CHAPTER 2
PHENOTYPIC PREDICTORS OF CHROMOSOME 22q11 DELETION

2.1 INTRODUCTION

The DiGeorge syndrome/velocardiofacial syndrome is a complex developmental disorder associated with a wide variety of clinical manifestations. A broad spectrum of clinical features ranging from subtle isolated feature to severe multiorgan defects is found in individuals with 22q11 deletion syndrome [132]. These phenotypic features can be found in various combinations in patients with similar size of deletion, in monozygotic twins and also within members of same family with chromosome 22q11 deletion [77,78]. The individuals with this syndrome often shows congenital heart disease mainly conotruncal malformations and craniofacial defects [12]. Fertility is generally unaffected in patients with 22q11 deletion syndrome. It is difficult to provide genetic counselling and prognostic information to families, since the syndrome is associated with a broad range of clinical features and wide disparity in reported information regarding the dysmorphic facial features. Hence, it is not very easy for clinicians to find who should be offered genetic testing. The best method to confirm the chromosome 22q11 deletion is FISH technique. However, genetic testing on a regular basis for children with congenital heart defect is not feasible, since, it is expensive, time consuming and genetic facilities are unavailable in most places.

Although classical phenotypic features of 22q11 DS are shown in the literature, but due to the striking variability in the clinical expression and absence of genotype phenotype correlation in patients with 22q11 DS [76], the clinical diagnosis of 22q11 deletion is a challenge. Hence, in such situations, the phenotypic predictors of deletion may be useful for patient management and counseling [67]. Nevertheless, this information will be helpful to families when early intervention and preventive treatment is accessible to help the affected once.
2.1.1 CLINICAL FEATURES IN 22q11 DELETION SYNDROME

The 22q11 deletion syndrome is the most common microdeletion syndrome in man. The phenotype features are highly variable with over 180 manifestations described in association with it [65]. The reduced gene dosage on autosome 22q11 is characterized by a large spectrum of congenital defects including DiGeorge syndrome (DGS; OMIM # 188400), Velocardiofacial syndrome (VCFS; OMIM # 192430) and Conotruncal anomaly face syndrome (CAFS; OMIM # 217095).

DiGeorge syndrome is found to be linked with developmental anomaly of the derivatives of the third and the fourth pharyngeal pouches. It is characterized by hypoplasia or aplasia of the thymus gland resulting in immune deficiency, neonatal hypocalcemia resulting in seizures or tetany due to hypoplasia of the parathyroid glands and vulnerable to infections due to a deficiency of T cells. In case of velocardiofacial syndrome cleft palate & lip, cardiac anomaly, speech delay, hypernasal speech, learning disability, bulbous nose and square nasal tip are commonly seen. DiGeorge syndrome, Velocardiofacial syndrome and Conotruncal anomaly face syndrome are found to have a common genetic etiology [47,55,57,58,59].

In 22q11 DS, a broad range of clinical findings either alone or in combination are seen, they are:-

The commonly observed features include congenital heart disease mainly conotruncal malformations, characteristic facial features, palatal abnormalities, hypocalcemia, immune deficiency and learning difficulties. Less common features include psychiatric illness, autoimmune disease, growth hormone deficiency, severe dysphagia and hearing disorder.
2.1.2 CARDIOVASCULAR DEFECTS

Nearly 75% of patients with 22q11 DS are found to have congenital heart disease. Conotruncal malformations (tetralogy of Fallot, interrupted aortic arch, ventricular septal defect, and truncus arteriosus) and right-sided aortic arch are usually seen in them [12,74]. It is the major reason for high mortality (>90% of all deaths). In most of the cases of 22q11 DS, cardiac defect is found in infants, however, patients without such defects are usually unnoticed until mid or late childhood or adolescence. In older patients, overlapping of features is seen and frequency of heart defect is found to be comparatively less. The patients with CHD and microdeletion are reported to have extracardiac features, hence clinical assessment should be done minutely in them.

