Discussion
5. Discussion

The twentieth century witnessed great advances in the diagnosis, treatment, and prevention of birth defects and developmental disabilities and in the quality of life and life expectancy of people living with disabilities. The development of advanced surgical techniques and better clinical management of selected birth defects, such as congenital heart disease and spina bifida has resulted in marked increases in survival of children and adults with these conditions.

Chromosome rearrangements are a notable cause of embryonic lethality and birth defects. Identifying the genes that underlie the pathogenesis of chromosome deletion and duplication syndromes is a challenge because the involved chromosomal segment can contain several detrimental genes. The identification of these genes that are relevant to such disorders often requires the analysis of individuals that carry rare, microdeletions, translocations or single gene mutations (Gowde and Patel, 2007). With the advent of sensitive and specific technique of FISH, the diagnosis of micro-deletions has become simpler, and more accurate which has resulted in a significant increase in the diagnosis of patients (Pinkel et al., 1986). Currently, FISH is considered as the method of choice for detection of microdeletions as it helps in analyzing relatively large number of cells and is very specific for every microdeletion.

Extensive data is available for prenatal diagnosis of chromosomal abnormalities using FISH from the western countries (Ward et al., 1993; Jalal et al., 1998; Verlinsky et al., 1998) but there are very few reported studies from an Indian set
Most of the Indian studies were retrospective studies and were limited to screening of aneuploidies by FISH (Verma et al., 1990; Kabra et al., 1996; Jobanputra et al., 2002; Ashok et al., 2003). The incidence of congenital malformations in the newborn ranges from 1.63% to 3.7% (Bhat and Babu, 1998; Patel and Adhia, 2005).

An attempt has been made in the present study to understand the etiology of Congenital heart defects (CHDs), Congenital Diaphragmatic hernias (CDHs) and agenesis of corpus callosum (ACC) in both prenatal and postnatal cases from southern state of India (Karnataka). In the present study the overall incidence rate of congenital malformation could not be calculated as it was a selected group of samples excluding common chromosomal abnormalities like Down syndrome and other aneuploidies.

Out of the total samples collected, 55% were prenatal sample and 45% were postnatal sample. From amongst the total samples, the majority obtained were CHD samples with 74% followed by 14.67% of ACC samples and 11.33% of CDH samples. Thus in general, the distribution of samples also represents the reported incidence rate of CHD, CDH and ACC as described in the review chapter. The incidence of CHD is more and the incidence of CDH is comparatively rare.

5.1 Congenital heart defect (CHD)

In this day and age, about 85-95% of the children with CHD survive into adulthood due to better surgical techniques (Morris and Menashe, 1991; Okita et al., 1995). Therefore, the number of adults with a CHD is on the rise. Once these patients enter the reproductive age group, knowledge of heritability of such defects is essential. Isolated congenital heart defects are most frequently sporadic. Despite
the sporadic nature, a genetic component is still very likely to contribute to the occurrence of these defects and there is a higher recurrence risk amongst siblings and offspring of patients with CHD (Sanchez-Cascos, 1978; Loffredo et al., 2004).

Among the samples obtained with CHDs, 54% were prenatal samples and 46% were postnatal samples. In the prenatal samples, amniotic fluid sample formed the majority with 55% followed by 38% of cord blood and 7% of chorionic villi samples. This correlates with the fact that maximum abnormalities are picked up during the mid-trimester anomaly scan. However, our hospital being a tertiary center, majority of patients is referred late for second opinion or confirmation. Within CHDs, Ventricular septal defect (VSD) was the most common defect, around 42% in prenatal, 27% in postnatal and overall around 35%. This was followed by Tetrology of fallot (TOF) which was 27% in prenatal, around 33% in postnatal and overall around 31%. Pulmonary atresia (PA) or Pulmonary stenosis (PS) was the third common, with 17% in prenatal, around 8% in postnatal and overall around 13%.

Consanguinity was observed in about 11% of CHD patients which is low compared to around 46% of Ramegowda and Ramchandra (2006) study. Consanguinity is common in South India but ours is metropolitan population with less consanguinity. Only 3.65% of mothers with advanced maternal age had a child with CHD. On the other hand, around 73% of mothers in the ages between 21-30 years had child with CHD. This difference observed may be due to the fact that maximum number of deliveries occurs in this age group. Most of the pregnancy is assumed to be normal and not screened in detail.
Heart development, occurs early in the embryonic stage leading to significant mortality and morbidity thus affecting the clinical outcome of the affected individuals. CHD, being a complex trait, are thought to be multifactorial (Bruyere et al., 1987). Even though, extensive knowledge of the genetic control of cardiogenesis in animals is available, this has not translated into an equivalent amount of clinical knowledge of the genetic determinants of CHD in humans.

Following are the microdeletion probes screened for CHD samples-

5.1.1 DiGeorge Syndrome (DGS) - 22q11.2

The genetic predisposition to cardiac malformation may be influenced by in utero environmental or genetic background. The importance of genetic factors in the cause of congenital heart defects has been shown by previous studies (Ferencz et al., 1987; Johnson et al., 1997). Microdeletion of chromosomal region 22q11 is an important cause of selected conotruncal cardiac defects of the heart and account for about 6.9% to 68% of cases (Driscoll et al., 1993; Goldmuntz et al., 1993; Momma et al., 1996; Alikapıfoðlu et al., 2000; Giray et al., 2003).

