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Medical Genetics

Study guide

Under the editorship of S. V. Popov

Recommended by the Academic Council of Sumy State University

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This study guide covers information about basic principles of diagnosis and treatment of patients with genetic pathology.

For English-speaking students of higher educational institutions of III–IV levels of accreditation, postgraduates, family physicians, pediatricians, internists.

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1. TASKS OF MEDICAL GENETICS. SYNDROMOLOGICAL ANALYSIS. BIRTH DEFECTS

I. A few words about Medical Genetics

Medical genetics is the specialty of medicine that involves the diagnosis and management of hereditary disorders. Medical genetics differs from human genetics in that human genetics is a field of scientific research that may or may not apply to medicine, but medical genetics refers to the application of genetics to medical care. For example, research on the causes and inheritance of genetic disorders would be considered within both human genetics and medical genetics, while the diagnosis, management, and counseling of individuals with genetic disorders would be considered part of medical genetics.

In contrast, the study of typically non-medical phenotypes such as the genetics of eye color would be considered part of human genetics, but not necessarily relevant to medical genetics (except in situations such as albinism). Genetic medicine is a newer term for medical genetics and incorporates areas such as gene therapy, personalized medicine, and the rapidly emerging new medical specialty, predictive medicine.

Medical genetics encompasses many different areas, including clinical practice of physicians, genetic counselors, and nutritionists, clinical diagnostic laboratory activities, and research into the causes and inheritance of genetic disorders. Examples of conditions that fall within the scope of medical genetics include birth defects and dysmorphology, mental retardation, autism, and mitochondrial disorders, skeletal dysplasia, connective tissue disorders, cancer genetics, teratogens, and prenatal diagnosis. Medical genetics is increasingly becoming relevant to many common diseases. Overlaps with other medical specialties are beginning to emerge, as recent advances in genetics are revealing etiologies

for neurologic, endocrine, cardiovascular, pulmonary, ophthalmologic, renal, psychiatric, and dermatologic conditions.

Medical genetics consists of several parts:

1. Clinical genetics. Clinical genetics is the practice of clinical medicine with particular attention to hereditary disorders. Referrals are made to genetics clinics for a variety of reasons, including birth defects, developmental delay, autism, epilepsy, short stature, and many others. Examples of genetic syndromes that are commonly seen in the genetics clinic include chromosomal rearrangements, Down syndrome, DiGeorge syndrome (22q11.2 Deletion Syndrome), Fragile X syndrome, Marfan syndrome, Neurofibromatosis, Turner syndrome, and Williams syndrome.

2. Metabolic/biochemical genetics. Metabolic (or biochemical) genetics involves the diagnosis and management of inborn errors of metabolism in which patients have enzymatic deficiencies that perturb biochemical pathways involved in metabolism of carbohydrates, amino acids, and lipids. Examples of metabolic disorders include galactosemia, glycogen storage disease, lysosomal storage disorders, metabolic acidosis, peroxisomal disorders, phenylketonuria, and urea cycle disorders.

3. **Cytogenetics.** Cytogenetics is the study of chromosomes and chromosome abnormalities. While cytogenetics historically relied on microscopy to analyze chromosomes, new molecular technologies such as array comparative genomic hybridization are now becoming widely used. Examples of chromosome abnormalities include aneuploidy, chromosomal rearrangements, and genomic deletion/duplication disorders.

4. **Molecular genetics.** Molecular genetics involves the discovery of and laboratory testing for DNA mutations that underlie many single gene disorders. Examples of single gene disorders include achondroplasia, cystic fibrosis, Duchenne

muscular dystrophy, hereditary breast cancer (BRCA1/2), Huntington disease, Marfan syndrome, Noonan syndrome, and Rett syndrome. Molecular tests are also used in the diagnosis of syndromes involving epigenetic abnormalities, such as Angelman syndrome, Beckwith-Wiedemann syndrome, Prader-Willi syndrome, and uniparental disomy.

5. **Mitochondrial genetics**. Mitochondrial genetics concerns the diagnosis and management of mitochondrial disorders which have a molecular basis but often result in biochemical abnormalities due to deficient energy production.

One of the most important threats for human's health is the genetic diseases.

Genetic disease is a disorder caused by genetic factors and especially abnormalities in the human genetic material (genome).

Types of (human) genetic diseases are:

1. Single-gene/monogenic genetic diseases.

In this category the starting point is a mutation/change in one gene. Some of these are sickle cell anemia, cystic fibrosis, Aicardi Syndrome, Huntington's disease.

