probably represents the severe end of the Bochdalek hernia spectrum rather than a distinct entity. It is further sub-typed depending upon intact or absent rim of posterior and lateral musculature.

About 85% of Bochdalek hernias occur on the left side, about 10% on the right, and approximately 5% are bilateral.
NORMAL DIAPHRAGM

ABNORMAL DIAPHRAGM

Fig.10 Types of CDH

Ref.: http://www.cdhsupport.org/types.php
2.6.2.2 Non posteriolateral hernia or Non Bochdalek hernia

Anterior defects of the diaphragm can occur in the midline, on the left side, or the right side.

Morgagni hernias: It can result in the herniation of liver or intestines into the chest cavity and comprise approximately 2% of all CDH. It often does not cause symptoms in the newborn period.

Other anterior hernias: These rare and severe types of hernias, possibly derived from septum transversum are found in individuals with Pentalogy of Cantrell (which also includes defects in supraumblical midline abdominal wall, lower sternum, diaphragmatic pericardium and heart.

Central hernia: This rare diaphragm defect involves the central muscular portion of the diaphragm. The entire rim of diaphragmatic musculature is present.

2.6.3 Embryology

The development of human diaphragm occurs between 4th and 12th wks of gestation. The normal development of the diaphragm is not well understood and traditional views suggest that the diaphragm arises from four different structures (Rottier R et al., 2005). The Septum transversum gives rise to the central portion of the diaphragm, the pleuroperitoneal folds (PPFs) gives rise to the posteriolateral section of the diaphragm, the dorsal mesentery gives rise to the portion of the diaphragm posterior to the esophagus, and the elements of the thoracic body wall contribute to the rim of musculature around the diaphragm’s periphery (Holder et al., 2007).
2.6.4 Maternal Age

Maternal age does not appear to influence risk for diaphragmatic hernia; nor does parity but risk appears to be higher among multiple births (David and Illingworth, 1976; Torfs et al., 1992; Robert et al., 1997; Forrester and Merz, 1998).

2.6.5 Consanguinity

Consanguineous parents with isolated CDH child may have higher recurrence risk (Arad et al., 1980; Janik et al., 1996; Mitchell et al., 1997; Ding et al., 2005)

2.6.6 Etiology

CDH can occur as an isolated defect, in combination with multiple congenital anomalies or as a part of defined syndrome (Fryns et al., 1979; Enns et al., 1998). Little is known about the etiology of CDH. However there is an increase in evidence for a genetic cause of CDH. The Fig.11 explains the classification of cases with CDH.

2.6.6.1 Isolated CDH or Non Syndromic CDH

In addition to defining the location and type of CDH determining the overall status of the CDH patients is important. CDH is the only birth defect in 60% of cases and are classified as isolated CDH. Although additional problems like pulmonary hypoplasia, cardiac dextroposition and left heart hypoplasia coexists with CDH, they are usually considered as a part of CDH. The mortality rate of isolated CDH is 10%-20% low as that of Syndromic CDH due to increase in CDH expertise (Pober, 2008).
Fig. 11 Etiological classification of CDH.

Ref.: Pober, 2008 P20
2.6.6.2 Non isolated or Syndromic CDH

40% of the CDH cases have additional malformations in which chromosomal abnormalities are seen in 10% of CDH cases. The most common abnormalities are trisomy 18 and isochromosome 12p (Pallister-Killian syndrome or PKS). Other common aneuploidies are Trisomy 13, Trisomy 21 and 45, X (Tibboel and Gaag, 1996; Holder et al., 2007). Structural abnormalities like deletions, duplications, inversions and translocations have also been described in association with CDH (Enns et al., 1998; Lurie, 2003; Holder et al., 2007).

Common Chromosomal Anomalies Associated with CDH are as follows (Table 3.)

**Tetrasomy 12p (OMIM: 601803):**

**(Isochromosome 12p or Pallister-Killian syndrome, PKS)**

The presence supernumerary isochromosome consisting of 2 copies of short arm of chromosome 12 confirms the diagnosis of PKS. The signs leading to diagnoses of PKS in children are linear streaks of skin hyper pigmentation, sparse hair bitemporally, coarse facies, mental retardation and seizures, while prenatal triggers include the presence of multiple major malformations(such as CDH and Brain Malformation) along with polyhydraminos, edema, and relative limb shortening.
Table 3. Common chromosomal anomalies associated with CDH.