Prenatal diagnosis of chromosome 22q11.2 microdeletion by FISH analysis was first reported in 1995 (Puder et al., 1995; Van Hemel et al., 1995). Since then, numerous studies have confirmed the high occurrence of 22q11.2 microdeletion after prenatal detection of cardiac anomalies (Volpe et al., 2003; Bretelle et al., 2010; Liu et al., 2010). Three genes namely TUPLE1, TBX1 and N25 are responsible for 22q11.2 deletion syndrome.

The prevalence of 22q11.2 microdeletion is more in prenatal period than the postnatal period (Iserin et al., 1998; Boudjemline et al., 2001). Perhaps, this
difference may be accounted for by perinatal death of fetuses/neonates due to very complex CHD forms and/or low birth weight (Volpe et al., 2003) or termination of pregnancy after detection of microdeletion in our country.

Volpe and group (2003) reported 141 cases of malformations of the outflow tracts or IAA from 1150 prenatal cases of heart defects diagnosed over a period of 10 years. 22q11 microdeletion was detected in 28 out of 141 fetuses (19.8%). IUGR, additional aortic arch anomalies and thymic hypoplasia were significantly more frequent in fetuses with 22q11 microdeletion. Most often, IUGR appeared to be associated with the worst prognosis. Prenatal ultrasound thymus examination, showed 75% sensitivity and 94% specificity. The combination of these two predictors, namely, thymus defects and IUGR associated with additional aortic arch anomalies was more than 90% sensitive and 100% specific. Liu and group (2010) studied 37 prenatal cases of which 23 were cases of fetal cardiac malformation and 14 of non-cardiac malformation for 22q11.2 deletion syndrome. The prevalence rates of the TUPLE1 gene deletion in the amniotic fluid cells from fetuses with cardiac malformations and fetuses without cardiac malformations were 43.5% and 57.1% respectively.

Bretelle and group (2010) reported a retrospective study of 883 cases for 22q11.2 deletion over a period of 12 years. Antenatally, 22q11.2 microdeletion was detected in 8 fetuses (4.7%) from among 169 pregnancies, all presenting with conotruncal anomalies. During the same period, postnatal 22q11.2 deletion was diagnosed in 81 out of 714 patients aged from day1 to 42 years (11.3%). In their study, abnormal fetal heart at USG was the basis of 22q11.2 testing and the overall rate of 22q11.2 deletion detection was almost 5%, which is concordant with previous studies strongly depending on the type of CHD. In contrast 49%
prevalence of cardiac defects found in their postnatal studies indicate that a significant number of 22q11.2 deletion syndrome patients do not have a CHD. This figure was an exception and much lower than the 84% they reported in their first series of study before 1997 (Levy-Mozziconacci et al., 1997) and the 75% commonly admitted in the literature (Kobrynski and Sullivan, 2007). A wide variety of non-cardiac malformations such as overt cleft palate, renal and limb abnormalities, neural tube defects and polyhydramnios are identifiable prenatally, and have been reported to occur in association with 22q11.2 deletion syndrome (Ryan et al., 1997; Wu et al., 2002). Few authors have raised the possibility of considering prenatal 22q11.2 deletion studies in the event of non-cardiac USG findings. In a recent review on genetic counseling for 22q11.2 deletion syndrome, McDonald-McGinn and Zackai (2008) argued that, as such findings lead to systematic prenatal diagnosis of aneuploïdies, the addition of 22q11.2 deletion studies to standard cytogenetics should be considered. Similarly, isolated increased nuchal translucency (NT), a powerful marker of fetal CHD and aneuploidies or isolated intrauterine growth retardation without major CHD do not deserve deletion testing (Chen et al., 2006; Donnenfeld et al., 2006; Lautrup et al., 2008). Increased NT, polyhydramnios, IUGR, pulmonary arterial abnormalities, aortic arch anomalies and thymic hypo/aplasia were found to be more frequent in fetuses with deletion (Boudjemline et al., 2002; Volpe et al., 2003). In a study of 95 fetuses with CHD in which the status for 22q11.2 deletion was known, Chaoui and group (2002) concluded that the marker thymic hypo/aplasia performed with a sensitivity of 85% and a specificity of 97%. Bretelle and group (2010) proposed to take advantage of these observations to set out guidelines to improve the prenatal detection of 22q11.2 deletion syndrome in fetuses with normal hearts (Fig.42).
These results suggest that deletion studies could be justified in fetuses with non-cardiac prenatal USG findings that have been reported in association with 22q11.2 deletion syndrome. 22q11.2 microdeletion is one of the primary conditions leading to intrauterine growth retardation and congenital heart malformation (Aslan et al., 2005). Therefore, high-risk fetuses showing growth retardation and malformation should receive screening for 22q11.2 microdeletion. FISH test of the key gene TUPLE1 is still considered the gold standard for the diagnosis of 22q11.2 microdeletion syndrome (Liu et al., 2010).