2.1.3 CRANIOFACIAL FINDINGS

Characteristic craniofacial findings include auricular abnormality, nasal abnormality, hooded eyelids, ocular hypertelorism, cleft lip and palate, asymmetric crying facies and craniosynostosis [356]. Microcephaly is noticed in approximately 40% of patients with hemizygosity of chromosome 22q11 region [357]. Dental abnormalities include enamel hypoplasia, hypodontia, aberrant tooth shape and delayed tooth eruption [358]. Velopharyngeal insufficiency may also predispose to otitis media and conductive hearing loss.

A characteristic pattern of mild facial dysmorphology is observed in patients with this syndrome which includes low-set ears, minor ear lobe anomalies, hypertelorism, short palpebral fissures, hooded eyelids, square nasal tip, thin alae naseae, microstomia and micrognathia [78,359].

2.1.4 ENDOCRINOLOGIC FINDINGS

The endocrine disorders most often associated with DGS/VCFS are short stature, hypocalcemia, thymic related immunodeficiency and less commonly observed autoimmune and thyroid disorders. The developmental defect of derivatives of
various pharyngeal arches and pouches results in absent or small parathyroid glands as found in 22q11 DS, it is normally observed in early life. Hypoparathyroidism can be late onset, transient, latent and recurrent [360]. In patients with this syndrome parathyroid glandular dysfunction is noted in the form of hypocalcemia with hypoparathyroidism to normocalcemia with normal parathyroid hormone levels [360-362]. The hypocalcaemic condition results in convulsions or seizures (fits). It is usually serious in the neonatal period.

Hypothyroidism (underactive thyroid), autoimmune hyperthyroidism, and growth hormone deficiency may be observed in individuals with 22q11 deletion [108,357,363]. In 20% of young adults with this syndrome hypothyroidism is seen.

2.1.5 OTHER FEATURES OF 22q11 DS

Besides the typical features of 22q11 DS, other features found to be linked to this syndrome are abnormal skeletal problems like polydactyly of hands and feet, genitourinary tract anomalies, vascular ring, ophthalmic abnormalities, central nervous system anomalies like neural tube defect, seizures, asymmetric crying facies, gastrointestinal anomalies, renal agenesis and in some carcinomas like renal cell carcinoma and Wilm's tumor.

Cleft palate

The cleft palate is found to be an associated feature of 22q11 DS. Most of the individuals with or without overt cleft palate typically have hypernasal speech complicated by upper airway asymmetry, adenoid hypoplasia, platybasia, muscle hypotonia and other neuroanatomical abnormalities [364]. The prevalence of cleft palate in individuals with 22q11 deletion depends on the careful investigation of palate and the centre where study is carried out. Hence, a wide variation in frequency (1.8%- 98%) of cleft palate is observed in different studies with 22q11 DS [74,357, 365].
Audiologic findings

In approximately 40% to 60% of patients with chromosome 22q11 deletion hearing impairment (conductive, sensorineural, or mixed hearing loss) is noticed [11,366].

Genitourinary findings

Renal anomalies are observed in 10% to 36% of individuals [12,345]. A prospective study using renal ultrasonography in 80 patients with the 22q11 DS who had no prior history of uropathy showed 31% with renal or other genitourinary abnormalities [367]. Utero-vaginal aplasia (Mayer-Rokitansky-Kuster-Hauser syndrome) has been reported in few patients with 22q11 DS [368].

Immunologic defects

Recurrent infections caused by defect with the immune system which occur as a result of aplasia or hypoplasia of thymus are a noted feature. Autoimmune disorders like rheumatoid arthritis & Grave’s disease are found in patients with this syndrome. Patients with 22q11DS exhibited decreased T-cell numbers. A low FoxP3+ natural regulatory T (nTreg) cells were found indicating a relation between nTreg cell population and thymic function. These patients also had a significantly decreased proportion of memory B cells in the B-cell pool [369].

