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Genetic Approach to Congenital Malformations

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Introduction

Development of a multicellular organism from a single cell is a very complex, co-ordinated process governed by many genes and their timely expression in various organs at various stages of embryogenesis. It is not surprising that in some embryos some things go wrong during development leading to structural abnormalities of various organs. Major defects in very early stages of embryogenesis may not be compatible with survival and lead to spontaneous abortions. Most of the structural abnormalities/anomalies also known as malformations of internal or external organs occur during the first trimester (dysmorphogenesis), though they may be detected in the second trimester of pregnancy by fetal ultrasonography or after birth during the neonatal period or in infancy. Some malformations involving abnormalities of the size of an organ like microcephaly or obstruction causing hydronephrosis or hydrocephalus may become obvious during the later part of pregnancy or during infancy. Though all the structural defects are congenital, i. e. present since birth, internal anomalies may not be detected till they manifest with some symptoms. Malformations of internal organs can be diagnosed by various imaging techniques like ultrasonography, echocardiography, CT scan or Magnetic Resonance Imaging (MRI) which are used when there are some symptoms indicating the possibility of internal malformations. Individually each malformation is rare but 3% of neonates have one major malformation and around 0.7% of neonates have multiple malformations. This is a brief review about the various birth defects and malformations and a systematic clinical approach to diagnose the same.

Types of Birth Defects

The various types of structural abnormalities of organs are given in Table 1 and Figure 1.

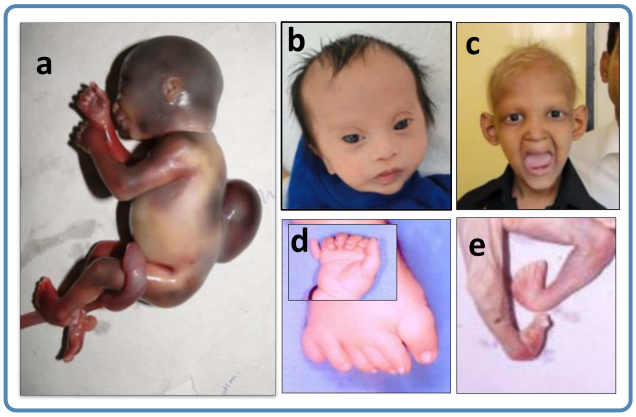

Figure 1 [a] Meningo-myelocele – a major malformation [b] Hypertelorism and upslant of eyes in a child with characteristic facial phenotype of a child with Down syndrome due to trisomy 21[c] Ectodermal dysplasia showing absence of teeth and sparse, light coloured hair [d] Polydactyly – a minor malformation [e] Talipoequinovarus – an example of deformation.

Patterns of Congenital Malformations

A malformation or structural defect of an organ can be an isolated anomaly or may be associated with major or minor anomalies or variants of other systems. Multiple malformations are classified into syndromes, sequence and association. This gives an idea about the etiopathogenesis and is useful in clinical approach to the management.

- **Syndrome:** A syndrome is often used to describe a pattern of malformations occurring together and definitely or presumably caused by a common etiology. The syndrome may be due to a chromosomal etiology (e. g. Down syndrome due to trisomy 21), a microdeletion syndrome (e. g. Williams syndrome due to a submicroscopic deletion

Table 1 Types of Birth Defects.

Type of birth defect	Description	Examples	Comment
Major malformation (single major anomaly in 3% of births, multiple malformations in 0.7% neonates)	Morphological defects that occur due to errors in the normal development and differentiation of an embryo.	Meningocele, posterior urethral valve, ventricular septal defect, cleft lip	If untreated, will lead to significant impairment of the function of the organ leading to mortality or morbidity
Minor malformation (14% and 3% of neonates have a single and multiple minor malformations respectively)		Polydactyly, ear tag, Single palmar crease	Only of cosmetic importance, but of use in diagnosis of etiology
Normal variants	Clinical features which are at the far end of normal distribution	Low set ears, hypertelorism	May be present in normal individuals but in association with other birth defects can be of help in the diagnosis of a syndrome
Deformation	Abnormal shape of a normally formed structure due to distortion caused by mechanical forces	Talipes equinovarus in a child with amyoplasia or congenital myopathy due to oligohydramnios or intrauterine crowding	May have good prognosis with postnatal intervention. Risk of recurrence in the family is usually not increased if the causative factor does not recur
Disruption	Damage or dissolution of a part following normal development	Amputation of digits due to damage by constricting amniotic band or thrombo-embolic phenomenon	Usually do not recur in the family
Dysplasia	Morphological defects caused by abnormal maturation and organization of cells into tissue	Achondroplasia (involvement of bones), Ectodermal dysplasia (hair, teeth, sweat glands and nails are involved)	Involvement of multiple organs in the body where the particular tissue is present. Mostly are single gene disorders.

