Chapter

Introductory Chapter: Epidemiology, Evaluation and Risk Assessment of Congenital Anomalies

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1. Introduction

Congenital anomalies (CA) are the leading cause of infant mortality in the USA (**Figure 1**) [1]. The five most common birth malformations recorded in the USA are club foot (1 in 593 births), Down syndrome (1 in 707 births), pulmonic stenosis/atresia (1 in 1052 births), cleft palate (1 in 1687 births), and limb defects (1 in1943 birth). Congenital heart disorders, neural tube defects, and Down syndrome are the three commonest causes of mortality due to CA in that order

One in every 33 children born in the USA suffers from a birth anomaly, and 1 in 5 deaths among infants is due to morbidities related to them [2]. Globally about 7.9 million children are born with a major birth malformation every year, and CA is the fourth leading cause of neonatal death worldwide [3]. The annual cost of hospitalization due to congenital malformations is reported to be $22.9 billion in the USA [4]. The cost of care of infants suffering from CA is relatively higher than any

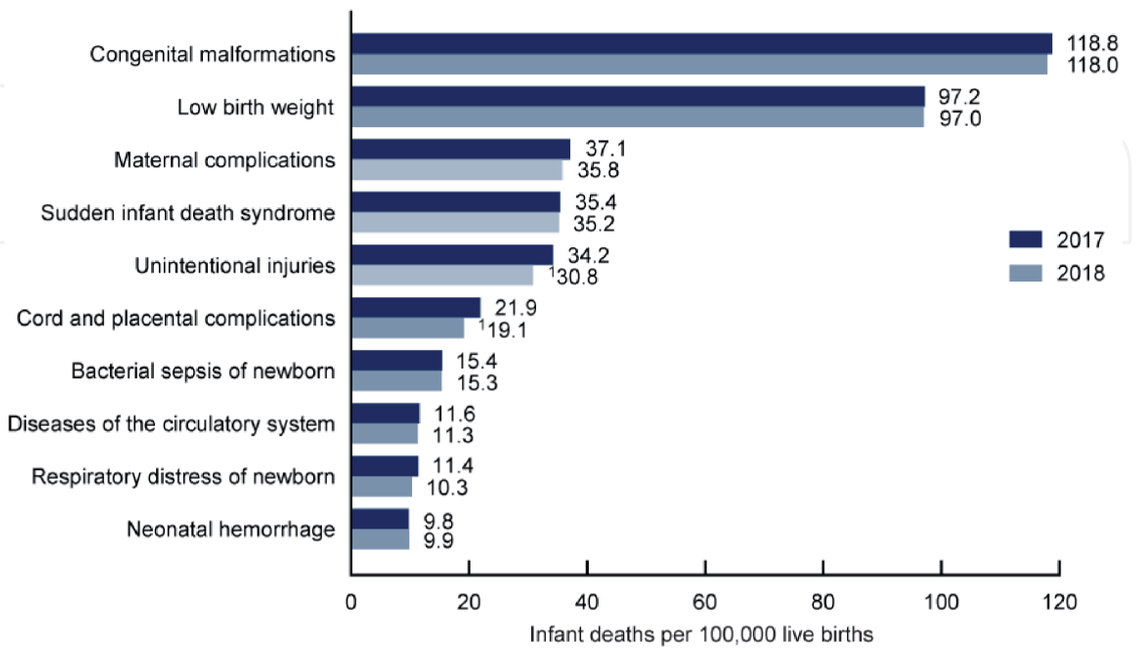

Figure 1.
Infant mortality per 100,000 live births in the United States.

other childhood morbidity. While 3.0% of all hospitalizations are attributed to birth anomalies, the diagnosis accounts for 5.2% of total hospital costs. The cost of care is highest in the children who are <1 year of age and accounts for 35.0% of the total hospitalization costs among the pediatric population. The congenital cardiac defects register the highest CA-associated hospitalization costs across all age groups at 26.6%, while only 14.0% of CA–associated hospitalizations are attributed to this system.