Musculoskeletal findings

The skeletal system defects mainly include scoliosis, abnormal vertebrae and limb abnormalities including talipes equinovarus, polydactyly and syndactyly [12]. Inguinal and umbilical hernias are also frequently noticed in 22q11 deletion syndrome. In a study on 120 cases with 22q11 DS, short stature was present in 40% and thin long fingers was found in approximately 60% of individuals [357].
2.1.6 AIM AND RESEARCH HYPOTHESIS

A broad spectrum of phenotypic manifestations is found to be associated with 22q11 deletion syndrome. The facial features like prominent nasal root, abnormal ears and eyes and small mouth are ‘classical’ features, but none of features is completely characteristic, which results in under recognition of this syndrome. In addition, in most developing and underdeveloped nations, there is limited availability of genetic facilities and even if genetic test is done, it takes several days for conventional cytogenetic and FISH results on metaphase chromosomes. Most of the studies on 22q11 DS are on oriental children and those of European ancestry. Hence in this study, it was tried to identify specific phenotypic features which can be predictor of chromosome 22q11 deletion in Indian children with conotuncal defects.

Specific aim

To find the phenotypic predictors of 22q11 deletion which can help clinicians to provide more appropriate interventions in case of unavailability of genetic facilities?
2.2 METHODS

2.2.1 SUBJECT AND METHODS

In this part of the study, a comprehensive genetic and clinical evaluation was done in 226 out of 254 (89%) children, in remaining 28 cases due to the patient’s condition or logistic reason the detailed clinical examination could not be performed. Missing data and paucity of clinical details in some of the sections may be due to death of child or due to unavailability of phenotypic features in some cases. This study was conducted at a tertiary care hospital of south India. Patients were referred mainly from different parts of Kerala (Southern part of India). The settings for the study were department of Human Cytogenetics, Pediatric Cardiology and Paediatric Genetics of AIMSRC. The children with conotruncal defect and age at diagnosis ranging from 1 day - 2 years (mean 6.5 months) were included in the project. The children with this age were selected, since the phenotypic features are subtle in this age group.

Patients recruited into the study based on cardiac abnormalities were then underwent independent evaluation for extracardiac features by Pediatric Clinical Geneticist. It included detailed physical examination for dysmorphology and other physical defects. The extra cardiac features included dysmorphic facial features, mental retardation, cleft palate, cleft lip and hypocalcaemic status. Informed parental consent was obtained for all patients. Specialized imaging for organ defects were not routinely performed and done wherever needed. Nasal endoscopy was not performed routinely to detect submucous cleft palate. Developmental assessment was done for all patients, but detailed evaluation for intellectual capacity and psycho-behavioral problems could not be performed. Besides these, the clinical history was taken and clinical evaluation of the parents and siblings for phenotypic abnormalities were also considered. Parents/family members were counseled in detail both before and subsequent to the cytogenetic testing.
2.2.2 STATISTICAL ANALYSIS

Univariate and multivariate logistic regression analysis was performed to ascertain extracardiac features (16 physical features and hypocalcemia) helpful in identifying high-risk patients with chromosome 22q11 deletion. The extracardiac features included microcephaly, low set ears, dysplastic flared pinna, short palpebral fissures, hypoplastic ala nasii, bulbous nasal tip, prominent hooked nose, micrognathia, microstomia, high arched palate, thin long fingers, posteriorly placed pinnae, asymmetric ears, hypertelorism/telecanthus, facial weakness/asymmetry and abnormal eye slant.
2.3 RESULTS & DISCUSSION

A significant aspect of the 22q11 deletion syndrome is its variability in phenotypic features, with over 180 features [65]. Some of the characteristic features of 22q11 DS are age-dependent whereas other features like facial abnormalities are more biased [193]. The clinical prediction of 22q11 DS is difficult due to phenotypic variability and subtle features especially in young children. Therefore, in the current study children with 2 years or less than 2 years of age were selected. In this part of study specific phenotypic features which can be predictor of 22q11 deletion from a group of clinical features, associated with 22q11 DS were identified.