on chromosome 7q11), or mutation in a gene (eg. Apert syndrome due to mutation in *FGFR2* gene).

- **Sequence:** A single malformation may lead to a chain of events leading to multiple anomalies and such a group of anomalies is known as sequence. An example is congenital absence of kidneys leading to oligohydramnios; which in turn leads to flattening of face, lung hypoplasia and clubfeet, called the Potter sequence.
- **Association:** The co-occurrence of a group of malformations together more frequently than can occur by chance, without an identified common etiology, is labelled as an association. As genomic techniques are identifying etiologies, some of the cases previously described as associations are being regrouped as syndromes. E.g. some cases of CHARGE (Coloboma, Heart defect, Atresia choanae, Retarded growth, Genital and Ear anomalies) association are now found to be due to mutation in the *CHD7* gene.

Clinical Presentation of Malformations

Many malformations such as neural tube defects manifest at birth either because they are obvious on external examination or may present with symptoms due to the resulting functional defect e.g. tracheoesophageal fistula. Some internal anomalies like atrial septal defect, hydronephrosis due to pelviureteric junction obstruction or ectopic kidney may go undetected for many years. Nowadays, many major malformations are getting detected prenatally due to routine antenatal ultrasonographic evaluation. Some fetuses with malformation(s) may get spontaneously aborted or are stillborn. A neonate with a major malformation is an emergency and evaluation for associated major or minor malformations for making an etiological diagnosis is essential in addition to supportive care and appropriate curative surgery.

Importance of Etiological Diagnosis of Congenital Birth Defects

Most of the congenital birth defects are genetic in origin; though in some cases the definite genetic defect is not still identified. Other causes are teratogens. For some malformations, especially isolated malformations like neural tube defects and pyloric stenosis, multifactorial etiologies encompassing multiple genetic variations interacting with environmental factors like folic acid intake are hypothesized. One type of congenital malformation may be caused by different etiologies and the etiological diagnosis can be made by evaluating for associated features and genetic tests. Table 2 shows one example where the enlargement of lateral ventricles in brain can be due to various causes and risk of recurrence depends on the etiology. Figure 2 shows brain imaging showing

Table 2 Etiologies associated with ventriculomegaly.

Disorder	Etiology	Risk of recurrence in sibling	Comment
Aqueductal stenosis	Monogenic - *CCDC88C* gene (Autosomal recessive)	25%	Clinically may not be differentiable from X- linked
Aqueductal stenosis	Monogenic- *L1CAM* gene (X - linked)	50% of male offspring, if mother is a carrier	Adducted thumb, if associated, is confirmatory of X- linked variety
Retinoic acid intake by mother	Teratogenic	Nil, if drug intake is avoided	History of intake by mother during pregnancy
Cytomegalovirus (CMV) infection in fetus	Transplacental transmission of virus from infected mother (History of symptoms of infection in mother may or may not be present)	Nil as recurrence or reactivation of infection in mother does not cause symptomatic infection in the fetus	Brain imaging may suggest the diagnosis due to periventricular calcification. Molecular testing for CMV virus is confirmatory
Aase-Smith syndrome	Autosomal dominant	Nil if parents are normal	Cleft palate, joint contractures, Dandy Walker malformation
Lissencephaly	Many different causes	Depends on etiology	MRI brain confirms lissencephaly
Hydrolethalus syndrome	Monogenic - *HYLS1* or *MPDZ* gene (Autosomal recessive)	25%	Polydactyly, cleft lip, cardiac anomalies are seen
Holoprosencephaly	Many different causes including chromosomal, single gene, syndromic etiologies	Depends on etiology	Carrier parent of a single gene mutation may have a single central incisor as a very mild feature (forme fruste) of holoprosencephaly