2. Abnormal morphogenesis

Normal morphogenesis is a systematic process that involves a combination of several simultaneously or sequentially occurring histo-physiological activities at the cellular and molecular levels. These processes are cell migration, aggregation of identical cell types, the interaction between neighboring tissues, controlled cell death, mechanical forces, and hormonal effects. The congenital anomaly is an outcome of abnormal morphogenesis and is defined as any structural or functional anomaly detected at birth that interferes with the performance or appearance of the subject. The etiopathogenesis of congenital anomalies is broadly divided into genetic and non-genetic groups, and the latter one is classified into several major sub-categories. Some anomalies remain idiopathic in etiopathogenesis, and others may be multifactorial, i.e., have both genetic and environmental influences. Congenital anomalies are categorized as major and minor as per their clinical implications. While major anomalies have significant adverse medical consequences, minor malformations may be of cosmetic significance only. The prevalence of major malformations is reported to range from 2 to 4 percent according to the population studied, while minor anomalies may be as high as 35% in certain populations [5]. The presence of 3 or more minor malformations is associated with an increased risk of a major anomaly or syndrome. Major anomalies are generally the consequence of molecular defects that interfere with normal morphogenesis processes like apoptosis, intracellular signaling, migration of neural crest derivatives, and chromatin remodeling. Some of the genes (e,g. Homeobox genes in Synpolydactyly, microphthalmia, and holoprosencephaly), transcription factors (e.g., deletions of T-box 1 in Conotruncal heart defects of DiGeorge syndrome), fibroblast growth factor receptors (in Craniosynostoses syndromes), and enzyme defects (such as cholesterol biosynthesis leading to Smith-Lemli-Opitz syndrome) have been identified as etiopathogenic factors for specific major malformations.

2.1 Patterns of abnormal phenotypes

Abnormal morphogenesis may manifest in various forms at the macroscopic and microscopic levels [6]. Following are some of the common patterns and terminologies used in the description of an abnormal phenotype.

- Anomaly is defined as a significant morphological or anatomic variation in the phenotype from the standard reference population. The difference may be macroscopic or microscopic. Anomalies are categorized into major and minor. Major anomalies have significant adverse implications in functional, anatomical, psychological, social, or cosmetic wellbeing, and minor anomalies are variants with no significant medical or major cosmetic consequences. The presence of three or more minor anomalies suggests significant defects in morphogenesis. Even though detected in only 0.5% of births, almost 90% of such cases have ≥1 major anomaly and might eventually end up with the diagnosis of some associations or syndrome.

- Malformation is an inherently non-progressive morphologic anomaly brought about primarily by an intrinsic error in the developmental process at the cellular or molecular levels of an organ or a body part. Malformations may be isolated, or part of a syndrome and are causally heterogeneous. The aberration can happen due to gene mutations, exposure to teratogens, or a combination of the two. For example, the limb anomaly in Holt–Oram syndrome is genetic in origin, whereas in thalidomide embryopathy, it is due to teratogenic exposure.

- Deformation alludes to a distortion of the shape or size in an otherwise normal body part of a normally developing or developed fetus, caused by some aberrant extrinsic or intrinsic mechanical forces. Examples include craniofacial asymmetry, arthrogryposis, and metatarsus adductus (**Figure 2**). Uterine anomalies and abnormal fetal positions are important setups for the generation of fetal deformation. Deformations are causally heterogeneous and may result in the loss of alignment or abnormal positioning and distorted configuration. Deformations are generally reversible after birth but may be difficult if the transduction of abnormal mechanical forces is prolonged. Deformation generally happens after organogenesis, but if in early gestation, it may permanently alter the structural relationships. If mechanical transduction happens during embryogenesis, anomalies in the neural tube, tendons, and joints may be produced.