2.3.1 PREVALENCE OF EXTRACARDIAC FEATURES IN CHILDREN WITH 22q11 DELETION

Our study shows that, in spite of wide spectrum of extracardiac features, children with conotruncal defects owing to 22q11 microdeletion were more likely to have extracardiac features like dysmorphic facial features, thin long fingers and hypocalcemia. Mental retardation and hernia were observed in two children each and seizure in one patient with 22q11 deletion. The cause of this may be developmental anomalies due to monosomy of genes within 22q11.2 region [164].

Facial features

The subtle dysmorphic facial features that are supposed to be typical of the 22q11 DS were noted during clinical examination. In this study, 226 children were evaluated for extracardiac features, which showed that low set ears, dysplastic flared pinna, short palpebral fissures, bulbous nasal tip, microstomia, high arched palate were mainly noticed in individuals with hemizygosity of chromosome 22q11.2 region (figure 2-1). Another study carried out for a period of 8 years in 334 children from Free State and Northern Cape provinces of South Africa showed long, narrow mid-facial area and small ears as the most marked facial defects in 22q11 DS [192].

Figure 2-1. Low set ears, dysplastic /flared pinna, short palpebral fissures, bulbous nasal tip, microstomia and thin long fingers in patients with deletion.
Whereas, a study on African-American individuals with 22q11 deletion showed absence of typical facial features in 7/33 (21.2%) patients and 3/33 (9.1%) showed three of the four typical characteristics including a bulbous nasal tip, prominent nasal root, hooded eyelids, and auricular abnormalities. This was compared to a similar group of Caucasian patients where none of the 11/204 (5.4%) had typical facial features and 63/204 (30.9%) had three of the four features. Hence the clinical suspicion of 22q11 deletion was found to be lower in African-American population. Hence, it is difficult to make clinical diagnosis of deletion based on facial features, especially in individuals from African-American heritage [356].

The facial features are found to be subtle in neonates or adults, but are most distinct during childhood. Even though it is comparatively easy to diagnose patients with typical facial appearance, some patients only have a few characteristic facial features making the clinical diagnosis difficult. However, it is suggested that facial features like presence of low set ears, dysplastic/flared pinna, short palpebral fissures, bulbous nasal tip, microstomia and high arched palate are significant in clinical diagnosis of 22q11 deletion syndrome.

**Cleft palate**

The 22q11 deletion is significantly associated with the prevalence of cleft palate and a submucosal cleft palate. The cleft palate is found in 1.8% - 98% of individuals with a deletion of chromosome 22q11 region [74,357,365]. Discrepancies between reported series probably reflect ascertainment bias with higher frequencies of cleft palate in those studies originating from craniofacial clinics. The prevalence of cleft palate ranged from 12% to 35% in the 22q11 DS in studies not specifically from cleft palate centres [10-12]. The geographical location was found to be linked with the prevalence of palatal anomalies, with Europe showing less prevalence rate [12], as compared to North America. In the present study cleft palate was found in 5% (2/44) cases with 22q11 deletion. However the frequency of cleft palate in the present study could be higher than reported, since nasal endoscopy could not performed routinely to detect submucous cleft palate.
A cleft lip is rarely found in patients with 22q11 deletion [11,12], which is in accordance with this study, where none of the patient with deletion had cleft lip. In one of the previous study on 100 patients with 22q11 DS, isolated cleft palate was noticed in six children and in six others a cleft lip or cleft lip and palate was found [106].

**Hypocalcemia**

In patients with chromosome 22q11 deletion, underdevelopment of parathyroid gland leads to low parathyroid hormone (PTH). This hormone is important for the maintenance of calcium. It is observed that individuals with deletion may have hypocalcemia which may lead to symptoms like seizures. In 20% to 63% of patients with 22q11 deletion syndrome, hypocalcemia is reported between birth and 3 months of age [74,323,357]. About 10% of them show hypocalcemic-related seizures [61], with increasing age calcium homeostasis typically normalizes, although recurrence of hypocalcemia in later childhood has been noticed during illness and/or puberty. In some cases, children on treatment of infantile hypocalcemia, may be missed with the diagnosis of 22q11 DS until school age.