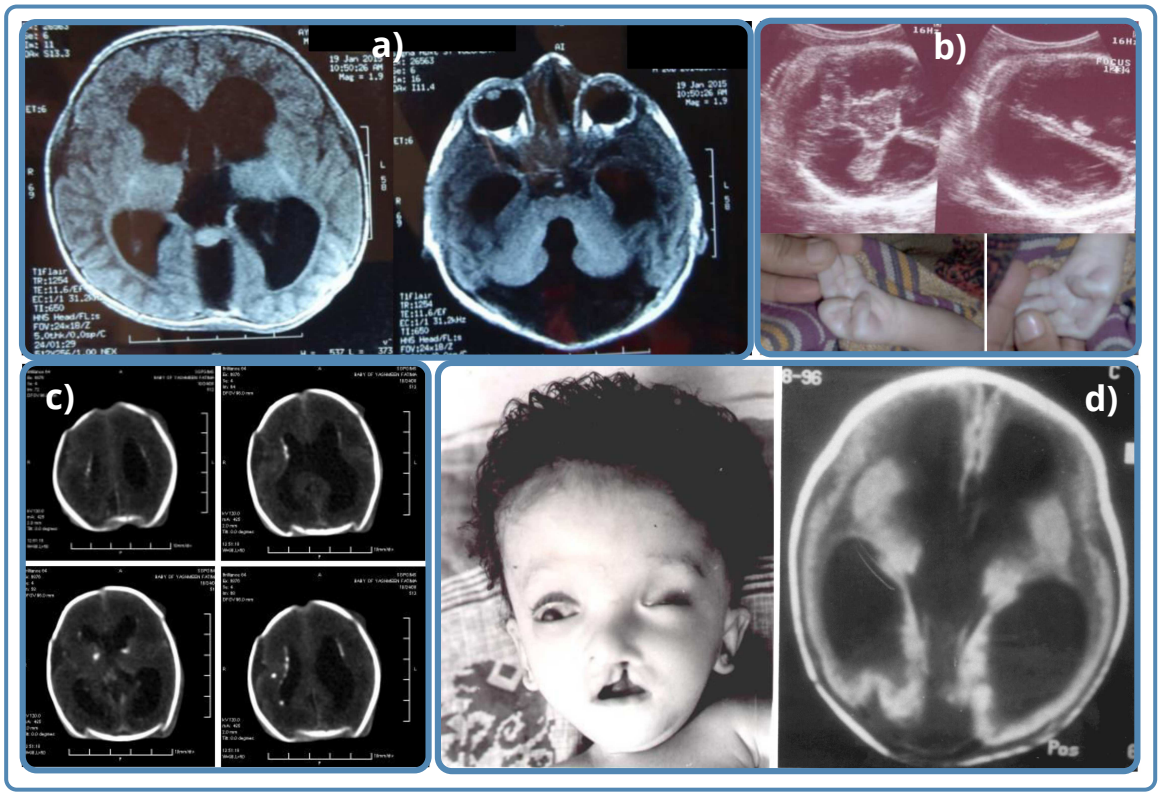

Figure 2 Neuroimaging showing enlargement of brain ventricles due to different etiologies [a] Dandy Walker malformation [b] X linked aqueductal stenosis associated with hypoplastic palmar flexed thumbs [c] Pseudo-TORCH syndrome showing periventricular calcification in a fetus from a consanguineous family with a previous child diagnosed with ventriculomegaly [d] Walker-Warburg syndrome with lissencephaly, microphthalmia and cleft lip.

dilatation of ventricles of the brain due to different etiologies.

As seen in Table 2, risk of recurrence of the malformation and the prognosis varies depending on the etiology. Etiological diagnosis also helps in early detection of treatable complications by surveillance and in assessing the risk of recurrence in subsequent offspring in the family.