- Disruption is defined as a static morphologic abnormality brought about in-utero by some destructive mechanical forces acting upon an otherwise normally developing or developed fetal tissue or physical part. It results in the destruction of the involved body part and may cause the developmental arrest of the adjacent tissues, thereby leading to a secondary malformation (**Figure 3**). It can be an initial event in a sequence of events if it occurs early in gestation. It is causally heterogeneous and may be isolated or part of a syndrome or other broader patterns. A disruption can impart a particularly distinctive appearance because of the loss of tissue and aberrant differentiation of adjacent tissues with or without the production of adhesions. The process results in cell death and tissue destruction, and the mechanisms may include vascular compromise, anoxia, teratogens exposure, infections, or mechanical forces. Clinical entities such as missing digits or limbs are examples of disruption. The process

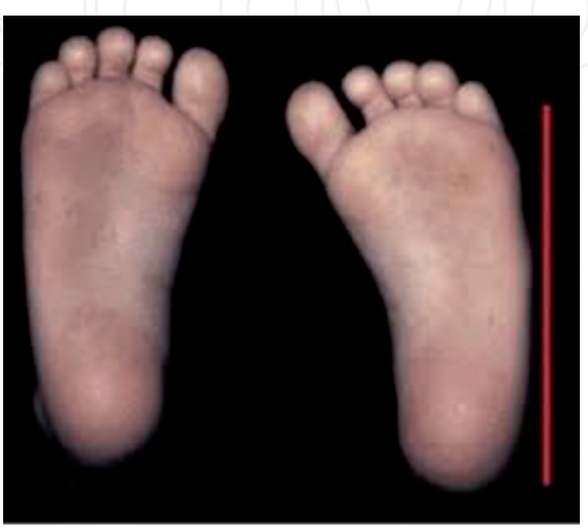

Figure 2.
Metatarsus adductus as an example of deformation due to intrauterine positioning or space constraints.

Figure 3.
Amputation of digits with a constricting amniotic band as an example of disruption.

characteristically affects several tissue types in a specific anatomical region, and the phenotypic abnormalities may cross cell lines of embryonic development. Some pathological developmental processes can cause both disruption and deformation. For example, constriction rings at the tip of a finger associated with bands (fibrous strands of tissue) are often used as an example of disruption, but fibrous bands can also cause deformation. "Amniotic bands" encircling a limb are one possible mechanism of disruption of an extremity.

- Dysplasia is an abnormality in the growth and development of cell or tissue histology, or the anatomical structure or physiological function resulting from such growth. Tumors and malignancies are the results of a dysplasia process. Dysplasia can be isolated in occurrence or be a part of broader patterns. Dysplasias are causally heterogeneous and can be triggered by genetic factors, teratogens exposure, or metabolic disorders. The presentation may involve one or multiple germ layers and single or multiple organs. It may be localized or generalized; unilateral or bilateral; focal or multifocal; benign, malignant or premalignant; static or progressive; or evanescent. Dysplasia may be associated with malformations. It can happen at the cellular level (microscopic), examples being BPD and fibrous epithelial dysplasia, or in organs (macroscopic) such as renal dysplasia. It may be a dynamic or an ongoing process and maybe widespread or confined to a single organ. It can occur both prenatally and postnatally.

- Sequence refers to the phenomenon of a single or multiple morphological anomalies cascading from a single primary anomaly, which might be malformation, disruption, dysplasia, or deformation in character. The resultant anomaly is not an essential and direct derivation of the primary cause, as happens in genetic aberration cases. A sequence can occur as an isolated phenomenon or as a component manifestation of broader patterns such as syndromes; and like malformations is causally heterogeneous. A common example is the Pierre Robin sequence, in which a small mandible, the primary anatomical defect, leads to protruding tongue, which in turn may interfere with the palatal closure and consequently create a cleft palate. In describing a sequence, sometimes it may be difficult to distinguish between the primary and the consequential effects.