<table>
<thead>
<tr>
<th>CHROMOSOMAL ABNORMALITY/LOCUS</th>
<th>INCIDENCE OF CDH IN THIS DISORDER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pallister-Killian Syndrome/</td>
<td>Approx. 30%</td>
</tr>
<tr>
<td>Isochromosome or Tetrasomy 12p</td>
<td></td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>Rare</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>1 to 2 %</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>Rare</td>
</tr>
<tr>
<td>Del(4)(p16)/ Wolf-Hirschhorn Syndrome</td>
<td>Rare</td>
</tr>
<tr>
<td>+der(22)(t(11;22)(q23;q11)</td>
<td>5% to 10%</td>
</tr>
<tr>
<td>Del(15)(q26.1)</td>
<td>Majority</td>
</tr>
<tr>
<td>Del(1)(q41-q42)</td>
<td>5%</td>
</tr>
<tr>
<td>Del(8)(p23.1)</td>
<td>Approx. 15%</td>
</tr>
</tbody>
</table>

Ref.: Pober, 2008 and Holder et al., 2007.
The isochromosome is rarely found in peripheral blood karyotyping but can be documented in 5%-100% of amniocytes, chorionic villi, and skin fibroblasts (Doray et al., 2002). CDH is present in approximately 10%-20% of postnatally diagnosed cases (Mathieu et al., 1997; Schaefer et al., 1997) and in 33% of prenatally diagnosed cases (Doray et al., 2002). The mechanism underlying in the formation of isochromosome is not completely understood but improper division of centromere during or before maternal meiosis has been suggested (Struthers et al., 1999). In the 400 genes which are thought to reside on 12p, which confers risk for CDH is unknown.

+ der(22)t(11;22)(q23;q11) (OMIM: 609029) and trisomy 22

Cases of CDH, occurring with additional anomalies, have been reported in patients with trisomy 22 as well as in patients with ‘partial trisomy 22q’ (Kim et al., 1992; Pober, 2008). Individuals trisomic for 22q11 commonly have growth retardation, intellectual disability, cardiovascular malformations, craniofacial anomalies (including preauricular tags or sinuses, micrognathia, cleft palate), and abnormal ears. One recent report suggests that the CDH causal gene in persons with t(11;22) results from three copies of a gene located on the long arm of chromosome 11, rather than on chromosome 22 (Klaassens et al., 2006).

del(1)(q41q42): Array CGH demonstrates deletion in chromosome (1)(q41q42.12) in unrelated individuals whose phenotype overlaps with Fryns syndrome. This finding, in conjunction with previous reports of cytogenetic abnormalities in multiple malformed infants with CDH, suggests that the locus contains a gene,
possibly *DISPL*, important for diaphragm development (Kantarci *et al.* 2006, Shaffer *et al.* 2007).

**Del(15)(q26.1-q26.2) (OMIM: 142340, DIH1)—** There is compelling evidence from more than two dozen cases that loss of the 15q26.1-q26.2 interval significantly increases the risk for abnormal diaphragm formation. Loss of this interval can result from one of several mechanisms such as *de novo* deletions, unbalanced translocations, or formation of a ring chromosome 15. All have had intellectual disability, growth retardation, and/or additional birth defects such as craniofacial anomalies, cardiovascular malformations, hypoplastic genitalia, or cryptorchidism, overlapping facial appearance with some ‘Fryns syndrome’ (Klassens *et al.*, 2006; Pober, 2008). Mortality among patients with deletions encompassing this interval remains very high. *Chick ovalbumin upstream promoter-transcription factor II (COUP-TFII)* resides within this region (Holder *et al.*, 2007).

**Del(8)(p23.1) (OMIM: 222400, DIH2)—** Another CDH ‘hot spot’ revealed by cytogenetic and molecular cytogenetic studies is (8)(p23)(Borys and Taxy, 2004; Shimokawa *et al.*, 2005; Lopez *et al.*, 2006; Pober 2008). This region contains the candidate gene GATA4, known to be important for heart, lung, and diaphragm development (Jay *et al.*, 2006; Ackerman *et al.*, 2006). These individuals have cardiovascular malformations, intellectual disability, mild facial dysmorphology, and renal anomalies.
Single Gene Disorders

Some of the more common monogenic syndromes in which CDH occurs are listed in Table 4; a few of these syndromes are presented in greater detail below. Other syndromes have CDH as an occasional finding; examples of these are Apert, Beckwith-Weidemann, CHARGE, Coffin-Siris, Goltz, Perlman, and Swyer syndromes.

Fryns syndrome (OMIM: 229850): It is a multiple malformation/intellectual disability condition with CDH. Additional features includes characteristic coarse facial appearance (often with widely spaced eyes, a broad nasal bridge, and macrostomia), hypoplasia of the nails and/or terminal phalanges, pulmonary hypoplasia, ACC, genitourinary anomalies (renal dysplasia and cysts, bicornuate uterus), ocular abnormalities (cloudy corneas), cardiovascular malformations, and orofacial clefting (Slavotinek, 2004). Lymphatic malformation resulting in cystic hygroma identified prenatally in some affected fetuses would account for the short broad neck observed postnatally in some infants. Polyhydramnios occurs in over 50% of pregnancies with Fryns syndrome. The prognosis is poor, with most affected infants succumbing soon after birth; a few long-term survivors demonstrate varying degrees of intellectual disability.