2. Multifactorial/polygonic genetic diseases.

The second type of human genetic diseases is caused by mutations in more than one gene. Many well-known chronic diseases are multifactorial genetic diseases. Everybody knows Alzheimer, diabetes, obesity and arthritis. Besides many cancer types are caused by multi mutations.

3. Chromosomal genetic diseases.

Chromosomes are big DNA molecules composed from genes. The chromosomes are located in the cell nucleus. Abnormalities in the structure, number (and not only) of the chromosomes can cause some of the most dangerous genetic disorders. This type of disorders seems to be much easier to observe because they are, sometimes, detected by examination with microscope. Down syndrome is the most well-known

disease caused by chromosomal abnormalities. There is a third copy of chromosome – 21 in this disorder (there are two copies of each chromosome in the cells of healthy people). Chromosomal diseases can be also caused by segments and joins of parts of chromosomes.

4. Mitochondrial genetic diseases.

It is not a common situation. Mitochondrial DNA is a DNA molecule found in the mitochondria (out of the nucleous) – a necessary organelle for cellular respiration. Mutations in the mitochondrial DNA can also cause undesirable abnormalities.

II. Phenotype

The human genome has approximately 38 000 genes which are the individual units of heredity of all traits. The genes are organized into long segments of deoxyribonucleic acid (DNA), which, during cell division, are compacted into intricate structures with proteins to form chromosomes. The function of genes is the production of structural proteins and enzymes. This occurs through a series of events, termed transcription, processing, and translation.

Phenotype is the total observable physical traits of an individual (organism or cell). Mayr notes that these observable features include anatomical, physiological, biochemical, and behavioral characteristics. The term can also be used in reference to one particular trait or characteristic that is measurable and is expressed in only a subset of individuals within that population. For example, blue eye color, aggressive behavior, bilateral symmetry, and length of antennae are phenotypic traits.

The phenotype of a developing or developed organism is held to be the result of interaction between the inherited genotype (the genetic makeup of the individual), transmitted epigenetic factors (those changes in genome function that do not alter the nucleotide sequence within the DNA), and non-

hereditary environmental variation. Some phenotypes are controlled entirely by the individual's genes. Others are controlled by genes but are significantly affected by non-genetic or environmental factors. Still other phenotypes are entirely non-genetic, for example, a person's language or physical traits that were altered by surgery.

Each human being has a unique phenotype. Even identical twins, who have the same genotypes, exhibit differences (such as fingerprints or behavioral characteristics) because of non-genetic factors. The process of sexual reproduction, crossing over, mutations, and environmental and other non-genetic influences all help assure that individuals throughout history are unique. Religions also emphasize the importance of one's spiritual aspect (soul, spirit) and spiritual environment (such as the history of past actions) as influences on the nature of a person, versus an over-emphasis on genotype and physical influences. From the point of view of religion, as a unique manifestation of God's nature, each person can offer a unique joy to God and to others.

Geneticists use easily observable phenotypes to deduce an organism's genotype, and analyze complex phenotypes to help hypothesize about how individual genes function.

Genotype and phenotype

The terms "genotype" and "phenotype" were created by Wilhelm Johannsen in 1911. A genotype is the genetic makeup (set of genes) of an individual organism or cell. Genes are the units of heredity in living organisms and are encoded in the organism's genetic material – those segments of DNA that cells transcribe into RNA and translate, at least in part, into proteins.

An organism's genotype is a major (the largest by far for morphology) influencing factor in the development of its phenotype, but it is not the only one. For many traits, the genotype may set the potential and limits for phenotypic expression, but environmental influences can be major.

Although there has been a historical debate regarding the prominence that should be given to “nature” (genes) versus “nurture” (environment), the consensus is that most characteristics of an organism are affected by both factors. For example, the presence or absence of nutrients will affect plant growth and health. The phrase norm of reaction refers to the amplitude of variation of a phenotype produced under different environmental conditions.

Many phenotypes also are determined by multiple genes. Thus, the identity of one or a few alleles of an organism does not always enable prediction of its phenotype.

Even two organisms with identical genotypes normally differ in their phenotypes. One experiences this in everyday life with monozygous (i. e. identical) twins. Identical twins share the same genotype, since their genomes are identical; but they never have the same phenotype, although their phenotypes may be very similar. This is apparent in the fact that their mothers and close friends can tell them apart, even though others might not be able to see the subtle differences. Furthermore, identical twins can be distinguished by their fingerprints which are never completely identical. Of course, personality differences can be substantial.