- Association is defined as a pattern of morphological anomalies which are not causally related but occur together more often than would be expected by

chance only. Some associations may be syndromes with overlapping features. Such cases may eventually be identified to have a pathological etiology and then moved to the category of a syndrome. In the case of CHARGE association, after the causative gene was identified, the nomenclature was changed to CHARGE syndrome.

- A syndrome is a combination of causally but not necessarily pathogenically related anomalies that are characterized into a specific condition. The anomalies can be malformations, deformations, disruptions, sequences or dysplasia, major or minor, or functional, such as those affecting the neurological, cognitive, sensory, or behavioral performances.

2.2 Anomalies of genotype

Genetically determined and inheritable diseases can occur as a result of chromosomal or single-gene (Mendelian) abnormalities. Some of the inherited disorders are multifactorial in origin, and others may be attributed to defects in the mitochondrial chromosomes [7]. The last category has the highest phenotypic variability due to heteroplasmy and the fact that mitochondrial DNA has high incidence of mutation [8]. As the mitochondrial DNA is inherited from the mother, the genetic transmission occurs from the affected mother only. Single gene disorders (SGD) may present with autosomal dominant, autosomal recessive, X-linked recessive, and X-linked dominant patterns of inheritance. Cystic fibrosis is the commonest SGD reported in the Caucasian population.

Chromosomal anomalies may result from maldistribution (numerical aberrations) or rearrangements (structural abnormalities) of chromosomes. A numerical error in the array of chromosomes is termed aneuploidy, which presents as polyploidy with an addition or monosomy with a reduction in the number of chromosomes (**Figure 4**). Most of these conditions result from the failure of chromosomes to disjoin during meiosis (non-disjunction). Some of the common examples of aneuploidy are the trisomy syndromes or Turner and Klinefelter syndromes.

Structural chromosome disorders result from the breakage and rearrangement of segments or parts of a chromosome (**Figure 5**).

- Deletion refers to the loss of a piece or section of chromosomal material. If too small to be visualized under a microscope, it is termed microdeletion. Deletions can be terminal if only one break is present at the end or interstitial if two pieces of chromosome material are lost from within the chromosome. There is only one copy of a particular chromosome segment instead of the usual two copies in deletion syndromes.

- Duplication presents with an extra copy of a segment of a chromosome. So there are three copies of a particular chromosome segment instead of the usual two.

- Mutation indicates a change in the DNA sequence that leads to a change in its function. A mutation may be silent (no overt clinical signs or symptoms as amino acids may be encoded on different codons); missense (the codon is changed by a new nucleotide); nonsense (new nucleotide changes the codon to a STOP codon so that the mRNA translation is stopped) or splice-site when mutation at splice site prevents removal of an intron.

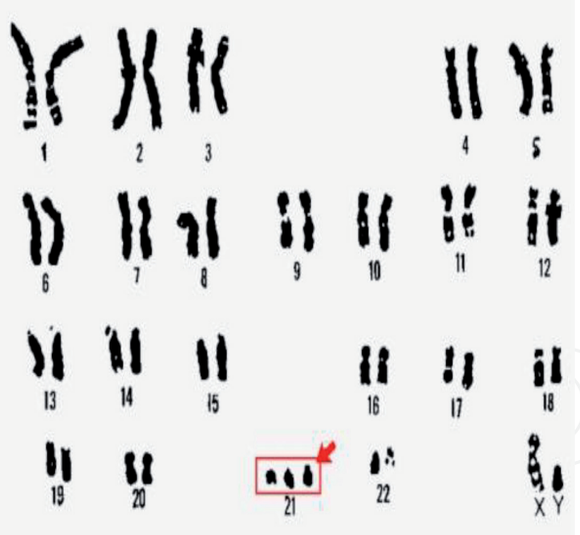

Figure 4.
An example of aneuploidy with Trisomy 21 showing an extra chromosome 21.