The genetic basis of Fryns syndrome is unknown, but has been considered to follow an autosomal recessive pattern of inheritance based on reports of sibling recurrences and parental consanguinity. However, de novo microdeletions at 1q41-q42 and 15q26.2 detected in some individuals with Fryns syndrome, or a Fryns-like phenotype, suggest that genetic heterogeneity may underlie this phenotype (Slavotinek et al, 2006; Kantarci et al, 2006).
Table 4. Monogenic syndromes associated with CDH, the locus and the gene involved.

<table>
<thead>
<tr>
<th>SYNDROME</th>
<th>OMIM NO/ INHERITANCE</th>
<th>LOCUS</th>
<th>GENE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornelia de Lange</td>
<td>122470/AD 300560/XL</td>
<td>5p13.1 Xp11.2</td>
<td>NIPBL SMC1A</td>
</tr>
<tr>
<td>Craniofrontonasal Dysplasia</td>
<td>304110/XL</td>
<td>Xq12</td>
<td>EFNB1</td>
</tr>
<tr>
<td>Donnai-Barrow Syndrome</td>
<td>222448/AR</td>
<td>2q24.3-2q31.1</td>
<td>LRP2</td>
</tr>
<tr>
<td>Fryns Syndrome</td>
<td>229850/UNK</td>
<td>UNK</td>
<td>UNK</td>
</tr>
<tr>
<td>Mathew-Wood Syndrome</td>
<td>601186/AR</td>
<td>15q24.1</td>
<td>STRA6</td>
</tr>
<tr>
<td>Jarcho-Levin Syndrome</td>
<td>277300/AR</td>
<td>19q13,</td>
<td>DLL3</td>
</tr>
<tr>
<td>Simpson-Golabi Behmel</td>
<td>312870/XL</td>
<td>Xq26</td>
<td>GPC3</td>
</tr>
<tr>
<td>WT1-Opathies (Denys-Drash, Frasier, and Meacham Syndromes)</td>
<td>194080, 136690, 608978/AD</td>
<td>11p13</td>
<td>WT1</td>
</tr>
</tbody>
</table>

AD- Autosomal Dominant, AR- Autosomal Recessive, XL- X Linked, UNK- Unknown

Ref.: Pober, 2008, P22
Donnai-Barrow syndrome (DBS) (OMIM: 222448): It is a rare autosomal recessive disorder and common abnormalities include ocular hypertelorism, myopia, sensorineural hearing loss, omphalocele or umbilical hernia, enlarged anterior fontanel, ACC, mildly impaired cognitive development, and a characteristic pattern of low molecular weight proteinuria. Initially reported as separate entities, recent work shows that DBS and facio oculo acoustic renal syndrome (FOAR) are the same autosomal recessive disorder caused by mutations in the low-density lipoprotein receptor-related protein 2 gene (LRP2). (Gripp et al 1997; Chassaing et al 2003; Kantarci et al 2007; Pober, 2008.)

Matthew-Wood syndrome (OMIM 601186): It is a rare autosomal recessive disorder with a striking phenotype, most often including microophthalmia or anophthalmia, pulmonary hypoplasia or agenesis, and diaphragmatic defects. Mutations in STRA6 (a member of the stimulated by retinoic acid gene family) have been found in some cases (Pasutto et al., 2007).

Candidate Pathways and Genes

Though the etiology of the CDH cases remains unknown, there are number of evidence that shows that there are certain genes and pathways which play a role in the development of CDH. The retinoid signaling pathway and genes like COUP-TFI, FOG 2, GATA 4, WTI and SLIT 3 are the ones which are thought to be involved in the development of CDH (Holder et al., 2007). Preliminary evidence that retinoids may play a role in the development of CDH in human comes from a small study in which the levels of plasma retinol and retinolbinding protein in the cord blood of infants with CDH was found to be 50% lower than those in age-
matched controls (Major et al., 1998). Congenital diaphragmatic hernia, DIH1, has been mapped to chromosome 15q26, and another, DIH2, to chromosome 8p23.1. DIH3, mapped to 8q23 is associated with mutation in the ZFPM2 gene.

**Retinoid Acid (RA) Signaling Pathway (Fig.12)**

Vitamin A (retinol) and its derivatives like retinoid are essential for embryonic development. Deficiency in vitamin A is thought to lead to the development of CDH. This was observed in pregnant rats supplied with diet deficient in vitamin A. The developmental proportion of CDH was reduced when vitamin A was reintroduced in the diet. The pathway is explained as follows.