In the present work, hypocalcemia was noticed in 39% (17/44) children with microdeletion of chromosome 22q11, it was mainly presented in neonatal period (71%; 12/17). The present data was comparable with 35% in 208 Chilean patients [371]. In a previous study on 158 patients, 49% patients had confirmed hypocalcemia [11]. In another series of 340 individuals with 22q11 DS, 60% (203/340) had hypocalcemia [12].

The cause of hypocalcemia in 22q11 DS is abnormal development of third and fourth pharyngeal pouch, resulting in defective organogenesis or absence of the parathyroid glands [372-374]. In an earlier study on 43 patients, FISH test showed deletion in 5 cases, all individuals with microdeletion had hypocalcemia [375]. Patients with hypocalcemia can be treated with calcium supplements and 1,25-cholecalciferol. The present study indicates a need for calcium testing in patients with syndromic conotruncal defects. The knowledge of hypocalcemia in individuals with deletion is important for the management of seizures.
2.3.2 COMPARISON OF EXTRACARDIAC FEATURES IN PATIENTS WITH AND WITHOUT DELETION

In the current study, all children with monosomy of chromosome 22q11 showed one or more extracardiac features of 22q11 DS, which suggests that children with deletion and conotruncal malformations have classic or subtle extracardiac features, hence an accurate clinical assessment of patients with conotruncal defect is needed before declaring that the defect is isolated. Based on history and clinical examination, it was observed that extracardiac features including low set ears, dysplastic flared pinna, bulbous nose, micrognathia and thin long fingers were more common in chromosome 22q11 haploinsufficient children as compared to those without deletion (Fig 2-2). However the combination of these features varied with case.

Figure 2-2. Comparison of extracardiac features in patients with and without deletion
2.3.3 GENOTYPE PHENOTYPE CORRELATION

A significant aspect of this syndrome is individuals with homozygous deletion of chromosome 22q11 are found to manifest heterozygous clinical features. The features associated with the 22q11 deletion are extensive and highly variable from patient to patient. Majority of studies have shown no correlation between deletion size and phenotypic features in 22q11 DS [76,122,147].

In our cohort of 226/254 children, 17 extracardiac features that are generally associated with 22q11 DS were studied (Table 2-1). The clinical prediction of 22q11 DS is difficult due to phenotypic variability and subtle features especially in young children. An incomplete penetrance and therefore marked variability in clinical expression between different patients make clinical diagnosis difficult [46]. Clinical assessment misses 1/4 patients with a 22q11.2 deletion if genetic test is not performed on a routine basis. Hence clinical assessment is not suitable for detecting patients to be tested for 22q11.2 deletion [376]. In this study, it was tried to study minutely the phenotypic features which can be indicator of deletion.

In current cohort of 226/254 children, 17 extracardiac features that are generally associated with 22q11 DS were studied (Table 2-1). For interpreting the results with respect to statistical significance, the maximum value for \( p < 0.05 \) has been fixed in the univariate analysis. However, in the multivariate logistic regression analysis all variables which were found to be statistically significant at \( p = 0.2 \) were included not to miss any variable which was found to be significant at borderline in univariate analysis. This is usual practice in multivariate analysis. While interpreting the results from multivariate analysis type I error (\( p \) value) was taken as 0.05. The twelve characteristics (which included 11 physical features and hypocalcemia) including microcephaly, low set ears, dysplastic/flared pinna, short palpebral fissures, hypoplastic ala nasii, bulbous nose, prominent hooked nose, micrognathia, microstomia, high arched palate thin long fingers and hypocalcemia were found to have statistically significant correlation with presence of deletion by FISH test as compared to those without the deletion (Table 2-1).
Table 2-1 Genotype phenotype correlation by univariate analysis