Clinical Approach to a Patient with Congenital Malformation(s)

A patient, usually a child or a neonate is brought for evaluation due to presence of a major malformation or due to presence of multiple anomalies (often it may be just different looking facial features or facial dysmorphism) which may or may not be associated with developmental delay/ intellectual disability or other neuro-developmental disorders like autism. The clinician should evaluate for associated major malformations, functional disabilities like developmental delay or deafness and most importantly attempt to identify the etiology. The process of diagnosis may be long drawn in many cases. The clinician needs to take into account the understanding and expectations of the parents, their psychosocial and educational background and talk to the family with empathy, so that the family is convinced about the desire of the clinician to help the patient. Good communication with the patient and family helps to give them confidence and get their co-operation and commitment which are needed for the evaluation, which often involves a series of tests, some of which may not give any diagnostic results. Children with dysmorphic features and their parents may be sensitive to the 'different' looks and affected individuals often have a poor body image. The dysmorphic features are of diagnostic importance and need to be noted by careful examination of the affected child and sometimes parents also. However, during the clinical examination, utmost care should be taken not to hurt the family by using unfriendly/ insensitive descriptive terms. Photographic documentation is important but should be done only after written consent of the patient or the parents.

Clinical Evaluation

The clinical approach to a child with malformation or dysmorphism includes detailed clinical history and thorough head-to-toe and systemic examination. The prenatal history and family history play very important roles (Table 3).

Examination of a dysmorphic child

The initial part of evaluation is anthropometric measurement of the height / length, weight and head circumference and comparison with age/sex matched controls. The head-to-toe examination should be carried out by careful evaluation after removing clothes. Facial dysmorphism, hair pattern, nails, neck, fingers and toes, body hair, and pigmentary abnormalities need to be noted. Some features may be present in either of the parents and suggest that the feature is a familial character and may not be related to the etiology of developmental delay / intellectual disability or a syndrome. The American Journal of Medical Genetics had published a special issue on the definitions and descriptions of various human phenotypic variations and this issue is freely available online http://onlinelibrary.wiley.com/doi/10.1002/ajmg.a.v149a:1/issuetoc. A standardized vocabulary for phenotypic variations has been developed and is known as the Human Phenotype Ontology (HPO). At present there are more than 11000 terms. The uniformity of nomenclature helps in clinical diagnostics based on clinical features (Phenomyzer - http://compbio.charite.de/phenomizer/) and also research on relationships between clinical phenotyping and molecular, cellular and biochemical abnormalities and pathways.

Search for syndromic diagnosis

Once the clinical assessment of phenotypic variations is done, the next step is to search for the etiological diagnosis or syndrome. There are two processes for the diagnosis of a syndrome. There are some syndromes with characteristic phenotypes and especially the common syndromes can be diagnosed based on the typical dysmorphic features (gestalt). The key features, which are diagnostic clues for such syndromes, are known as 'Pearls of dysmorphology'. Figure 3 shows typical cases of some common syndromes with malformations.

Use of Databases for Syndrome Search

More than 5000 multiple malformation syndromes are described in literature and many new ones are getting delineated. It is impossible to remember all the features of each syndrome and to clinically diagnose the rarer conditions, even for a dysmorphology expert. For this purpose, there are databases of multiple malformation syndromes (Table 4) and these can help experts and newcom-

Table 3 Points to be noted in the history in the clinical evaluation of a patient with congenital malformation(s).

Prenatal history	Drugs, infection, exposure to radiation
	Maternal illnesses like diabetes mellitus, epilepsy
	Prenatal ultrasonogram records
	Antenatal complications like polyhydramnios/ oligohydramnios
Family history	A three-generation pedigree to note relatives with similar malformations /clinical problems
	Age of mother
	Consanguinity
	Recurrent abortions, stillbirths
Present / past history	Growth and development
	Symptoms related to malformations of other systems e. g. convulsions, dyspnea, visual or hearing problems
	Symptoms to suggest possibility of dysplasia or inborn errors of metabolism like progressive course, changing pattern of dysmorphism etc.