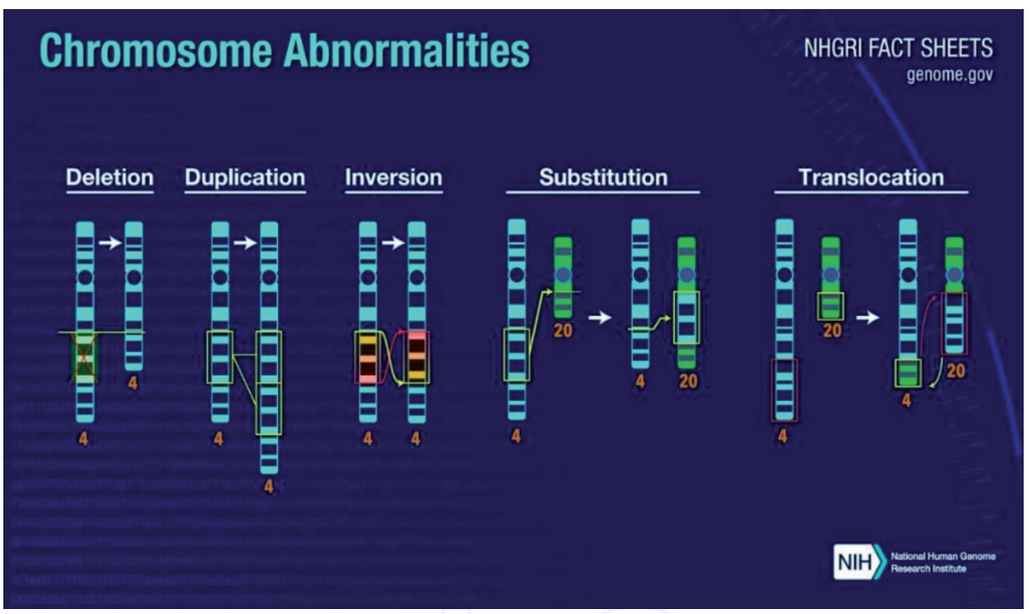

Figure 5.
Common structural chromosomal anomalies resulting in abnormal genotype.

- Translocation happens when genetic material is exchanged between two chromosomes. It may be balanced with no gain or loss of DNA and no anomalies or unbalanced when the process results in gain or loss of chromosomal material. Even though there may not be any phenotypical anomalies, balanced translocations might have implications for the offsprings of the patient.

- Reciprocal translocation is an anomaly in which the phenotype is normal despite the break and exchange of material between 2 chromosomes.

- Robertsonian translocation is another example in which the phenotype may be normal. It involves only selected chromosomes, such as 13,14,15,21,22. In this anomaly, the short arm is lost, and long arms fuse at the centromere.

- Inversion is described when a piece of a chromosome breaks at two places and then is reinserted in the opposite direction. Inversions may be paracentric if it does not involve centromere or pericentric if the centromere is involved.

- Isochromosomes result from abnormal mitosis in which a break at centromere results in two short (p) arms or two long (q) arms from the same side, both carrying identical genes.

- Dicentric chromosomes are defined as the abnormal fusion of two chromosome pieces, both having a centromere.

- Ring chromosomes form when the deletion happens at the ends of both arms of the same chromosome and the remaining chromosome join, making a ring-like shape. The chromosomes may be eventually lost, resulting in monosomy.

3. Evaluation of an infant with congenital malformations

The clinical evaluation of a child born with an anomaly begins with a detailed history of the extended family and a comprehensive history of the parents, with special attention to age, gravidity, parity, miscarriages, pregnancy-related complications, history of prescription drugs intake, substance abuse, significant illnesses and consanguinity among others. This is followed by a thorough physical examination, which should consist of a detailed assessment of craniofacial profile for dysmorphology and of individual organ systems, including the vertebral column, extremities, and skin. The histopathological features of the placenta and umbilical cord should be noted. The next step includes the performance of specific diagnostic tests, which are selected on the basis of the results of history and physical findings and should be individualized according to the case. Referral to a geneticist is indicated if one major or more than two minor anomalies are present.