Indian postnatal studies of chromosome 22 microdeletions for isolated congenital heart disease showed the microdeletion in 6/105 (5.71%) (Gawde et al., 2006) and 4/23 (17%) (Madon et al., 2010) patients. In the present study, chromosome 22 microdeletions were seen in prenatal samples 2/60 (3.33%), in postnatal sample 3/50 (6%) and 5/110 (4.5%) amongst the total samples. Out of five patients, four had a de novo deletion and conotruncal defect. Only one of the patient had an affected mother with non-cardiac defect and velocardial feature. To the best of our knowledge this is the first prenatal study with 22q11.2 deletion syndrome from India. The detection rate in our prenatal sample was 3.33% from randomized CHD samples while Brettle and group (2010) had 4.7% detection rate with conotruncal anomalies.
Fig. 42 Decisional charts proposal for prenatal 22q11.2 DS testing according to ultrasound finding (US) and aneuploidy screening tests.

Ref.: Bretelle et al., 2010, P369
In our study, the mean gestational age for diagnosis of CHD was 20 wks. Out of 111 patients of CHD, 71 (64%) had out flow tract abnormalities. The most frequently occurring CHD were VSD (35%) and TOF (31%) which are similar to the results observed in the study by Bretelle and group (2010). In our study CHD77case had a familial 22q11.2 deletion syndrome where the pregnant mother had distinct velocardial facial feature and had terminated the previous pregnancy due to CHD. She had 22q11.2 deletion without any CHD and prenatal diagnosis in the fetus and successive pregnancy also showed 22q11.2 deletion in both the fetuses with no major CHD detected antenatally and postnatally. CHD 111 case which had deletion of 22q13.3 was included as interesting case due to presence of CHD with deletion of 22q.

Therefore, Increased NT, IUGR and other non-cardiac malformations of 22q11.2 deletion syndrome should be screened carefully for thymus hypo or aplasia and fetal echo including vascular ring. If either of one (thymus or cardiac abnormality) is present, then the chances of 22q11.2 deletion detection are more. Prenatal findings of 22q11 micro-deletion enable clinicians to provide informative counseling to the couple. The couple should ideally be provided information about 22q11 deletion phenotypic findings, recurrence risk, disease variability and the prognosis. Couples, who decide not to undergo termination of pregnancy, should be informed of good neonatal care, so they can plan accordingly for treatment in a tertiary care centre both for cardiac intervention and the management of associated clinical features such as immunodeficiency and hypocalcaemia.
5.1.2 DiGeorge locus II (DGSII) - 10p14

DiGeorge-like phenotype is seen with the deletion in the short arm of chromosome 10, at or near 10p13 (Schuffenhauer et al., 1998). This deletion was first reported by Elliott and group (1970) although the association with DGS anomalies was not described until 1984 (Herve’ et al., 1984). Characteristics of patients with the deletion at 10p13p14 comprise of many of the features of DGS/VCFS, including cardiac defects, T-cell deficiencies, transient hypoparathyroidism/hypocalcemia, facial anomalies such as low-set and small ears, micrognathia, and facial clefts, hypertelorism, short nose with anteverted nares, abnormally shaped skull, microcephaly, hand and foot abnormalities, genitourinary anomalies, hearing loss, and severe psychomotor retardation (Shapira et al., 1994; Schuffenhauer et al., 1995; Schuffenhauer et al., 1998; Van Esch et al., 1999). Deletions of chromosome 10p may also result in a DGS/VCFS-like phenotype. However, in addition to the DGS/VCFS anomalies, deletions in this region often present with renal anomalies and hearing deficits as well (Schuffenhauer et al., 1998; Van Esch et al., 1999).

Van Esch and group (1999) reviewed 36 patients with a deletion of 10p and compared with the classic DiGeorge syndrome. Thirty-one out of the 36 patients had a terminal deletion, four patients had an interstitial deletion and one had a reciprocal insertional translocation. The variability of clinical anomalies seen in 22q11.2 deletions also exists for monosomy of 10p. They compared the phenotypes observed in both the condition which is presented in the Table 26.
Table 26. Comparison of DGSI and DGSII feature

<table>
<thead>
<tr>
<th></th>
<th>Del 10p</th>
<th>Del 22q11</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Van Esch et al</td>
<td>Our case</td>
</tr>
<tr>
<td>Mental Retardation</td>
<td>Severe</td>
<td>moderate</td>
</tr>
<tr>
<td>Renal anomalies</td>
<td>54%</td>
<td>No</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>56%</td>
<td>No</td>
</tr>
<tr>
<td>Heart Malformation</td>
<td>47%</td>
<td>Yes</td>
</tr>
<tr>
<td>Perceptive Deafness</td>
<td>41.5%</td>
<td>No</td>
</tr>
<tr>
<td>Facial Dysmorphism</td>
<td>Not specific</td>
<td>No</td>
</tr>
<tr>
<td>Growth Retardation</td>
<td>86.5%</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Schuffenhauer and group (1998) gave evidence for a reduced penetrance of DGSII, which is also well known for DGSI. Renal abnormalities have been observed in 36% of patients with partial monosomy 22q11 and in 67% of DGSII patients in their study. Thus, abnormalities of the urinary tract might be more common in DGSII than in DGSI. Heart defects in monosomy 10p involve TOF, ASD, VSD, aortic valve stenosis and PS, and thus appear to be more heterogeneous than in patients with partial monosomy 22q11. Hearing loss was found in three of eight patients in a study and has been described in at least four other patients with partial monosomy 10p (Kinoshita et al., 1992; Obregon et al., 1992; Shapira et al., 1994). Because several patients have been reported at an age before hearing loss might be obvious, and no information on this feature is given in other reports, Schuffenhauer and group feels that hearing loss might be more common in partial monosomy 10p than reported to date. The hearing loss in deletion 10p is sensorineural in contrast to hearing loss in 22q11 deletion syndrome which is mostly conductive and related to palate deformities and velopharyngeal insufficiency. Growth and severe mental retardation was seen in all patients. (Van Esch et al., 1999).