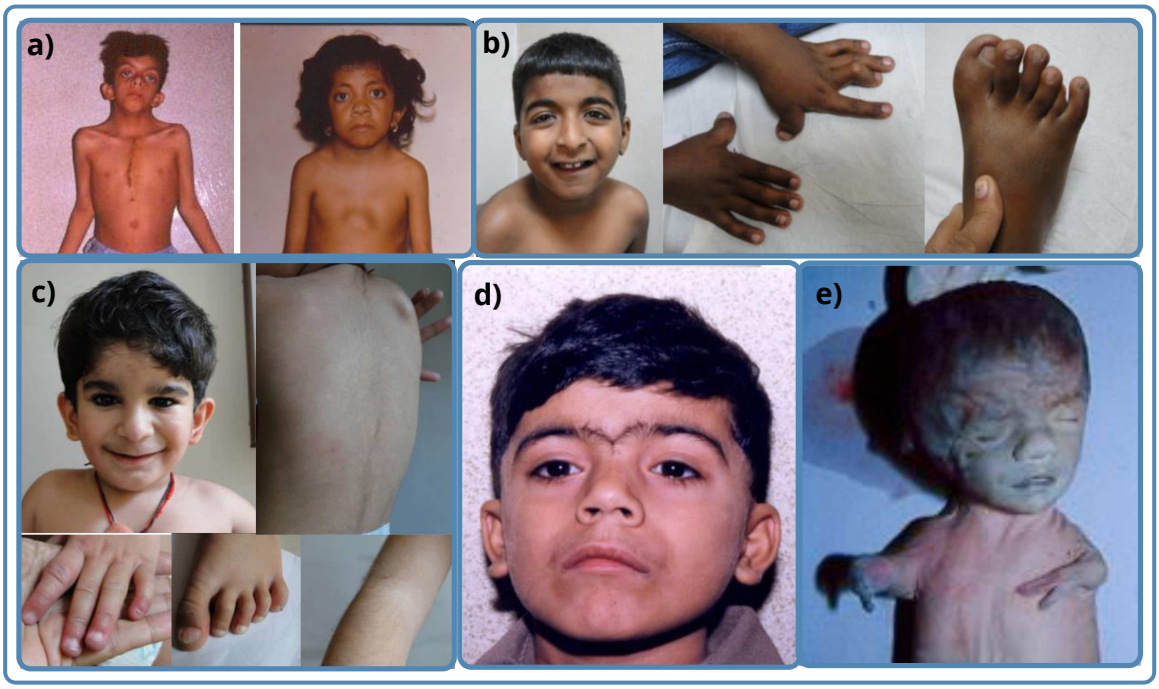

Figure 3 [a] Noonan syndrome – note upturned nose, ptosis, webbing of neck, pectus excavatum in lower part along with scar following operation for cardiac anomaly; [b] Rubinstein-Taybi syndrome with characteristic overhanging columella, grimacing smile and broad thumbs; [c] Coffin- Siris syndrome – hypoplastic terminal phalanx and nail of fifth finger and increased body hair; [d] Cornelia de Lange syndrome – note arched eyebrows meeting in the midline with an upturned nose; [e] A fetus with Cornelia de Lange syndrome with severe upper limb involvement in the form of ectrodactyly. Note the similarity of facial features with the face of the child in Figure 3[d].

ers in dysmorphology to get differential diagnoses for each case. The phenotypic abnormalities which are used as search handles in any database have to be specific and uncommon. Eg. midline cleft lip, preaxial polydactyly, microphthalmia are good search handles while microcephaly, cleft lip, intellectual disability, short stature, hypertelorism are poor handles as they will give a long list of differential diagnoses.

As medical genetics is a very rapidly advancing specialty, literature search for latest developments should be a part of syndrome diagnosis and genetic counseling.

Role of Investigations in Dysmorphology Diagnosis

Investigations have two important roles in the evaluation of a child with dysmorphism. The first is to look for internal anomalies or associated treatable entities and surveillance for complications. Table 5 gives some representative examples. In some cases the presence of internal malformations also provides support to the clinical diagnosis as is the case in Marfan syndrome.