3.1 Physical examination

The physical examination starts with the standard measurements of weight, length, and head circumference. In infants suffering from short stature, skeletal dysplasia, or suspected Marfan syndrome, arm span and lower segment/upper segment ratio are useful variables to note. The following is a list of specific features and characteristics to seek during the general evaluation of an infant with congenital malformations: midface hypoplasia; prognathism , retrognathia, micrognathia; facial asymmetry; hypertelorism, hypotelorism; ophthalmoplegia; esotropia, exotropia; cataract; nystagmus; ptosis; inner canthal distance, outer canthal distance, interpupillary distance; palpebral fissures length; long, anteriorly or posteriorly rotated ears; low set ears; microtia; prominent, bulbous nasal tip; slit appearance of nose; anteverted nares; long or smooth philtrum; macrostomia, microstomia, high arched palate, cleft lip/palate; cleft uvula,; macroglossia, protruding tongue ; wide or short neck , neck webbing ; pectus excavatum or pectus carinatum; wide-spaced nipples; scoliosis, deep sacral dimple, sacral hair tuft, sacral tag; sirenomelia; limited range of motion of extremities, contracture; polydactyly syndactyly, brachydactyly, arachnodactyly, broad thumbs and toes; clubfoot; breast development; Tanner staging. Ambiguous genitalia; micropenis; cryptorchidism; hypoplasia of labia or vaginal hypoplasia/atresia, sparse or excess body hair; abnormally light hair; skin hyperpigmentation /hypopigmentation; albinism; nail dystrophy. In addition to these, a detailed systemic evaluation is an essential and integral part of the physical examination of a child born with anomalies. The physical examination should be standardized and performed by a trained dysmorphologist [9].

3.2 Specific tests and laboratory investigations

As mentioned earlier, the need and selection of testing depend upon the results of the history and physical examination. Specific tests commonly include computed tomography (CT), and magnetic resonance imaging (MRI) scans of the central nervous system (CNS), echocardiogram, skeletal survey, and autopsy if the patient expires. An ultrasound evaluation of the genitourinary system is indicated in the conditions of ambiguous genitalia in order to detect the presence of abdominal gonads and uterus and to assess the anatomy of kidneys, ureters, bladder, and testicles. Fundoscopy may be done to detect ocular anomalies, such as retinal colobomas, optic disc defects, and chorioretinitis. TORCH infections and other significant viral infections should be ruled out by performing specific tests as clinically indicated.

3.3 Tests for suspected genetic disorders

The selection of chromosomal studies is guided by the clinical presentation. Exome sequencing helps in the identification of rare single-gene disorders and is indicated in the conditions of multiple, complex congenital anomalies with no otherwise identified genetic defect. Molecular-based chromosome microarray studies (array comparative genomic hybridization or aCGH) are utilized to diagnose microdeletions. aCGH is the first line of testing in children with multiple anomalies and intellectual disabilities [10]. It has now replaced Giemsa banding karyotype (G-banding) and fluorescent in situ hybridization (FISH) studies. aCGH testing may be indicated in the following conditions: one or more major anomalies, three or more minor anomalies, unexplained intellectual deficiency, dysmorphism with or without tone and intellectual deficits, unexplained failure to thrive, family history of birth defects, and multiple miscarriages [11]. The FISH test may be used to confirm a microdeletion or microduplication detected by aCGH and is generally used in the prenatal screening of cells in amniotic fluid. In the conditions of consanguinity or suspected uniparental disomy (UPD), where the chromosome material is coming from one parent only, single nucleotide polymorphisms (SNPs) is indicated. Another test, called Whole-exome sequencing (WES), is used in children with multiple structural anomalies associated with intellectual disability and/or seizures [12]. This technique uses next-generation sequencing and can simultaneously analyze the coding regions of almost 19,000 to 20,000 genes. While WES cannot be used for the detection of microdeletions or microduplications, whole-genome sequencing (WGS) can detect larger deletions or duplications, as well as triple repeat expansions; and mutations in deep intronic regions, regulatory regions that are outside of the coding regions, and untranslated gene regions.