The concept of phenotypic plasticity describes the degree to which an organism’s phenotype is determined by its genotype. A high level of plasticity means that environmental factors have a strong influence on the particular phenotype that develops. If there is little plasticity, the phenotype of an organism can be reliably predicted from knowledge of the genotype, regardless of environmental peculiarities during development. An example of high plasticity can be observed in larval newts – when these larvae sense the presence of predators, such as dragonflies, they develop larger heads and tails relative to their body size and display darker pigmentation. Larvae with these traits have a higher chance of survival when

exposed to the predators, but grow more slowly than other phenotypes.

In contrast to phenotypic plasticity, the concept of genetic canalization addresses the extent to which an organism's phenotype allows conclusions about its genotype. A phenotype is said to be canalized if mutations (changes in the genome) do not noticeably affect the physical properties of the organism. This means that a canalized phenotype may form from a large variety of different genotypes, in which case it is not possible to exactly predict the genotype from knowledge of the phenotype (i. e. the genotype-phenotype map is not invertible). If canalization is not present, small changes in the genome have an immediate effect on the phenotype that develops.

Phenotypic variation

Phenotypic variation (due to underlying heritable genetic variation) is a fundamental prerequisite for a population's adaptation to its environment due to natural selection. The "fitness" of an organism is a high-level phenotype determined by the contributions of thousands of more specific phenotypes. Without phenotypic variation, individual organisms would all have the same fitness, and changes in phenotypic frequency would proceed without any selection (randomly).

The interaction between genotype and phenotype has often been conceptualized by the following relationship:

genotype + environment → phenotype

A slightly more nuanced version of the relationship is:

genotype + environment + random-variation → phenotype

An example of the importance of random variation in phenotypic expression is Drosophila melanogaster in which the number of eyes may vary (randomly) between left and right sides in a single individual as much as they do between

different genotypes overall, or between clones raised in different environments.

A phenotype is any detectable characteristic of an organism (i. e., structural, biochemical, physiological, and behavioral) determined by an interaction between its genotype and environment. According to the autopoietic notion of living systems by Humberto Maturana, the phenotype is epigenetically being constructed throughout ontogeny, and we as observers make the distinctions that define any particular trait at any particular state of the organism's life cycle.

The concept of phenotype can be extended to variations below the level of the gene that effect an organism's fitness. For example, silent mutations that do not change the corresponding amino acid sequence of a gene may change the frequency of guanine-cytosine base pairs (GC content). These base pairs may have a higher thermal stability ("melting point") than adenine-thymine, a property that might convey, among organisms living in high temperature environments, a selective advantage on variants enriched in GC content.

III. Physiologic Basis of Birth Defects

The development of birth defects is greatly dependent on the gestational age, nature of the teratogens and the intensity and duration of exposure. The reader is strongly encouraged to review human development, particularly embryology as it relates to organogenesis, to better understand how and when environmental factors may influence fetal development. Organ systems differ in the timing and duration of formation which results in marked differences in susceptibility. For example, the cardiovascular system undergoes a lengthy and complex developmental phase which probably explains why this organ system has the highest incidence for birth defects. Also as general rule, significant early insults (less than 8 gestational weeks) result in spontaneous miscarriages, whereas exposure later in the gestation (typically after organogenesis or

approximately 14–16 weeks of gestation) has the least effect. There are, however, many exceptions to these basic rules.

It is essential to understand the pathophysiologic mechanisms for fetal maldevelopment which may be divided into **malformation, deformation, disruption or dysplasia**.

Malformation

A malformation is a primary structural defect occurring during the development of an organ or tissue. Most malformations have occurred by 8 weeks of gestation.

An isolated malformation, such as cleft lip and palate, congenital heart disease or pyloric stenosis can occur in an otherwise normal child.

Multiple malformation syndromes comprise defects in two or more systems and many of them are associated with mental retardation.

Disruption

A disruption defect implies that there is destruction of a part of a fetus that had initially developed normally. Disruptions usually affect several different tissues within a defined anatomical region.