Yatsenko and group (2004) reported a case of a 4-year-old boy with craniofacial dysmorphism, developmental delay and ASD with interstitial deletion of 10p. They reviewed 19 patients with congenital heart defects and deletions involving 10p and propose that ASD is a common cardiac anomaly associated with DiGeorge II syndrome. They suggested that the presence of an ASD in patients with DGS or a DGS-like phenotype may potentially be used to differentiate between DGSII and DGSI. Bartsch and group (2003) studied a series of 295 patients with suspected DGS/VCFS and identified 58 subjects with 22q11 deletion, but none with 10p deletion. Berend and group (2000) reported 412 patients referred for DGS or VCFS
and analyzed their samples using the dual-probe FISH assay. Deletion of the DGSI locus at 22q11.2 was found in 54 (13%) patients, and one patient had a deletion of the DGSII locus at 10p13p14 (0.24%).

In our study 10p deletion was detected in 1 out of 110 cases (0.91%). This suggests that deletion for the DGSII locus on chromosome 10 may be 50 times less frequent than the deletion on chromosome 22. The incidence for deletions of 22q11.2 has been estimated to be 1 in 4,000 newborns (Devriendt et al., 1998). Based on this frequency, the deletion of chromosome 10p resulting in the DGSII phenotype can be estimated to be about 1 in 200,000 newborns.

At the beginning of this study there were no diagnostic probes available for DGSII region but recently two commercial companies, Cytocell, UK and Kreatech, Netherlands have come up with the diagnostic probe for this region. This is in conformity with this study about the importance of DGSII region in a patient with CHD.

Many patients with the mild end of the DGS/VCFS spectrum have been referred to the cytogenetics laboratory by the physicians for FISH for the deletion on 22q11.2, and high resolution G-banded analysis has been requested for only those patients with a more severe presentation, although the most effective method for detecting all possible cytogenetic abnormalities would be to perform a complete chromosome analysis along with the FISH studies. Other chromosomal abnormalities can help in detecting new gene loci similar to all other genes which were identified based on chromosomal abnormality. Even though the deletion on 10p is relatively rare deletions of DGSI and DGSII result in similar phenotypes,
and hence, it is still beneficial to screen patients referred for DGS and VCFS for both DGSI and the DGSII loci (Berend et al., 2000).

5.1.3 Deletion 8p23.1 (GATA4)

Pettenati and group (1992) reported a family in which three members presented with minimal phenotypic abnormalities, normal intelligence to mild mental retardation, and a cytogenetically terminal chromosome deletion at band 8p23.1. An Unaffected father passed an 8p23 deletion on to his 11 year old daughter who had mild learning difficulties and 7 year old son who was more severely affected with moderate learning difficulties.

Pehlivan and group (1999) described four individuals with del (8)(p23.1) and CHD with haploinsufficiency of the GATA4 gene by FISH. FISH analysis on cells from 48 individuals with CHD and normal karyotypes failed to detect any submicroscopic deletions at the GATA4 locus. They concluded that haploinsufficiency at the GATA4 locus is often seen in patients with del (8)(p23.1) and CHD.

Reddy (1999) described a case with del (8)(p23.1) in amnionocyte culture with no cardiac anomalies. The father carried the same 8p23 deletion and was unaffected. The pregnancy was continued and resulted in the birth of a baby girl. The child was normal at six months of age and was unaffected by the deletion. Giglio and group (2000) studied 12 patients (7 had CHD and 5 did not) with distal 8p deletions from 9 families by defining their chromosome rearrangements at the molecular level by FISH and short-tandem repeat analysis. GATA4 was not
deleted in two of the patients. Their results showed that a causal relationship does not seem to exist between GATA4 haploinsufficiency and 8p-CHD (deletion 8p).

Gilmore and group (2001) presented a female infant with a distal deletion of 8p (8p23.1 → pter) whose development was monitored over a 5-year period from 12 months of age. Despite initial delays in gross motor and language skills, cognitive development (assessed with the Bayley Scales of Infant Development) and intellectual ability (measured on the Stanford-Binet Intelligence Scale) were within the average range. The results were consistent across all assessments. This finding contrasts with most previously reported 8p- cases, which have described individuals with mild to moderate intellectual disability. It is not certain whether the deletion arose as a de novo event because the parents decided not to have their chromosomes studied at that time. The child has no cardiac defects or other medical conditions and her facial features display no obvious dysmorphic characteristics.