The other important role of investigations in evaluation of a case with dysmorphology is identification or confirmation of etiology.

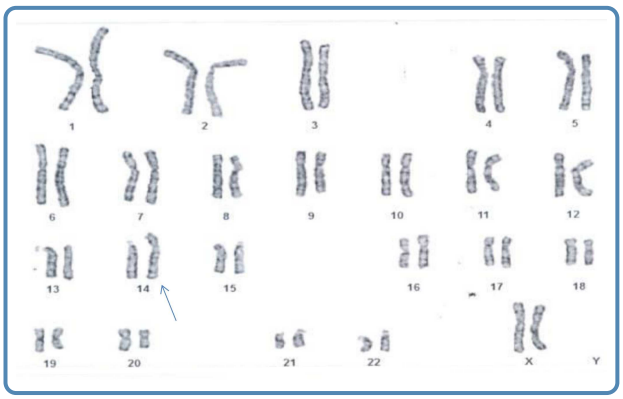

Figure 4 Traditional karyotype showing trisomy 21 due to translocation of an extra copy of chromosome 21 to chromosome 14.

Table 4 List of databases that help in syndrome diagnosis.

Database	Source	Features
Baraitser-Winter Dysmorphology Database (LDDB) Winter-Baraitser Neurogenetics Database (LNDB)	London Medical Databases (LMD) http://www.lmdatabases.com/	- Gives pictures; user friendly; needs to be purchased - Online version available - FACE2GENE LMD application
Pictures of Standard Syndromes and Undiagnosed Malformations (POSSUM)	http://www.possum.net.au/	Gives pictures; user friendly; needs to be purchased
Online Mendelian Inheritance in Man (OMIM)	http://omim.org/ Provides detailed information about phenotypes as well as mutations	A catalogue of all genes and genetic disorders; updated, comprehensive information with clinical synopsis and external useful links
Phenomyzer	http://compbio.charite.de/phenomizer/	Uses Human Phenotype Ontology terms

Table 5 Representative examples of investigations suggested for some malformations or syndromes.

Malformation / Syndrome	Associated malformation / problem	Investigations
Turner syndrome	Cardiac anomalies, deafness, hypothyroidism, horseshoe kidney	Echocardiogram, ultrasonography, thyroid hormone profile, audiometry
Congenital absence of depressor anguli oris	Cardiac anomalies	Echocardiogram
Ear tag	Deafness	Evaluation of hearing
Marfan syndrome	Aortic root dilatation, dislocation of lens	Echocardiogram, Ophthalmologic evaluation
Bardet-Biedl syndrome	Retinitis pigmentosa, deafness, renal problems	Hearing and ophthalmologic evaluation, renal function tests
Vertebral segmentation and rib cage abnormalities	Restrictive lung disease	Pulmonary function tests

Genetic Tests

Genetic disorders are classified into chromosomal disorders, single gene (also known as Mendelian as they follow Mendel's laws of inheritance) disorders and multifactorial. There is no confirmatory laboratory investigation for multifactorial disorders but chromosomal abnormalities may need to be ruled out in some malformations before labeling the malformation as multifactorial. Chromosomes have been studied by traditional karyotyping for more than 5 decades (Figure 4). However, now techniques using DNA principle of complementary regions (A to T and G to C) annealing each other are used to study small regions on chromosomes which are beyond the resolution of traditional karyotyping. These molecular cytogenetic techniques are Fluorescence In Situ Hybridization (FISH), Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR), Multiplex Ligation Probe Amplification (MLPA) and Cytogenetic MicroArray (CMA). Table 6 gives principles and appropriate indications of these tests (Figure 5).