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Clinical features</th>
<th>Deletion (44)</th>
<th>No deletion (182)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Microcephaly</td>
<td>25 (57%)</td>
<td>51 (28%)</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>Low set ears</td>
<td>35 (80%)</td>
<td>89 (49%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>Dysplastic/flared pinna</td>
<td>35 (80%)</td>
<td>51 (28%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>Short palpebral fissures</td>
<td>21 (48%)</td>
<td>27 (15%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>Hypoplastic ala nasii</td>
<td>13 (30%)</td>
<td>17 (9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>6</td>
<td>Bulbous nose</td>
<td>39 (89%)</td>
<td>68 (37%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7</td>
<td>Micrognathia</td>
<td>35 (80%)</td>
<td>68 (37%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8</td>
<td>Microstomia</td>
<td>18 (41%)</td>
<td>19 (10%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>9</td>
<td>High arched palate</td>
<td>27 (61%)</td>
<td>44 (24%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10</td>
<td>Thin long fingers</td>
<td>33 (75%)</td>
<td>43 (24%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>11</td>
<td>Prominent hooked nose</td>
<td>6 (14%)</td>
<td>7 (4%)</td>
<td>0.032</td>
</tr>
<tr>
<td>12</td>
<td>Hypocalcemia</td>
<td>17 (39%)</td>
<td>10 (6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>13</td>
<td>Posteriorly placed pinna</td>
<td>2 (5%)</td>
<td>16 (9%)</td>
<td>0.904</td>
</tr>
<tr>
<td>14</td>
<td>Asymmetric ear</td>
<td>5 (11%)</td>
<td>21 (11%)</td>
<td>1.00</td>
</tr>
<tr>
<td>15</td>
<td>Hypertelorism/Telecanthus</td>
<td>18 (41%)</td>
<td>70 (39%)</td>
<td>0.899</td>
</tr>
<tr>
<td>16</td>
<td>Eye slant</td>
<td>2 (5%)</td>
<td>16 (9%)</td>
<td>0.533</td>
</tr>
<tr>
<td>17</td>
<td>Facial weakness/asymmetry</td>
<td>3 (7%)</td>
<td>4 (2%)</td>
<td>0.270</td>
</tr>
</tbody>
</table>

Other phenotypic features like posteriorly placed pinnae, asymmetric ears, hypertelorism/telecanthus, facial weakness/asymmetry and abnormal eye slant did not show statistically significant correlation with FISH positivity (Table 2-1).
The phenotypic features found to be statistically significant on univariate analysis were then subjected to multivariate logistic regression analysis, which showed that the presence of eight features i.e. low set ears, dysplastic /flared pinna, short palpebral fissures, bulbous nasal tip, microstomia, high arched palate, thin long fingers and hypocalcemia together were in 93.5% agreement with the presence of deletion by FISH test (Table 2-2), but there was clinical heterogeneity of these features in individuals with deletion.

Table 2-2 Extracardiac features found significant by multivariate analysis

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Clinical features</th>
<th>Deletion (44)</th>
<th>No deletion (182)</th>
<th>p Value</th>
<th>Odd’s Ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bulbous nose</td>
<td>39 (89%)</td>
<td>68 (37%)</td>
<td>0.003</td>
<td>10.3</td>
<td>2.2 – 49.0</td>
</tr>
<tr>
<td>2</td>
<td>Dysplastic/ flared pinna</td>
<td>35 (80%)</td>
<td>51 (28%)</td>
<td>&lt;0.001</td>
<td>20.9</td>
<td>4.4 – 99.7</td>
</tr>
<tr>
<td>3</td>
<td>Low set ears</td>
<td>35 (80%)</td>
<td>89 (49%)</td>
<td>0.001</td>
<td>12.73</td>
<td>3.01 – 53.8</td>
</tr>
<tr>
<td>4</td>
<td>Thin long fingers</td>
<td>33 (75%)</td>
<td>43 (24%)</td>
<td>0.026</td>
<td>4.42</td>
<td>1.2 – 14.8</td>
</tr>
<tr>
<td>5</td>
<td>High arched palate</td>
<td>27 (61%)</td>
<td>44 (24%)</td>
<td>0.027</td>
<td>4.4</td>
<td>1.2 – 16.6</td>
</tr>
<tr>
<td>6</td>
<td>Short palpebral fissures</td>
<td>21 (48%)</td>
<td>27 (15%)</td>
<td>0.002</td>
<td>11.75</td>
<td>2.5 – 55.3</td>
</tr>
<tr>
<td>7</td>
<td>Microstomia</td>
<td>18 (41%)</td>
<td>19 (10%)</td>
<td>&lt;0.001</td>
<td>21.9</td>
<td>4.1 – 119.1</td>
</tr>
<tr>
<td>8</td>
<td>Hypocalcemia</td>
<td>17 (39%)</td>
<td>10 (6%)</td>
<td>&lt;0.001</td>
<td>33.2</td>
<td>6.0 – 183.9</td>
</tr>
</tbody>
</table>