Another study described a case where in a 22 year old unaffected mother passed an interstitial 8p23.1p23.2 deletion on to a son, who was also unaffected, and a daughter who had moderate learning difficulties, deafness and a heart condition (Devriendt et al., 2006).

Schellberg and group (2004) investigated a group of 376 children over a period of 7 years with different types of CHD, to assess the presence of chromosomal aberrations. Analysis of microdeletions was performed using FISH in 335 of the 376 patients and in total; they detected 51 microdeletions (15%). According to the clinical phenotype, they used locus specific probe 22q11.2 in 321 cases, 7q11.23 in 16 cases, 8p23.1 in 11 cases, 10p13 in 2 cases, and 4p16.3 in 3 cases. In 43 out of
312 cases investigated, they found a microdeletion of 22q11.2 (14%), but no microdeletion was reported in the 12 cases screened for 8p23.1, or in the 2 cases screened for 10p13.

In this study, case no. CHD1 Karyotype showed del (8)(p11.2p21) wherein GATA4 (8p23.1) gene was not deleted. This confirmed that it is an interstitial deletion and not a terminal deletion. NKX2 transcription factor related, locus 6 (NKX2-6) (OMIM 611770) is localized to 8p21.2. NKX2-6 is one of several genes that are coexpressed with the gene T Box-1 (TBX1) (OMIM 602054) in the second heart field. Another candidate gene for congenital cardiac malformations is the gene Neuregulin-1 (NRG1) (OMIM 142445) in 8p12. NRG1 is involved in two different aspects of cardiac development, namely trabeculation (development of the finger-like extensions of the heart’s wall) and valvuloseptal formation. CHD are common in interstitial deletion of the 8p12-p21 region and to some extent may be due to haploinsufficiency of NKX2-6 or NRG1 (Willemsen et al., 2009).

More recently, the chromosome 8p23 deletion syndrome was outlined as a distinct syndrome including mild to severe mental retardation, microcephaly, growth retardation, congenital heart defect and facial anomalies including micrognathia and low-set ears and the same major features as were previously attributed to the clinical entity terminal 8p-syndrome (Giglio et al., 2000; Pae’ z et al., 2008; Willemsen et al., 2009). However, several phenotypic features of so called terminal 8p-syndrome were also described in patients with a proximal interstitial deletion not encompassing this region between 8p21.3 and 8p23, for example, in a patient with a deletion in 8p11.21-p11.23 (Tsukahara et al., 1995; de vries et al.,
However, our case had only IUGR and cleft lip in addition to CHD. As it was prenatal case, we do not know if there was any mental deficiency and dysmorphic feature.

Giglio and group (2000) presented evidence that not all subjects with 8p deletions and CHD had a deletion of GATA4 gene. From this it appears that GATA4 may not play a role in causing CHD in 8p23 deletion syndrome. However, in 2003, Garg and group (2003) reported GATA4 mutations affecting all 16 individuals in 5 generations of a family with congenital heart defects, predominantly ASD but also had AVSD and various valvular abnormalities. It is thus clear that GATA4 haploinsufficiency does contribute to CHD in at least some individuals with 8p23 deletions, although it is likely that at least one other gene in the region also contributes to the cardiac phenotype, given the findings of Giglio and group (2000).

5.1.4 Deletion 5q35.1 (NKX 2-5)

Elliot and group (2003) studied cohorts of 146 individuals with secundum ASD, PFO or HLHS. The single mutation detected in their ASD cohort occurred in an individual with a family history of ASD; none were found in 90 sporadic cases. Their findings suggest that NKX2-5 mutations are a relatively infrequent cause of sporadic ASD and HLHS. Screening for NKX2-5 mutations may be warranted in individuals with ASD and a positive family history, irrespective of the presence or absence of AV conduction block.
McElhinney and group (2003) tested 608 patients with conotruncal anomalies (n=370), left-sided lesions (n =160), secundum ASD (n =71), and Ebstein’s malformation (n =7) for NKX2-5 mutations. Only 18 patients were detected to have NKX2-5 mutations from amongst 608(3%) patients and they came to a conclusion that NKX2-5 mutations occur in a small percentage of patients with various CHD.

Further, the 3.5 Mb 5q subtelomeric deletion syndrome is apparently very rare. The case reported by Rauch and group (2003) was the only one detected by subtelomeric FISH in a series of 570 patients with mental retardation of unknown origin (Rauch and Dorr, 2007). Cytogenetically visible pure constitutional deletions restricted to the very distal band of the long arm of chromosome 5, that is, 5q35, have only been reported in five instances. Draus and group (2009) studied a cohort of patients with ASD (n=13), VSD, (n=5), and atrioventricular canal defects (n=10). No evidence of somatic mutations was found in this study. Somatic mutations in NKX2-5 do not represent an important etiologic pathway in pathologic cardiac development in patients with cardiac septal defects.