Deformation

Deformations are due to abnormal intrauterine moulding and give rise to deformity of structurally normal parts. Deformations usually involve the musculoskeletal system and may occur in fetuses with underlying congenital neuromuscular problems such as spinal muscular atrophy and congenital myotonic dystrophy. This is illustrated in the characteristic pattern of abnormalities including the abnormal facies, pulmonary hypoplasia, and limb contractures that result from prolonged oligohydramnios, either secondary to renal agenesis (Potter syndrome) or premature rupture of membranes (Potter sequence).

Dysplasia

Dysplasia refers to abnormal cellular organisation or function within a specific organ or tissue type. Most dysplasias

are caused by single gene defects, and include conditions such as skeletal dysplasias and storage disorders from inborn errors of metabolism.

IV. The physical examination in clinical genetics

The physical examination is a valuable tool in medical practice that provides an objective supplement to historical information. To understand the special nature of the "genetic" physical exam, one must first recognize that the primary goal of a medical genetic evaluation is to identify a unifying etiology for seemingly unrelated birth defects, developmental problems, or other abnormal findings present in a fetus, child, or adult. Some may question the benefits of making a genetic diagnosis, as there are very few "cures" for such conditions. However, it is only by establishing a correct diagnosis that appropriate clinical management can be provided, along with accurate prognostic and recurrence risk counselling. Understanding the pathogenesis of a patient's problems can further help families begin to cope with guilt they may feel about "why" their child has a particular problem and can direct families to contact appropriate support groups.

The genetic physical exam relies heavily on the art of dysmorphology, defined as the study of abnormal form. A detailed analysis of body structure is employed to detect potential embryologic deviations from normal development. While the actual physical examination is similar to the general medical examination, much closer attention is given to form, size, proportion, positioning, spacing, and symmetry. Indeed, precise observation and accurate description of all physical features is the cornerstone of the genetic dysmorphology exam. A genetic diagnosis may subsequently be reached through careful collation, interpretation, and categorization of any physical differences present.

Categorization of physical abnormalities

Physical exam findings that are indicative of a genetic diagnosis may be of varying clinical relevance. Major anomalies are structural alterations arising during embryologic development that have severe medical or cosmetic consequences and typically require therapeutic intervention. Examples include congenital heart defects, neural tube defects, and cleft lip or palate. Minor anomalies, in contrast, are medically and cosmetically insignificant departures from normal development that do not require significant surgical or medical treatment and have no risk for long-term sequelae. Minor external anomalies most commonly are found in areas where structures are most complex and variable, such as in the face, auricles, hands, and feet. Examples include wide-set eyes, low-set ears, or brachydactyly (short fingers or toes). A third category of physical findings is minor, or normal, variants. While these findings may also represent medically insignificant departures from normal development, they occur at a low frequency among the normal general population. Examples include cafe-au-lait macules, single palmar creases, and fifth finger clinodactyly. It is important to appreciate that minor anomalies and variants are significant only when taken in context. For example, while preauricular ear pits are common in the general population, they may lead to the diagnosis of branchiootorenal syndrome when identified in a patient with sensorineural hearing loss and renal anomalies.

Although major anomalies are more obvious and tend to draw attention to the patient more quickly, it is important to recognize that they are not more important than minor anomalies or normal variants as clues to a diagnosis. Rather, it is often the most subtle findings, discernable only through a genetics physical exam, that lead to the diagnosis of a genetic syndrome. Learning to differentiate a minor anomaly from normal variation is vitally important, as it is through these subtle anomalies that most genetic diagnoses are made. In

particular, the rarer physical features may be the most useful in arriving at the correct differential diagnosis. Descriptions of normal features and variations can be found in excellent texts such as Diagnostic Dysmorphology or Smith's Recognizable Patterns of Human Malformation.

Guidelines to performing the genetic physical exam

The exam itself has two interrelated components: descriptive observation and careful measurement. Each physical feature should be evaluated, then normal and variant features should be accurately described using accepted standard terminology. While impressions are important (e. g., "the palpebral fissures look small"), they are insufficient alone. "No clinical judgment should ever be made on a measurable parameter without actually having measured and compared it to standard references" (Hall, 1993). For example, hypertelorism (widely spaced eyes) noted on casual observation may be an illusion caused by a widened nasal bridge or epicanthal folds; measuring interpupillary distance may reveal that eye spacing actually falls within the normal range. Distinguishing such differences may be of considerable importance when trying to determine if minor anomalies are present and consistent with a particular syndrome.