Some genetic disorders are metabolic in origin and require metabolic studies in addition to the genetic workup. These studies include tests for blood amino acids and urine organic acids, specific tests for the peroxisomal disorder, including liver biopsy in certain conditions, assessment of serum cholesterol precursors, and lactic acid and pyruvic acid levels in the blood and cerebrospinal fluid, among others. Metabolomics uses mass spectrometry to detect and quantify small molecules in plasma for the diagnosis of inborn errors of metabolism. This procedure scientifically studies the small molecule substrates (<1.5 kDa) called metabolites which are products of the biochemical processes in cellular metabolism. One of the cellular functions that entails mRNA, gene expression, and proteomic analyses leads to the release of gene products in the cell. Metabolomics meets the challenge of systems biology and functional genomics by integrating genomics, transcriptomic and proteomic information. Thus metabolomic information provides a better understanding of cellular biology.

3.4 Clinical care and outcome

The clinical care of a child is done via a multi-disciplinary approach and involves a team of pediatrician, geneticist, neurologist, cardiologist, surgeon, orthopedics surgeon, dermatologist, infectious disease specialist, and other specialties as indicated. The involvement of the social worker is important for family and social support. The natural course, complications, and eventual clinical outcomes depend on the anomaly and may range from inconsequential to severe. Some CAs are lethal in nature. Death from lethal anomalies can occur in-utero, at birth, during the perinatal period, or later at various stages of life. Others may have a normal span of life. The quality of life also is widely variable depending on the disorder.

4. Risk assessment of congenital anomalies

The assessment of risks for CA may not always be possible due to the lack of referable evidence and information. The known recurrence risks of some of the heritable disorders are presented in **Table 1**. Some of the risk factors for CA are well documented, such as parental age, subjects' gender, geographical location and exposure to drugs or toxins etc. Advanced maternal age is extensively reported to be associated with aneuploidies, such as trisomy 21, 13, and 18 and Klinefelter syndrome. Advanced maternal age has also been associated with non-chromosomal genitourinary anomalies,

Disease	Condition	Recurrence risk
Pyloric stenosis	Mother affected	19% risk for male, 7% for female offspring
	Father affected	5.5% risk for male, 2.4% for female
	1 child affected	4% risk to next male 2.4% to female child
Cleft lip	Unaffected parents, 1 child affected	4.5% risk for next child
	1 parent and 1 child affected	10% risk to next child
Cleft palate	1 child affected	2.6% risk to sibling
Congenital hip dysplasia	1 affected child	0.5% risk for male 6.3% for female child
Cardiac defects	1 child affected	3.4% risk for next child
	2 children affected	10% risk for next child
Neural tube defects	1 child affected	3.5% risk to next child
Trisomy 21	Mother with balanced translocation	10–15% risk for sibling
	Father with balanced translocation	5% risk for sibling
	1 child affected, no parental translocation	1% risk for sibling
Hirschsprung's disease	1 child affected	3–5% risk for next child
Club foot	1 affected child	2% risk if 1st child is male, 5% if female
	1 parent and 1 child affected	25% risk to next child

Table 1.
Recurrence risk of certain malformations. (adapted from Jones KL, Smiths recognizable patterns of human malformations, 5th edition, WB Saunders, Philadelphia, 1997; and Neonatology Review, 2nd edition, D. Brodsky & C. Martin, Hanley and Belfus Inc.2003.

as well as hips and feet deformities [13]. Advanced paternal age is associated with an increased incidence of de novo DNA mutations and chromosomal aberrations in the sperm, which may lead to miscarriage or genotypical and/or phenotypical anomalies in the fetus [14]. Like advanced maternal age, trisomy 21 is documented to be associated with advanced paternal age as well [15]. Other disorders reported to occur more commonly with advanced paternal age are achondroplasia, osteogenesis imperfecta, and some syndromes such as Apert, Waardenburg, Marfan, and Treacher Collins.