Rauch and group (2010) studied 230 patients with TOF by karyotyping, comprehensive 22q11.2 deletion testing, sequencing of TBX1, NKX2-5 and JAG1, as well as molecular cytogenetics in selected patients. Pathogenic genetic aberrations were found in 42 patients (18%), with 22q11.2 deletion as the most common diagnosis (7.4%) and NKX2-5 mutations were seen in two patients with non-syndromic TOF (0.9%). This is in contrast to earlier studies reporting NKX2-5 mutations in 1.7-4% of patients with TOF.
It is currently unknown as to what extent dominant or modifying NKX2-5 gene contributes to sporadic or familial CHD. Gene NKX2-5 and GATA4 account for a relatively small proportion of cases, and even when only individuals with a family history are considered, these genes are found in 10.7% and 7.8% respectively. Mutations in TBX20 contributing to human CHD, possibly account for another 5.1% of familial cases, leaving more than 75% of familial ASD unaccounted for, based on the figures for these three genes. Thus, the majority of the genetic basis of familial CHD is yet to be elucidated, and it would not be surprising if there were more genes yet to be discovered.

In this study, we didn’t get any patient in whom GATA4 and NKX2-5 genes were deleted. Although we had one case of with 8p deletion but GATA4 was not deleted. It was also negative for 22q11.2, 10p14 and 5q35.1 and it could be due to other gene like NKX2-6 or NRG1 responsible for the cardiac defect. This could be due to the very small incidence rate of the gene and requires more population screening. The other reason could be the type of cardiac defect, as this study involved generalized cardiac defect and did not demand patients with specific cardiac defect like conotruncal defect or ASD. Recent studies also suggest that mutation in GATA4 and NKX2-5 are responsible for CHDs.

A disadvantage of using a candidate gene screen for identification of mutations in human congenital heart defect is that it is hypothesis driven. Therefore, only genes known to be involved in cardiac differentiation will be investigated further whereas other genes, which might be implicated as well, remain undiscovered. Future research will adopt more advanced techniques to discover novel genes important for heart development, such as genome wide association studies (GWAs) and
whole genome sequencing. Advantages of these approaches are that they are not just hypothesis.

5.2 Congenital Diaphragmatic Hernia (CDH)

CDH described by Morgagni in 1761 and by Larrey in 1829 is a rare diaphragmatic anomaly that may be considered nearly always congenital (Bhandarwar et al., 2008). Much of the current research is focused on elucidating the genetics and patho-physiology contributing to CDH to develop more effective therapies. Latest data suggest that many cases of CDH are genetically determined and also indicate that CDH is etiologically heterogeneous (Pober, 2008). Research into the underlying causes of CDH has the potential to positively affect the clinical management of CDH in affected individuals and their families. The description of multiple genetic syndromes associated with CDH highlights the importance of a careful evaluation of patients with CDH (Holder et al., 2007) and the regions 15q26.1 and 8p23.1 may play an important role in the development of the diaphragm. A deletion of 8p23.1 or 15q26.1 should be considered whenever a CDH and/or a cardiac abnormality are detected on ultrasound (López et al., 2006).

In the present study of CDHs, 76% were prenatal samples and 34% were postnatal samples. In prenatal, cord blood sample formed majority of the samples with 54% followed by 38% of amniotic fluid and of chorionic villi samples with 8%. Isolated CDH was seen in 10/17 (59%) patients while 7/17 (41%) had additional malformations of CHD or neurological abnormalities. All CDH were observed to be left sided hernia. Consanguinity was seen in around 17.65% of CDH patients. None of the mothers with advanced maternal age had a baby with CDH. In fact, majority (94%) of mothers who had a child with CDH were in the age group of 21-
30 years. Deletion 15q26.1-q26.2 (DIH1 gene) was seen in one (7.77%) of the prenatal samples and overall 1/17 (5.88%). No deletion was seen for 8p23.1.

Prenatal diagnosis of isolated CDH either by ultrasound or by MRI scan should prompt a thorough genetic evaluation. In addition to collecting detailed pregnancy and family history, a standard chromosome analysis and a fetal echocardiogram should be performed. This workup is indicated even in cases with apparently isolated CDH because major as well as minor anomalies can escape prenatal detection. Such an evaluation may have an influence on decision making by the couple including decisions about the possible termination of pregnancy (Holder et al., 2007; Pober, 2008). Prenatal diagnosis allows patient education, potential identification of those cases at risk for worst outcome, and the opportunity for prenatal intervention.

Klaassens and group (2007) reported two patients with a deletion of 15q26 and phenotypes similar to other patients with CDH caused by 15q26 deletions. This phenotype consists of intra-uterine growth retardation, left-sided CDH, cardiac anomalies and characteristic facial features, similar to those seen in Fryns syndrome. They proposed that when this combination of birth defects is identified, either pre- or postnatally, further investigations to confirm or exclude a deletion of 15q26 are indicated, since the diagnosis of this deletion will have major consequences for the prognosis and, hence decision making. The finding of a deletion within 15q24-26 in a fetus with CDH has to be considered as a predictor of poor prognosis. It is worthwhile to investigate for subtle chromosomal lesions paying special attention to chromosome 15q in fetuses with CDH for enabling appropriate parental counseling (Schlembach et al., 2001).
If a fetus is diagnosed prenatally with isolated CDH and the pregnancy is not carried to term, every effort should be made to collect as much information as possible including fetal imaging (e.g. fetal MRI scan and echocardiogram), fetal karyotype, and a postnatal autopsy. A sample of the fetus should also be obtained and stored for future testing if possible.