Retinol travels to target cells via the blood and is taken up by receptors on the cell surface. Once in the cytoplasm, retinol is converted to retinal by retinol dehydrogenases and then to RA by retinal dehydrogenases, of which RALDH2 is the predominant enzyme. The action of RALDH2 (Retinal dehydrogenase-2) can be inhibited by teratogens, such as nitrofen. Several binding proteins are present in the cytoplasm, including retinol-binding proteins 1 and 2 (RBP1 and RBP2), which bind retinol and retinal, and cellular RA-binding proteins 1 and 2 (CRABP1 and CRABP2). When RA enters the nucleus, it mediates its effects by binding to RA receptors (RARs) and retinoid X receptors (RXRs). RARs and RXRs dimerize and regulate gene expression by binding to short DNA sequences, retinoid acid responsive elements (RAREs) and retinoid X–responsive elements (RXREs) are located in the vicinity of target genes. COUP-TFII expression is up regulated by RA. COUP-TFII can act as a repressor of this pathway by directly sequestering RXR, thereby preventing hetero dimerization to RAR and inhibiting gene transcription. This process may be a negative feedback system that precisely
Fig. 12 Retinoic acid (RA) signaling pathway and CDH candidate genes

Ref.: Holder et al., 2007, P836
balances the transcription of certain genes during diaphragm development. COUP-TFII has been shown to interact physically with FOG2, which, in turn, modulates the transcriptional activity of GATA4, GATA5, and GATA6.

**COUP-TFII (DIH1)(OMIM:142340)**

COUP-TFII (also known as NR2F2) is a transcription factor in the steroid/thyroid hormone receptor superfamily. The COUP-TFII gene is located on chromosome 15q26 in a region recurrently deleted in individuals with CDH (Lurie, 2003; Klaassens et al., 2005). Biggio and group (2004) cited numerous reports of either de novo deletion or unbalanced translocations involving the 15q24-q26 region, suggesting that this region is critical to normal development of the diaphragm. They described a patient with deletion of 15q26.1, the smallest isolated chromosomal aberration on distal 15q that had been reported to that time. In addition to diaphragmatic hernia, coarctations of the aorta and dysmorphic features were present.

Klaassens and group (2005) defined a minimally deleted region for CDH on chromosome 15q26 by use of FISH and array CGH data from patients with nonisolated CDH. Of the genes within this region, COUP-TFII was thought to be the strongest candidate because its expression had been shown previously to be regulated by retinoids and because COUP-TFII regulates gene transcription by influencing retinoic acid receptor or retinoid X receptor heterodimerization. This region has since been reduced to include COUP-TFII and only eight other known genes. Together, these data suggest that deletion of COUP-TFII is likely to play a key role in the development of CDH in individuals with 15q26 deletions (Holder et al., 2007).
GATA4 (DIH2) (OMIM: 222440)

GATA4 is a member of a family of DNA-binding proteins that recognize a consensus sequence (the GATA motif), which is found in the promoter regions of many genes (Arceci et al., 1993). GATA4 encodes a transcription factor that interacts with FOG2 during the morphogenesis of the heart (Crispino et al., 2001). GATA4 is located on chromosome 8p23.1, a region recurrently deleted in individuals with CDH. GATA4 is important for lung and diaphragm development in humans (Holder et al., 2007).

Wat and group (2009) described two individuals and a monozygotic twin pair discordant for anterior CDH with complex congenital heart defects caused by interstitial deletion of 8p23.1, demonstrated by array comparative genome hybridization. They clearly defined the CDH minimal deleted region on chromosome 8p23.1 and suggested that haploinsufficiency of other genes, in addition to GATA4, may play a role in the severe cardiac and diaphragmatic defects associated with 8p23.1 deletions.

FOG2 (DIH3) (OMIM: 603693)

FOG2 (also known as ZFPM2) is a zinc finger–containing protein that modulates the transcriptional activity of GATA proteins, which, in turn, play important roles in early embryogenesis. FOG2 is located on chromosome 8q23 in a region commonly deleted in individuals with CDH and that FOG2 interacts physically with COUP-TFII (Huggins et al., 2001).
2.6.7 Signs and symptoms

Infants with CDH often present in the neonatal period with severe respiratory distress; pulmonary hypoplasia is common. Presenting symptoms after infancy can be acute onset of respiratory or gastrointestinal distress or abdominal pain from chronic intestinal obstruction or pleural effusion from entrapment of the bowel in the chest.

Anomalies frequently found in patients with isolated CDH are Pulmonary hypoplasia, malrotation or incomplete rotation of bowel, PDA, Patent foramen ovale, heart hypoplasia and/or dextroposition, Tricuspid or mitral valve regurgitation, Undescended testes and Accessory spleen.