The other group of tests is for identifying mutations in genes causing monogenic disorders. Most of the mutations are variations in the DNA sequence and are identified by Sanger sequencing using automated sequencers (Figure 6). Before ordering the test the clinician should have a working diagnosis with a known causative gene. It needs to be noted that the identification of a causative gene confirms the diagnosis; absence of mutation in a

suspected gene does not rule out the diagnosis. The causes in such situation can be different like, inability of the technique to detect the type of mutation, mutation in other parts of gene like introns, promoter, etc. which are not usually tested, or the causative gene may be other than the one tested. It has been seen in a number of disorders that different genes may cause a clinically similar phenotype and some of the genes still may not have been identified. The updated information about all known genes and phenotypes is available on Online Mendelian Inheritance in Man (OMIM), a free database.

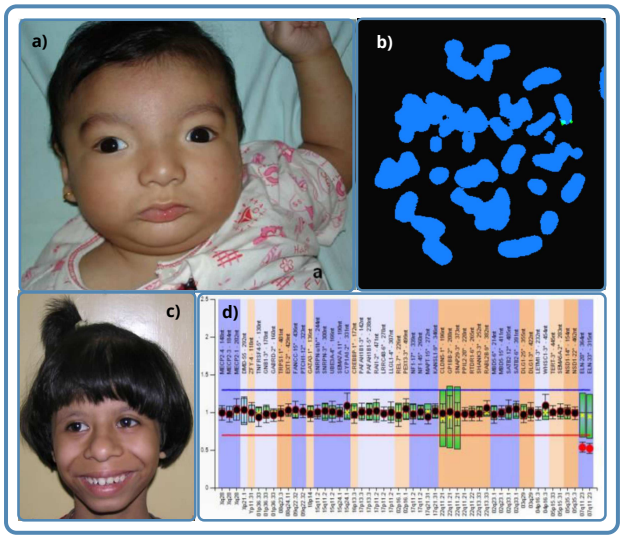

Figure 5 [a] Chromosome 5p microdeletion syndrome –also known as Cri-du-chat syndrome [b] FISH with probes for terminal region of chromosome 5 showing only one copy confirming the deletion on the other copy of 5p [c] Williams syndrome showing mild dysmorphism but with characteristic face – a case for gestalt diagnosis [d] MLPA showing decreased ratio (0.5) of probes on chromosome 7q11 region (patient's DNA sample to the DNA of a normal individual), while other probes show a normal ratio around 1.

For most of the Mendelian disorders, hundreds of mutations are identified and to identify the mutation, sequencing of all coding regions (exons) needs to be done. As many genes have 20 or more exons, the process is tedious and costly. The use of Sanger sequencing for diagnosis in clinical settings gets complicated by involvement of multiple genes causing the clinically indistinguishable phenotypes. This problem has been solved by the latest high throughput sequencing technique known as Next Generation Sequencing (NGS). NGS is routinely used in clinical settings to sequence multiple genes in one go (eg., genes in RAS- MAPK pathway which cause clinically overlapping phenotypes like Noonan syndrome, Cardio-facio-cutaneous syndrome, Costello syndrome, etc). It is also useful for sequencing large genes like the *FBN1* gene which causes Marfan syndrome. NGS based sequencing of coding regions (exons) of all genes known as Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS) is used in cases where identification of the candidate gene based on phenotype is not possible as the phenotype is novel or subtle. This helps in identification of the causative mutation in 30 to 50% of cases with developmental disorders and causative genes for many novel phenotypes are getting identified. As is the case in CMA, WES identifies thousands of variations in each individual and most of them are polymorphic and non-pathogenic. Dissecting the results of WES to identify the causative pathogenic mutation is a challenging task which needs high level of computational expertise, bioinformatic tools along with clinical correlation and functional studies of the variation. NGS based mutation detection in cases with malformation with or without developmental disabilities helps to identify new mutations and sometimes new genes, and also widens the phenotypic spectrum by identifying a mutation in a known gene which was not suspected on the basis of the clinical phenotype.