Robinson and Fitzgerald (2007) suggested that karyotype analysis should be performed in every child with CDH and additional malformations not directly caused by the hernia. By array CGH, Slavotinek and group (2005) screened patients with CDH and additional phenotypic anomalies consistent with Fryns syndrome for cryptic chromosomal aberrations. They identified submicroscopic chromosome deletions in 3 probands who had previously been diagnosed with Fryns syndrome and had normal karyotyping with G-banded chromosome analysis. Two female infants were found to have microdeletions involving 15q26.2, and one male infant had a deletion in band 8p23.1. Several recent reports describe non-isolated CDH cases with prior normal 46, XX or 46, XY routine karyotypes but abnormalities were detected on array CGH. The yield of aCGH in this setting is not currently known, but application of this technology should be considered in prenatally diagnosed cases of CDH (due to limitations in detecting all major malformations, dysmorphology, and minor anomalies) (Kantarci et al., 2006; Klaassens et al., 2007; Pober, 2008).

Outcome of neonates with CDH varies widely and the data from developing countries is inadequate. Reports from developed countries describe improved survival with high frequency ventilation, inhaled nitric oxide (iNO) and extracorporeal membrane oxygenation (ECMO), apart from delayed surgical repair (Sawyer et al., 1986; West et al. 1992). Many centers in developing countries lack
these advanced facilities, and hence, the outcomes expected would be different. The overall mortality rate for CDH remains high, despite increased prenatal detection, transfer to tertiary institutions for delivery, and advances in neonatal care (Bhat et al., 2008). Harrison and group (1994) in their prospective study, observed 58% mortality among CDH diagnosed before 24 weeks gestation despite optimal postnatal care. Colvin and group (2005) reported that prenatal diagnosis itself was an important predictor of mortality rates for live-born infants. Prenatally diagnosed CDH cases have 33% survival rate as compared with 67% survival rate for postnatally diagnosed infants.

In a study by Bhat and group (2008), the survival-to-surgery rate was 75%. Survival rate of surgical patients was also 75%. Prenatally-detected cases had a reduced survival to surgery rate compared to postnatally-detected rates (25% vs. 92%). More than 50% survival rate of neonates with CDH was observed in a centre with conventional ventilation. Poor outcome is likely to be associated with neonates who manifest within 12 hours of birth.

Understanding of the genetic factors associated with CDH may make it possible to devise preventive strategies or to improve therapeutic interventions. It is important to keep in mind that measures aimed at improving clinical outcome may not require the prevention or correction of the diaphragmatic defect itself. Instead, these strategies may focus on improvement of postnatal lung function, and, ultimately, prenatal modulation (such as tracheal occlusion procedures), since pulmonary hypoplasia and pulmonary hypertension are major contributors to both morbidity and mortality associated with CDH. With this information, it will be important to identify which CDH-related genes and pathways have direct affects
on normal diaphragm and lung development, because they may be particularly good therapeutic targets (Holder et al., 2007).

Genetic counseling for parents with offspring with CDH should be tailored to the situation. The familial form is less common, being estimated at approximately 1%-2% of all forms of CDH (Czeizel and Kovacs, 1985; Lipson and Williams, 1985; Puri, 1989). Familial CDH is so uncommon that it is not even mentioned in Smith's treatise on human malformations (Smith, 2006). Since over 98% of CDHs are sporadic occurrences with no clear teratogenic influence (Iritani, 1984; Janik et al., 1996), it is safe to conclude that this form of CDH is a developmental accident. If the results of the diagnostic studies performed are normal, then families should be counseled that the recurrence risk for apparently isolated CDH among first-degree relatives is approximately 1–2%. This risk of recurrence, extrapolated from available studies on CDH ‘precurrence’, is low but increased at least 20-fold over the CDH occurrence rate in the general population so that subsequent pregnancies should be monitored by fetal imaging (David and Illingworth, 1976; Czeizel and Kovacs, 1985; Pober et al., 2005).

To the best of our knowledge, this is the first study which encompasses prenatal or postnatal samples for CDH with micro-deletion and first case of prenatally diagnosed deletion 15q26.1 (DIH1 gene) from India. Bajaj and group (1991) and Dhir and group (2002) have reported only clinical diagnosed cases of postnatal CDH. At the beginning of this study there were no diagnostic probes available for CDH region but recently Kreatech, Netherlands a commercial company has come up with the diagnostic probe for this region. This is in concurrence with this study about the importance of chromosome 15q26.1 regions in patient with CDH.
5.3 Agenesis of Corpus Callosum (ACC)

ACC is the most common cerebral malformation (Jeret et al., 1987). Classical data indicate that 0.05-0.7% of the general population and 2.3% of children with developmental disabilities have ACC. Some children and adults with ACC are totally asymptomatic (Blum et al., 1990; Pilu et al., 1993; Vergani et al., 1994) and there is no correlation with partial or total agenesis, sex, family history or obstetric complications. A prospective study (8 years follow up) was carried out on 21 children, prenatally diagnosed to have apparently isolated ACC. Neuropsychological evaluation was performed each year and results at the ages of 2, 4, and 6 years were compared. First results show that nearly 80% of children are associated with a normal IQ (Moutard et al., 2003). However with age, IQ tends to deteriorate (in 22% of children at 4 years and 29% at 6 years).