2.6.8 Management

2.6.8.1 Diagnosis

Antenatal: CDH can be detected prenatally by an ultrasound examination performed during the second trimester in most affected infants.

Postnatal:

Physical Exam- Clinical examination reveals a scaphoid abdomen, diminished breath sounds ipsilateral to the side of the hernia, and displacement of the heart sounds contralateral to the hernia.

X-ray- Bowel gas is visible above the diaphragm accompanied by a mediastinal shift.
2.6.8.2 Treatment

Antenatal: Percutaneous Fetoscopic Endoluminal Tracheal Occlusion (FETO) Occlusion of the trachea prevents escape of the fluid produced by lungs and increases the pressure in the trachea. This increase can stimulate growth of the lungs.

Postnatal: Newborns with CDH are intubated immediately to avoid bag-mask ventilation and inflation of the bowel that has herniated into the chest. Extra-corporeal membrane oxygenation (ECMO) is used in some centers for neonates with critical cardiopulmonary deterioration. The ex-utero intrapartum treatment (EXIT) procedure transitions a newborn directly onto cardiopulmonary bypass when oxygenation and ventilation by intubation and mechanical ventilation are either not expected to be possible, or are likely to exacerbate pulmonary barotrauma. Other therapies that have been introduced in the acute neonatal treatment phase for CDH include nitric oxide (NO), delay of surgical repair, and use of surfactant and perflubron to stimulate the lungs to grow.

A diaphragmatic hernia is an emergency that requires surgery. Surgery is done to place the abdominal organs into the proper position and repair the opening in the diaphragm.
2.7 **Agenesis of Corpus Callosum (OMIM: 217990)**

"Corpus" means a body or structure. "Callosum" means a bridge. The corpus callosum is a white matter structure located in the midline and made up of thousands of nerve fibres (axons) which connect the two cerebral hemispheres (Fig.13). Each hemisphere of the brain is specialized to control movement and feeling in the opposite half of the body, and each hemisphere specializes in processing certain types of information (such as language or spatial patterns). Thus, to coordinate movement or to think about complex information, the hemispheres must communicate with each other. The corpus callosum is the main connector that allows that communication. Agenesis of the corpus callosum (ACC) is a birth defect in which the corpus callosum is partially or completely absent. Partial or complete absence of the corpus callosum, generally affects the posterior aspect of the structure (Pliu and Nicholaides, 1999).

### 2.7.1 Prevalence

Agenesis of the corpus callosum is among the most common brain malformations observed in humans and Frequency is 30-70 per 10,000 births for the general population and 2 to 3 % of those with mental disabilities (Freytag and Lindenberg 1967; Grogono, 1968; Jeret *et al.*, 1987). 

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Fig. 13 Structure of Corpus Callosum connecting to Cerebral Hemispheres.

www.mult-sclerosis.org/corpuscallosum.gif
2.7.2 Classification

Dobyns (1996) on the basis of the known embryology of the corpus callosum classified into two types, primary or 'true' types and secondary types.

2.7.2.1 Primary or True types

The first type includes defects in which exons form but is unable to cross the midline because of absence of the massa commissuralis and lead large aberrant longitudinal fiber bundles known as Probst bundles along the medial hemispheric walls. Probably the most common type of ACC, occurs in all ACC syndromes in which Probst bundles are seen.

The second type includes defect in which the commissural axons or their parent cell bodies is failed to form in the cerebral cortex. It occurs in congenital muscular dystrophy-dystroglycanopathy type A and in other types of lissencephaly in which Probst bundles are generally not seen despite absence of the corpus callosum (Sidman and Rakic, 1982).

2.7.2.2 Secondary types

The first type of callosal abnormality that may be mistaken for ACC is absence of the corpus callosum associated with major malformations of the embryonic forebrain prior to formation of the anlage of the corpus callosum. Examples include frontal encephaloceles and holoprosencephaly.

The second type consists of degeneration or atrophy of the corpus callosum, which results in striking thinning that may again be mistaken for true ACC (Dobyns, 1996).
2.7.3 Embryology

Formation of the cerebral commissures begins as early as 6 wks gestation with the formation of the primitive lamina terminalis. The most anterior portion, the rostrum, develops first and is followed by the genu, body and splenium. The axons destined to cross in the anterior commissure can be seen growing medially within the hemispheres. At 10 wks, the first fibers to reach the midline penetrate and cross the ventral portion of the lamina reuniens to form the anterior commissure. At 11 wks, fibers cross dorsal to the anterior commissure to form the hippocampal commissure. At 11-12 wks gestation, the first fibers cross the midline through the massa commissuralis, which is located between the anterior and hippocampal commissures, to form the corpus callosum. By 18-20 wks, a larger commissural plate is evident, and by 18-20 wks the corpus callosum has assumed the adult form except that it will continue to thicken and grow caudally for several months (Dobyns, 1996).