In some cases, a genetic syndrome can be diagnosed by the overall appearance, or "gestalt" of the facial appearance. This ability to instantaneously recognize a syndrome is powerful and impressive to one's colleagues. However, even an experienced dysmorphologist can be wrong if one is too quick to make a diagnosis as many findings are common to multiple disorders. Therefore, a careful and complete exam is warranted even in apparently obvious cases. Starting with the "big picture" is a useful approach, however, for examining each body region if followed by sequential evaluation of smaller component subunits within that region.

Components of the physical exam

When examining the patient's physical characteristics, it is important to be systematic and thorough. Growth parameters (i. e., head circumference, height, and weight) should be recorded on all patients including adults. The general appearance and behavior of a patient should also be carefully observed. Many genetic disorders have characteristic behavior that may have as much diagnostic importance as any physical finding.

A useful regional approach is to start with examination of the head and proceed downward. Greatest attention is given to examination of the head and face, as this is where the greatest variability of human features is seen. Assessing symmetry, placement, and proportions both of overall appearance and of all paired structures (e. g., eyes and ears) may detect differences not otherwise appreciated. The skull should be observed for symmetry and contour, then palpated for ridging of sutures and fontanel sizes. The forehead should be evaluated for contour and breadth. The placement, rotation, size, and configuration of the ears should be noted, as well as the presence of preauricular pits or tags. The eye distances should be measured. The external eye should be evaluated for unusual position or structural abnormalities of the lids, irises, or pupils. Ophthalmologic evaluation for cataracts or retinal abnormalities should be examined. The nose should be evaluated for the configuration of the nasal bridge and tip, as well as symmetry of the nasal septum. The configuration of the philtrum should be noted, along with the size of the mouth and any unusual features of the lips. The arch of the palate should be observed, along with any cleft of the palate or uvula. Teeth should be evaluated for size, placement, and abnormalities of enamel or configuration. In addition, the profile should be assessed for prominence or recession of the forehead, eyes, midface, and chin. Any redundancy of nuchal skin should be noted. Finally, a description of hair, eyebrows, and eyelashes

should include assessment of texture, colour, thickness, and length.

Evaluation of the trunk includes measuring relative proportions of upper and lower segment lengths (as divided at the symphysis pubis), sternal length, chest circumference, and internipple distance. The chest, clavicles, ribcage, and spine should be examined for deformity or abnormalities of contour. The positioning and form of the nipples and sternum should be observed. Any heart murmur, abdominal wall defect, or organomegaly should be noted. Sacral spinal defects such as hair tufts or pits should be noted. Examination of the genitalia should include assessment of proper proportions and positioning of structures, as well as Tanner stage.

Careful examination of the extremities can also yield useful diagnostic clues. The relative proportions and symmetry of the various segments of each limb should be noted, as specific abnormalities may indicate a particular skeletal dysplasia. All joints should be assessed for contractures, abnormal angulation, or hypermobility. Examination of the hands and feet require careful consideration, as they offer a wide range of insight into early fetal development. Measurements should be made of total hand, palm, middle finger, and foot lengths. Fingers and toes should be assessed for placement, contractures, webbing, and spacing. The contour of the soles of the feet should be noted. Careful observation should also be given to the texture and configuration of fingernails and toenails. For example, subungual fibromas may be the presenting sign of tuberous sclerosis in a patient with mental retardation and seizures.

The skin should be carefully examined in an effort to detect and describe any birthmarks or abnormalities in pigment, texture, elasticity, or wound healing. This may require using ultraviolet light in fair-skinned individuals. Observing dermatoglyphic patterns and skin creases is also important. Some conditions have characteristic dermatoglyphic patterns,

as exemplified by a predominance of fingertip whorls in Smith – Lemli – Opitz syndrome. More generally, abnormal skin creases reflect an abnormality in early fetal movement before 18 weeks' gestation.

Placing the physical exam in context

Information obtained from a detailed medical and family history will often guide the physical exam, allowing one to focus extra attention on specific findings. For example, a postpubertal male referred for evaluation of mental retardation should be assessed for large ear size and macroorchidism, i. e. features that would raise suspicion for fragile X syndrome. Similarly, using background knowledge will allow one to direct attention to features that may have been missed. For example, the examination of an infant with ambiguous genitalia should include careful analysis of the toes, as cutaneous syndactyly (webbing) between the second and third toes in this context would raise suspicion for Smith – Lemli – Opitz syndrome.