Green et al. have reported that the risks for cleft palate, diaphragmatic hernia, right ventricular outflow tract obstruction, pulmonary valve stenosis, anomalous pulmonary venous return, cataract, aortic coarctation, encephalocele, esophageal atresia, and multiple complex defects in the offsprings are enhanced with each unit year increase in the paternal age [14]. It is noteworthy that the effects of paternal age might vary in tandem with maternal age. While young maternal and paternal age are identified as independent risk factors for gastroschisis, young paternal age can become a risk factor for gastroschisis again if the mother's age exceeds 35 years. Omphalocele, spina bifida, orofacial clefts, and septal heart defects display associations with parental mating, which involves advanced paternal and young maternal age, and also between a young father and mother of advanced age.

Some diseases exhibit gender preferences [16, 17]. The diseases known to occur more commonly in males are pyloric stenosis, Hirschsprung's disease, imperforate anus, club foot, unilateral multicystic dysplastic kidney; cleft lip and palate, Poland sequence, ventricular septal defect, transposition of great vessels, aortic coarctation, hypoplastic left heart syndrome, subdiaphragmatic total anomalous pulmonary venous return, and pulmonic stenosis and atresia. Disorders identified to be more common in females are choanal atresia, choledochal cyst, congenital hip dysplasia, ureterocele, Trisomy 18, atrial septal defect, patent ductus arteriosus, anencephaly, meningomyelocele and congenital hypothyroidism. In a recent study of 12,795 cases with CA, male fetuses were found to be more susceptible to birth defects than females, however, with significant heterogeneity in the subtypes [16]. Sex organ anomalies are reported to be 8.5 times more common, and GIT defects 55%, whereas urinary tract anomalies 62% more prevalent in males than in females. Overall, the prevalence of major CA in males is 3.9% compared to 2.8% in females [17].

4.1 Calculation of carrier frequency and recurrence risk in a population

The Hardy-Weinberg equilibrium is utilized to predict carrier frequency in a given population and to calculate recurrence risk. The calculation assumes that the mating is random and there are no new mutations or natural selections.

4.1.1 Calculation of carrier frequency

Example: Normal gene frequency=P; Abnormal gene frequency =Q; P+Q =1 (i.e. 100%); 2PQ =carrier frequency; P^2= normal, non-carrier frequency; Q^2 = affected frequency.

Let us take the example of cystic fibrosis (CF), an autosomal recessive disease. The disease frequency of the morbidity is 1 in 2500 Caucasian births. I.e., Q^2 = 1/2500; therefore Q= 1/50. We know that P+Q=1. So P=1-Q or 1-1/50 = 49/50 ~ 1. Therefore 2PQ (carrier frequency) = 2 * 1 * 1/50 = 1/25.

4.1.2 Calculation of recurrence risk of CF

Example: a pregnant woman has a sister with CF. What are the chances of her having an affected child?

The Father's risk of being a carrier is 1/25, while the mother's chances of being a carrier are 2/3 as both of her parents are carriers. The chance of the offspring getting the gene from each parent is ¼. Therefore the chances of the child being affected by CF are 1/25X2/3X1/4=1/150.

5. Summary

Congenital anomalies are a heterogeneous group of disorders of abnormal morphogenesis, which present at birth and carry widely variable implications for morbidity and mortality. The basics of incidence, pathogenesis, and risk assessments of CA are discussed in this section.

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