2.7.4 Maternal age

In infants for whom a chromosomal anomaly was detected, advanced maternal age was associated with an almost six-fold increased risk for ACC. For cases without chromosomal anomalies, the effect of advanced maternal age, when adjusted for paternal age, was substantially lower (Glass et al., 2003).
2.7.5 Consanguinity

There is no report suggesting increase incidence of ACC with consanguinity. However, Familial cases of ACC have been reported suggesting Autosomal recessive inheritance (Young et al., 1985; Castro-Gago et al., 1993)

2.7.6 Etiology

2.7.6.1 Genetic causes

Evidence indicates that a combination of genetic mechanisms, including single gene Mendelian mutations, single-gene sporadic mutations and complex genetics (which may have a mixture of inherited and sporadic mutations) might have a role in the aetiology of ACC. Retrospective chart reviews and cross-sectional cohort studies report that 30–45% of cases of ACC have identifiable causes. Approximately 10% have chromosomal anomalies and the remaining 20–35% has recognizable genetic syndromes (Table 5.) (Bedeschi et al., 2006).

Chromosomal abnormalities

ACC has been observed in constitutional trisomies as well as in some consistent chromosomal rearrangements like del(6)(q23), dup(8)(p21p23), dup(11)(q23qter), del(X)(p22), Wolf-Hirschhorn syndrome and trisomies 8, 13 and 18. Other regions in which cytogenetic abnormalities have been associated with ACC include del(1)(q44), del(2)(q14), dup(5)(p15.3p13.1), dup(6)(p25), dup(14)(q23q24), del(15)(q13), and del(21)(q11q22.1) (Dobyns, 1996).
Table 5. Syndromes associated with ACC.

<table>
<thead>
<tr>
<th>Syndrome with identified genes</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andermann syndrome (KCC3)</td>
<td>ACC, progressive neuropathy and dementia</td>
</tr>
<tr>
<td>XLAG (ARX)</td>
<td>Lissencephaly, ACC, intractable epilepsy</td>
</tr>
<tr>
<td>Mowat Wilson syndrome (ZFHX1B)</td>
<td>Hirschsprung disease, ACC</td>
</tr>
<tr>
<td>ACC with fatal lactic acidosis (MRPS16)</td>
<td>Complex I and IV deficiency, ACC, brain malformations</td>
</tr>
<tr>
<td>HSAS/MASA syndromes (L1CAM)</td>
<td>Hydrocephalus, adducted thumbs, ACC, MR</td>
</tr>
<tr>
<td><strong>ACC seen consistently, no gene yet identified</strong></td>
<td></td>
</tr>
<tr>
<td>Acrocallosal syndrome</td>
<td>ACC, polydactyly, craniofacial changes, MR</td>
</tr>
<tr>
<td>Aicardi syndrome</td>
<td>ACC, chorioretinal lacunae, infantile spasms, MR</td>
</tr>
<tr>
<td>Chudley–McCullough syndrome</td>
<td>Hearing loss, hydrocephalus, ACC, colpocephaly</td>
</tr>
<tr>
<td>Donnai–Barrow syndrome</td>
<td>Diaphragmatic hernia, exomphalos, ACC, deafness</td>
</tr>
<tr>
<td>FG syndrome</td>
<td>MR, ACC, craniofacial changes, macrocephaly</td>
</tr>
<tr>
<td>Genitopatellar syndrome</td>
<td>Absent patellae, urogenital malformations, ACC</td>
</tr>
<tr>
<td>Temtamy syndrome</td>
<td>ACC, optic coloboma, craniofacial changes, MR</td>
</tr>
<tr>
<td>Toriello–Carey syndrome</td>
<td>ACC, craniofacial changes, cardiac defects, MR</td>
</tr>
<tr>
<td>Vici syndrome</td>
<td>ACC, albinism, recurrent infections, MR</td>
</tr>
<tr>
<td><strong>ACC seen occasionally (partial list)</strong></td>
<td></td>
</tr>
<tr>
<td>ACC with spastic paraparesis (SPG11)</td>
<td>Progressive spasticity and, thin corpus callosum</td>
</tr>
<tr>
<td>Craniofrontonasal syndrome</td>
<td>Coronal craniosynostosis, facial asymmetry, bifid nose</td>
</tr>
<tr>
<td>Fryns syndrome</td>
<td>CDH, pulmonary hypoplasia, craniofacial changes</td>
</tr>
<tr>
<td>Marden–Walker syndrome</td>
<td>Blepharophimosis, micrognathia, contractures, ACC</td>
</tr>
<tr>
<td>Meckel–Gruber syndrome</td>
<td>Encephalocele, polydactyly and polycystic kidneys</td>
</tr>
<tr>
<td>Microphthalmia with linear skin defects</td>
<td>Microphthalmia, linear skin markings, seizures</td>
</tr>
<tr>
<td>Opitz G syndrome</td>
<td>Pharyngeal cleft, craniofacial changes, ACC, MR</td>
</tr>
<tr>
<td>Orofaciodigital syndrome</td>
<td>Tongue hamartoma, microretrognathia, clinodactyly</td>
</tr>
<tr>
<td>Pyruvate decarboxylase deficiency</td>
<td>Lactic acidosis, seizures, severe MR and spasticity</td>
</tr>
<tr>
<td>Rubinstein–Taybi syndrome</td>
<td>Broad thumbs and great toes, MR, microcephaly</td>
</tr>
<tr>
<td>Septo-optic dysplasia (DeMorsier syndrome)</td>
<td>Hypoplasia of septum pellucidum and opticchiasm</td>
</tr>
<tr>
<td>Sotos syndrome</td>
<td>Physical overgrowth, MR, craniofacial changes</td>
</tr>
<tr>
<td>Warburg micro syndrome</td>
<td>Microcephaly, microophthalmia, microgenitalia, MR</td>
</tr>
<tr>
<td>Wolf–Hirschhorn syndrome</td>
<td>Microcephaly, seizures, cardiac defects, 4p–</td>
</tr>
</tbody>
</table>