Examining a patient's family members and/or reviewing their photographs can establish the background features that may assist the examiner in understanding where their patient's features might have originated. In particular, any parameter thought to be abnormal in the patient should be examined in their relatives.

A final point to consider is that physical findings change with time in many genetic syndromes. In Prader – Willi syndrome, for example, affected newborns are hypotonic and manifest failure to thrive, but within several years they become hyperphagic and obese. Periodic evaluations are important in cases in which a diagnosis cannot be reached, as physical examination findings may have changed into a recognizable pattern consistent with a known diagnosis. Similarly, reviewing photographs of a patient at various ages may detect phenotypic features classic for a particular condition that are no longer recognizable.

2. MONOGENIC DISEASES

Disorders caused by the inheritance of a single defective gene are known as monogenic diseases or single gene disorders.

There are more than 6 000 known single-gene disorders which occur in 1:200 births.

Some examples are cystic fibrosis, sickle cell anemia, Marfan syndrome, Huntington's disease, hereditary hemochromatosis, Duchenne muscular dystrophy, hemophilia, sickle cell anemia and Tay – Sachs disease.

The single-gene or monogenic diseases can be classified into three main categories: autosomal dominant, autosomal recessive, and X-linked.

Marfan syndrome

Marfan syndrome is an autosomal dominant disorder (25 % of cases are new mutations) affecting connective tissues caused by mutation in the gene encoding fibrillin 1 (FBN1). Fibrillin is the primary component of the microfibrils that allow tissues to stretch repeatedly without weakening. Because the patient's fibrillin is abnormal, the connective tissues are looser than usual, which weakens or damages the support structures of the entire body.

Antoine Marfan (1858–1942) first described it in 1896. Marfan syndrome affects three major organ systems of the body: the heart and circulatory system, the bones and muscles, and the eyes.

The disorder has an incidence of at least 1 in 10 000.

Signs and symptoms

Features include:

Musculoskeletal:

– tall stature;

– long limbs with significantly increased arm span and reduced upper to lower body segment ratio;

– long fingers (arachnodactyly);
– joint laxity and flat feet;
– long narrow face with deep set eyes;
– high narrow palate and dental crowding;
– kyphoscoliosis;
– spondylolisthesis;
– dural ectasia;
– pectus excavatum (the patient's breastbone or sternum is sunken inward);
– pectus carinatum (the sternum is pushed outward and narrowed). A few patients may have a pectus excavatum on one side of their chest and a pectus carinatum on the other;
– foot disorders (pes planus (flat feet) and chronic pain in feet);
– protrusio acetabulae (the acetabuli becomes deeper than normal during growth).

Cardiovascular:

About 90 % of Marfan patients have cardiac complications.
– mitral valve prolapse;
– aortic enlargement;
– aortic regurgitation;
– infective endocarditis.

Eye:

– ectopia lentis (dislocation of the lens, often upward);
– myopia (nearsightedness);
– retinal detachment (untreated can cause blindness);
– glaucoma;
– cataracts.

Other problems:

– spontaneous pneumothorax;
– obstructive sleep apnea;
– emphysema;
– striae (stretch marks of the skin).

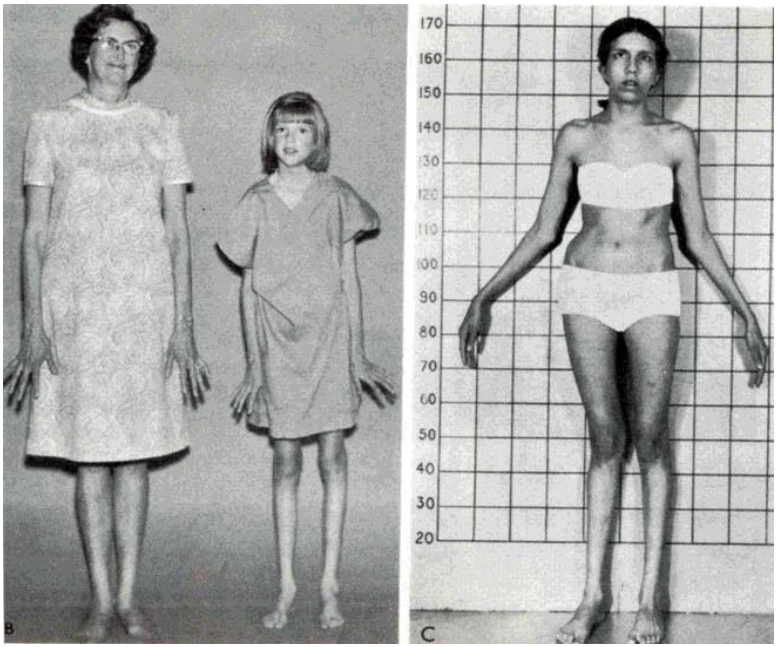

Figure 1 – Marfan syndrome

Diagnosis:

– ultrasound (to try to determine the length of fetal limbs in at-risk pregnancies);

– taking a family history;

– examination of the patient's eyes, heart, and bone structure.