The eight features found significant by multivariate analysis were present together in only one patient with deletion. In 12/226 (5%) cases, four features (low set ears, dysplastic flared pinna, bulbous nose and thin long fingers) were present together, out of them, 92% (11/12) had deletion. This shows variable clinical manifestation in patients with 22q11 deletion.

The population frequency of microdeletion of chromosome 22q11 is~ 1 in 4,000 live births [377]. The haploinsufficiency of some of the genes within the 22q11.2 region may play a role for characteristic psychiatric phenotype and cognitive functioning of schizophrenia [378]. Screening for changes in social
functioning and academic functioning besides typical symptoms, may assist in early diagnosis and treatment of major psychotic illness in 22q11.2DS [379]. In a recent study on 44 patients with copy number variations on 22q11.2, 43 individuals showed 22q11.2 heterozygous deletions, among which 40 had typical 3-Mb deletion, and 3 exhibited proximal 1.5-Mb deletion; no patient was found with atypical deletion on 22q11.2. All the 43 patients with 22q11.2 deletions displayed characteristic face but no difference in phenotypic spectrum was noticed between 3-Mb and 1.5-Mb deletions. The study proposes that the characteristic face can be used as a key indicator for direct diagnosis of 22q11.2 deletions in Chinese patients with VCFS [380].

The knowledge about 22q11 deletion is helpful for timely management of hypocalcemia. It is possible that genetic and/or epigenetic modifying factors may play role in such defects [381]. The malformations in various organs may be due to change in genetic makeup as a result of deletion, translocation, several mutations and polymorphisms which affect the function of protein. Most of the features of this syndrome show variable penetrance and expressivity caused by environmental factors during fetal development. Increased awareness and knowledge among clinicians who encounter these children early in life is essential to reduce the diagnostic delay [106]. The eight extracardiac features identified from a group of clinical features often associated with 22q11 DS may add to the expansion of the phenotypic criteria used in screening of patients at risk for 22q11 deletion.

2.3.4 ADVANTAGES AND LIMITATIONS OF STUDY

Owing to limited genetic centres and expensive FISH technique, chromosome 22q11 deletion testing in all patients with conotruncal defects is not possible in developing and underdeveloped countries. Besides this, it takes several days for conventional cytogenetic and FISH results on metaphase chromosomes, hence phenotypic predictors of deletion can be useful for management of individuals with conotruncal defects. The eight extracardiac features (six dysmorphic facial
features, thin long fingers and hypocalcemia) may be helpful in two conditions, firstly the extracardiac features may be useful to identify an increased risk of 22q11 deletion in patients with conotruncal defect and hence FISH test can be ordered in such cases, secondly, in taking clinical decision pertaining to specific perioperative interventions (irradiated blood transfusions), family counselling and to manage the associated complications of 22q11 deletion syndrome, even if advanced techniques like microarray, MLPA and FISH techniques are not available. The earlier diagnosis of 22q11 deletion helps in more specific and targeted management of the disease, particularly for the heart, brain and nervous system functions.

The ability of the phenotypic markers identified to prospectively predict the presence / absence of 22q11 deletion needs to be validated. The phenotypic markers can help to indicate high risk of 22q11 deletion, but confirmation is possible only with genetic study.