Ref. : Paul et al., 2007, P294
Single Gene disorder

Several syndromes that include ACC having Autosomal-dominant, Autosomal recessive and X-linked inheritance have been recognized (Dobyns, 1996). Some syndromes that frequently include ACC are

**Aicardi syndrome (OMIM: 304050):** It is classically characterized by a triad of features: agenesis of the corpus callosum, distinctive chorioretinal lacunae, and infantile spasms. Other features include characteristic facial features, microcephaly, gastrointestinal difficulties, small hands, vascular malformations and pigmentary lesions of the skin, increased incidence of tumors and mental retardation. It has X-linked dominant inheritance with lethality in males and is seen only in females and 47,XXY males.

**Acrocallosal syndrome (OMIM: 200990):** It is also known as Schinzel Acrocallosal syndrome and is a rare autosomal recessive syndrome characterized by corpus callosum agenesis, postaxial polydactyly, hallux duplication, multiple dysmorphic features, motor and mental retardation, and other symptoms.

**Andermann Syndrome (OMIM: 218000):** It is also known as Hereditary motor and sensory neuropathy with agenesis of the corpus callosum (HMSN/ACC). It is characterized by severe progressive sensorimotor neuropathy with resulting hypotonia, areflexia, and amyotrophy and variable degrees of dysgenesis of the corpus callosum. Mild-to-severe mental retardation and "psychotic episodes" during adolescence are observed. It is inherited in an autosomal recessive manner and *SLC12A6* is the only gene currently known to be associated with it.
Marden-Walker syndrome (OMIM: 248700): It is a rare autosomal recessive disorder which is characterized by blepharophimosis, microcephaly, micrognathia, multiple joint contractures, arachnodactyly, camptodactyly, kyphoscoliosis, and delayed motor development and is often associated with cystic dysplastic kidneys, dextrocardia, Dandy-Walker malformation and ACC.

Cerebro-oculo-facio-skeletal syndrome (COFS) (OMIM: 214150): It is rare autosomal recessive syndrome characterized by craniofacial and skeletal abnormalities, hypotonia, microphthalmia, microcephaly, micrognathia, arthrogryposis, ACC and mental retardation. Respiratory infections are frequent. A small number of individuals with COFS have a mutation in the ERCC6 gene.

A high frequency of ACC has been documented in infants with inborn errors of metabolism like maternal phenylketonuria (Levy et al., 1996).

Isolated ACC and developmental delay without detectable chromosomal changes have also been published with apparent autosomal dominant, autosomal recessive or X-linked modes of inheritance (Serur and Jeret, 1988; Dobyns, 1996).

2.7.6.2 Environmental causes

It is important to note that environmental factors might contribute to ACC as well. One clear example of environmental influences on callosal development is provided by fetal alcohol syndrome (FAS). The incidence of ACC in FAS is approximately 6.8%. Both clinical and experimental evidence indicates that alcohol exposure in utero decreases gliogenesis and glial-neuronal interactions, processes that are vital for corpus callosum development (Sowell et al., 2001; Paul et al., 2007). Various teratogens have also been implicated as a possible cause of agenesis of the corpus callosum, including prenatal exposure to infections such as
Rubella, CMV in early pregnancy and toxic exposure to certain drugs like valproate. Other cause of disruption of the normal development of the corpus callosum includes structural blockages (e.g. cysts) or other unknown factors.