The examination should include:

– echocardiogram taken by a cardiologist (only the echocardiogram can detect possible enlargement of the aorta);

– slit-lamp eye examination by an ophthalmologist;

– work-up of the patient's spinal column by an orthopedic specialist.

Treatment and management

The treatment and management of Marfan syndrome is tailored to the specific symptoms of each patient.

Cardiovascular system:

– monitoring with an echocardiogram every six months until it is clear that the aorta is not growing larger. After that, echocardiogram once a year;

– CT (computed tomography);

– MRI (magnetic resonance imaging);

– TEE (transesophageal echocardiogram).

Medications:

– beta-blockers propranolol (Inderal) and atenolol (Tenormin) to slow down the rate of aortic enlargement and decrease the risk of dissection by lowering the blood pressure and decreasing the forcefulness of the heartbeat;

– patients who are allergic to beta-blockers may be given a calcium blocker such as verapamil;

– prophylactic dose of an antibiotic (penicillin and amoxicillin) before having dental work or minor surgery.

Surgical treatment.

Surgery may be necessary if the width of the patient's aorta increases rapidly or reaches a critical size (about 2 in/ 5 cm). The surgical treatment involves replacing the patient's aortic valve and anticoagulant medication (warfarin).

Musculoskeletal system

Children should be checked for scoliosis by pediatricians at each annual physical examination and x rayed in order to measure the extent of scoliosis or kyphosis. Scoliosis between 20 °C and 40 °C in children is usually treated with a back brace. The child must wear this appliance about 23 hours a day until growth is complete. If the spinal curvature increases to 40° or 50°, the patient may require surgery. Surgical treatment of scoliosis involves straightening the spine with metal rods and fusing the vertebrae in the straightened position.

Spondylolisthesis is treated with a brace in mild cases. If the slippage is more than 30°, the slipped vertebra may require surgical realignment.

Dural ectasia can be distinguished from other causes of back pain on an MRI. Mild cases are usually not treated. Medication or spinal shunting is used in severe cases to remove some of the spinal fluid.

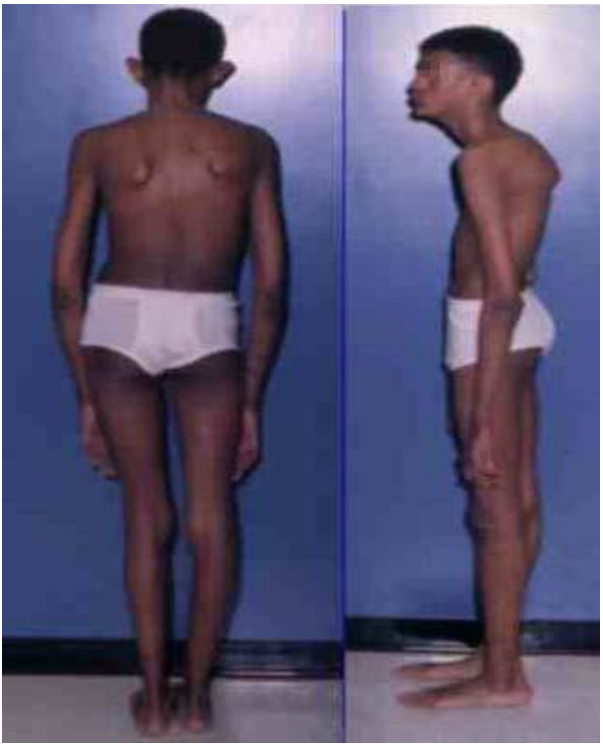

Figure 2 – Adult with Marfan syndrome

Pectus excavatum and pectus carinatum can be treated by surgery. In pectus excavatum, the deformed breastbone and ribs are raised and straightened by a metal bar. After four to six months, the bar is removed in an outpatient procedure.