Van Bon and group (2008) described the clinical presentation of 13 new patients with a submicroscopic deletion of 1q43q44, of which nine were interstitial. The clinical presentation of these patients has clear similarities with previously reported cases with a terminal 1q deletion. Corpus callosum abnormalities were present in 10 of our patient cohort. The AKT3 gene has been reported as an important candidate gene causing this abnormality. Although the AKT3 gene was not in their critical region, they sequenced this gene in another 19 patients with ACC without detecting any abnormalities. They also found AKT3 deletion in two sisters with ACC and their unaffected mothers. However, through detailed molecular analysis of the deletion sizes in their patient cohort; they were able to delineate the critical region for corpus callosum abnormalities to a 360 kb genomic segment which
contains four possible candidate genes, but excluding the AKT3 gene. Furthermore, a normal copy number of AKT3 with a more distal 1q44 deletion can still lead to corpus callosum abnormalities.

In the current study of ACC, around 45% were prenatal samples and around 55% were postnatal samples. Among the prenatal samples cord blood sample formed the majority with 70% followed by 30% of amniotic fluid. Consanguinity was seen in around 13.64% of ACC patients. None of the mothers with advanced maternal age had a child with ACC. In fact, majority (91%) mothers who had a child with ACC were in the age group of 21-30 years. In this study, 8/22 (36%) had only corpus callosal abnormality while 11/22 (50%) had other CNS abnormalities and 4/22 (18%) had Non-CNS features in addition to ACC. No deletion was seen in AKT3 gene (1q43-44). To the best of our knowledge; this is the first in prenatal or postnatal study for ACC with microdeletion from India.

Ghi and group (2010) studied nineteen fetuses with callosal underdevelopment, identified at a median gestational age of 22 weeks and confirmed at follow-up. It included fourteen with partial agenesis and five with hypoplasia. Among the 14 fetuses with partial agenesis, there were additional brain findings in 10, including two with absent cavum septi pellucidi, four with mild isolated ventriculomegaly and four with cerebellar abnormalities, two of which also had ventriculomegaly. Pregnancy was terminated electively in seven of the cases with partial agenesis and there was one neonatal death with additional extracranial anomalies as CHD who was diagnosed postnatally with Cri du Chat syndrome (5 p- deletion).

Among the six surviving infants, neurodevelopmental outcome was reported to be appropriate for age in three, while there were neurological abnormalities present in
the other three, including one case with mild motor delay associated with Dandy–Walker complex, one case with seizures due to multiple intracranial lipomas detected after birth and one case with severe mental retardation in whom CHARGE syndrome was recognized postnatally because of associated findings (choanal atresia, micrognathia). Among the five fetuses with prenatally diagnosed callosal hypoplasia, additional anomalies like hemimegalencephaly, ventriculomegaly, TOF, unilateral renal agenesis, esophageal atresia and IUGR were present in four. Two fetuses were terminated electively and three were born alive. Among them, apparently normal neurological development was observed in only one case and other two showed severe neurological impairment. An antenatal diagnosis of callosal underdevelopment is possible by expert USG. There is often association with other major anomalies and even in fetuses with apparently isolated findings, the prognosis is uncertain.

Volpe and group (2006) studied 20 cases of partial ACC (PACC) diagnosed by prenatal sonography and confirmed at pre or postnatal MRI and necropsy examinations. In their series the outcome of isolated PACC was not better than that of complete agenesis of the corpus callosum reported in other series. The relatively poor survival rate is due to the high rate of terminations and associated major anomalies. PACC can be diagnosed reliably and characterized in prenatal life. The sonographic sign present in most cases is colpocephaly. Prenatal MRI can be performed to confirm the diagnosis. It is particularly useful to demonstrate some additional cerebral anomalies. MRI may prove to be a useful second-line imaging modality in the prenatal diagnosis of ACC in fetuses (Manfredi et al., 2010).
Advanced maternal age is an important risk factor for ACC, especially for infants with an identified chromosomal abnormality. This risk was more than five times higher than that of women aged 25–29 years. Premature birth was more common among affected children: infants with callosal agenesis were 3–4 times more likely to be born prior to 37 weeks gestation (Glass et al., 2008). Boland and group (2007) hypothesized that haploinsufficiency of AKT3 gives rise to postnatal microcephaly and ACC and they sequenced the AKT3 gene in 47 patients and no mutations were found. They also sequenced a significant part of ZNF238 in 47 patients and again no abnormalities were found except for a heterozygous synonymous substitution in one patient. Poot and group (2007) reported a patient with vermis hypoplasia, dilatation of the fourth ventricle, enlarged cisterna magna and ACC. They identified a 5 Mb microscopic terminal deletion of 1q44 and no deletion of AKT3 and ZNF238. Caliebe and group (2010) described four patients with overlapping deletions in chromosomal region 1q44, who show developmental delay, in particular of expressive speech, seizures, hypotonia, CNS anomalies, including variable thickness of the abnormal corpus callosum in three of them. Two copies of AKT3 and ZNF238 were retained in two of their patients.

In this study, we did not get any patient in whom AKT3 gene was deleted. This could be due to the small sample size and requires a larger sample size. Other reason could be that it is not consistent with AKT3 as a candidate gene for ACC.