2.7.7 AKT3 Gene (OMIM: 611223) (v-akt murine thymoma viral oncogene homolog 3 gene)

Deletions of 1q42-q44 have been reported in a variety of developmental abnormalities of brain, including microcephaly and agenesis of the corpus callosum. The family of protein kinases called AKT has 3 isoforms, AKT1, AKT2 and AKT3. AKT1 and AKT2 are required for normal growth and metabolism, respectively. AKT3 does not appear to contribute significantly to the maintenance of normal metabolism but is critical for the attainment of normal organ size. However, AKT3 controls exclusively the mass of the mouse brain by influencing both cell size and number, most likely, at least in part, through the selective activation of downstream effectors in the mTOR pathway (Nakatani et al., 1999; Easton et al., 2005). Murthy and group (2000) mapped the AKT3 gene to chromosome 1q44 by FISH study.

De Vries and group (2001) reported two unrelated mentally retarded boys with clinical pattern of growth retardation (prenatal onset), severe progressive microcephaly, hypospadias, corpus callosum abnormalities, cardiac anomalies, and gastro-oesophageal reflux. One showed submicroscopic distal 1q deletion and the other with a partial submicroscopic trisomy of distal 13q in addition to a submicroscopic distal 1q deletion. Gentile and group (2003) described a case with minor facial anomalies, mental retardation, seizures, and partial agenesis of the
corpus callosum with de novo terminal chromosome 1q deletion. FISH showed the monosomy of 1q43 and 1q44 bands.

Boland and group (2007) who defined a 3.5 Mb critical region in 1q44 containing one or more genes leading to microcephaly and corpus callosum abnormalities when present in only one functional copy. Mapping of a balanced reciprocal t(1;13)(q44;q32) translocation in a patient with postnatal microcephaly and agenesis of the corpus callosum demonstrated a breakpoint in this region that was situated 20 kb upstream of AKT3, a serine-threonine kinase. The murine ortholog AKT 3 is required for developmental regulation of normal brain size and callosal development. Whole-mount in situ hybridization confirmed expression of AKT3 in the developing central nervous system during mouse embryogenesis.

Hill and group (2008) described a case distal deletions of chromosome 1q have a recognizable syndrome that includes microcephaly, hypoplasia or agenesis of the corpus callosum, and psychomotor retardation. Using microsatellite and single nucleotide polymorphism (SNP) markers, they mapped the deleted regions in seven patients with terminal deletions to 1q43-1q44. Andrieux and group (2008) reported a boy with developmental delay, growth retardation, facial dysmorphisms, vermis hypoplasia, micropolygyria, corpus callosum agenesis and normal karyotype. High resolution oligonucleotide array-CGH showed a de novo 6.9 Mb 1qter deletion / 4.4 Mb 18pter duplication. AKT3 represents an excellent candidate for developmental human microcephaly and agenesis of the corpus callosum, and suggested that haploinsufficiency causes
postnatal microcephaly and agenesis of the corpus callosum (Boland et al., 2007; Andrieux et al., 2008).

2.7.8 Sign and Symptoms

It varies greatly among individuals but some common characteristics include hypotonia, vision impairments, poor motor coordination, delays in motor milestones, low perception of pain, delayed toilet training, and chewing and swallowing difficulties. Even though IQ is normal, they may have some cognitive disabilities like difficulty in complex problem solving and social difficulties missing subtle social cues. The unusual social behavior in childhood is often mistaken for or misdiagnosed as Asperger syndrome or other autism spectrum disorders. Other characteristics associated with ACC include seizures, spasticity, early feeding difficulties and/or gastric reflux, hearing impairments, abnormal head and facial features, and mental retardation.

2.7.9 Management

2.7.9.1 Diagnosis

ACC can be diagnosed by CT scan or MRI (Fig.14). It can be diagnosed during pregnancy through routine prenatal USG. ACC can be accurately identified by targeted examinations performed at 18-20 wks or later. Failure to visualize the cavum septum pellucidum beyond 18 wks gestation should raise the suspicion of agenesis of the corpus callosum. ACC should be suspected when the posterior horn of the lateral ventricle is dilated, giving the ventricle a “tear drop” aspect. The definitive diagnosis of callosal agenesis depends upon direct demonstration of the absence of the complex formed by the corpus callosum and cavum septum.
Fig. 14 MRI of ACC

Ref.: Paul et al., 2007, P291
pellucidum by midsagittal or midcoronal views of the fetal brain.

### 2.7.9.2 Treatment

There is no specific treatment however individuals will benefit from early intervention services, supportive therapies, special education, and adult support services based on their individual needs. Evaluations and therapies should begin early in life and continue throughout childhood and into adult life.