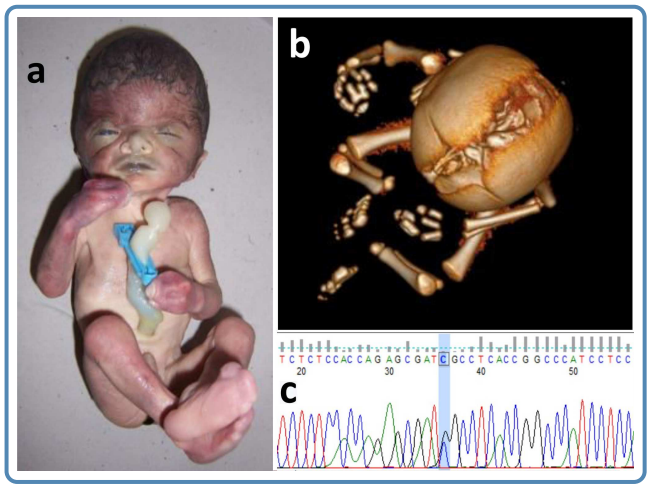

Figure 6 [a] A fetus with Apert syndrome showing tower skull and syndactyly [b] CT scan of the head showing closed coronal sutures and wide open sagittal suture [c] Sequencing results of a part of *FGFR2* gene showing the p.Ser252Trp mutation.

Table 6 Genetic investigations to study chromosomes and chromosomal disorders.

Technique / Investigation	Advantages	Limitations	Indication
Karyotype	Can look at all chromosomes in one go and the clinical suspicion of a specific diagnosis is not necessary	Level of resolution is low and sub-microscopic abnormalities cannot be detected. Needs living cells. Cell culture is needed for 3 to 10 days.	Clinical diagnosis of a chromosomal disorder Down syndrome, Turner syndrome, Trisomy 13 or 18; a fetus with hydrops fetalis or any major malformation; liveborn or a stillborn with multiple malformations; intellectual disability; short stature in a prepubertal girl; disorder of sex development
FISH	Can detect sub-microscopic deletions on chromosomes. No need of cell culture or live cells. Short reporting time of 1 or 2 days.	Clinical suspicion of a specific disorder(s) is needed, so that the probes for the appropriate region can be used. Can usually test 1 to 3 regions in one test.	Rapid prenatal testing for trisomy 21, 13 or 18; Microdeletion syndromes like Williams syndrome, Velo-cardio-facial syndrome etc.
QF PCR	Does not need cell culture. Reporting time one or 2 days	Can test up to 5 chromosomes in one go.	Rapid prenatal diagnosis for common aneuploidies on fetal sample (amniotic fluid).
MLPA (Probe sets are available for known microdeletion / duplication syndromes or for ends of all chromosomes)	Does not need live cells or cell culture. Rapid like FISH but can test 40 target regions in one test.	Can test for regions known for syndromic etiology and incorporated in the probe set used for MLPA.	Useful to test for many well delineated microdeletion syndromes in a child with intellectual disability.
CMA (Probes spanning the whole genome)	Does not need live cells or cell culture. Covers the entire genome. Has a high resolution.	Variants of uncertain significance identified	First tier of test for evaluation of a child with intellectual disability / dysmorphism if there is no obvious cause on clinical evaluation.

Note: FISH: Fluorescence In Situ Hybridization; QF-PCR: Quantitative Fluorescence Polymerase Chain Reaction; MLPA: Multiplex Ligation Probe Amplification; CMA: Cytogenetic MicroArray

Genetic Counseling and Prenatal Diagnosis

Congenital malformations are heterogeneous and as a group, a common entity. Counseling regarding prognosis, treatment options, and outcome with surgical and non-surgical interventions is important in prenatally as well as postnatally detected malformations. Genetic counseling about the risk of recurrence and prevention by prenatal diagnosis during the next pregnancy is an important part of management of a family with a child or a pregnancy with birth defect. Accurate genetic counseling needs correct etiological diagnosis of the proband (child or fetus with birth defect). Careful ultrasonographic evaluation of fetus at 18 to 20 weeks can prenatally detect most of the major malformations. Fetal autopsy has a major role in the evaluation of stillbirths and fetuses terminated after prenatal diagnosis of a malformation.

Conclusion

Evaluation of an individual with dysmorphism needs expertise and knowledge about syndromes, basic genetics and principles of genetic investigations and their interpretation. Etiological diagnosis helps in appropriate management of the affected individual as well as in accurate genetic counseling of the family.