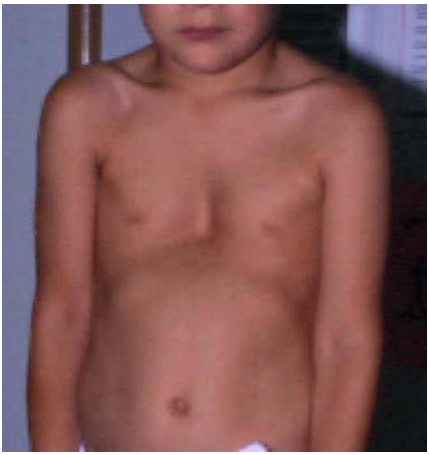

Figure 3 – Pectus excavatum

Protrusio acetabuli may require surgery in adult life to provide the patient with an artificial hip joint if the arthritic pains are severe.

Pain in the feet or limbs is usually treated with a mild analgesic (acetaminophen). Patients with Marfan syndrome should consider wearing shoes with low heels, special cushions, or orthotic inserts.

Visual and dental concerns

Patients with Marfan syndrome should have a thorough eye examination, including a slit-lamp examination to test for dislocation of the lens as well as nearsightedness. Dislocation can be treated by a combination of special glasses and daily use of 1 % atropine sulfate ophthalmic drops or by surgery. Glaucoma can be treated with medications or with surgery. Cataracts are treated with increasing success by implant surgery. All persons with Marfan syndrome should be taught to recognize the signs of retinal detachment (sudden blurring of vision in one eye becoming progressively worse without pain or redness).

Children with Marfan should be evaluated by dentist at each checkup for crowding of the teeth and possible misalignment, and referred to an orthodontist if necessary.

People with Marfan syndrome should avoid sports or occupations that require heavy weight lifting, rough physical contact, or rapid changes in atmospheric pressure (e. g., scuba diving).

Regular noncompetitive physical exercise, however, is beneficial for patients. Good choices include brisk walking, shooting baskets, and slow-paced tennis.

Phenylketonuria

Phenylketonuria (PKU) is an inherited, autosomal recessive disorder characterized by a deficiency in the enzyme phenylalanine hydroxylase (PAH). This enzyme is necessary to metabolize the amino acid phenylalanine to the amino acid tyrosine. The incidence of PKU is 1 in 15 000 births, but the incidence of this disease in Caucasian and Native American populations is higher than in African-American, Hispanic, and Asian populations.

Signs and symptoms

The affected infant is normal at birth. Mental retardation may develop gradually and may not be evident for the first few months. The IQ of PKU patients is generally lower than the IQ of their healthy peers.

Other symptoms can include:

– extreme patterns of behavior (hyperactive with purposeless movements, rhythmic rocking, and athetosis);

– delayed speech development;

– seizures (25 %) and electroencephalographic abnormalities (50 %);

– characteristic body odor of phenylacetic acid which has been described as musty or mousy;

– light body pigmentation (is due to a lack of melanin which normally colors the hair, skin, and eyes);

– vomiting;

– seborrheic or eczematoid rash (is mild and disappears as the child grows older);

– most infants are hypertonic with hyperactive deep tendon reflexes;

– microcephaly;

– prominent maxilla with widely spaced teeth;

– enamel hypoplasia;

– growth retardation.

Diagnosis

The primary diagnostic test for PKU is the measurement of phenylalanine levels in a drop of blood taken from the heel of a newborn baby's foot (Guthrie test). In this test, PKU is confirmed by the appearance of bacteria growing around high concentrations of phenylalanine in the blood spot.

Early diagnosis is critical. It ensures that the early treatment of PKU babies needs to develop normally and avoid the complications of PKU.

Figure 4 – Neonatal screening for PKU

PKU patients show high levels of phenylalanine and low levels of tyrosine in the blood.

Treatment

Treatment for PKU is recommended for babies that show a blood phenylalanine level of 7–10 mg/dL or higher for more than a few consecutive days.

Dietary therapy is the most common form of treatment for PKU patients. Phenylalanine is actually an essential amino acid. Diets provide very small amounts of phenylalanine and higher quantities of other amino acids, including tyrosine. The amount of allowable phenylalanine can be increased slightly as a child becomes older. PKU diets include all the nutrients normally required for good health and normal growth (carbohydrates, fats, vitamins, and minerals). High protein foods like meat, fish, chicken, eggs, nuts, beans, milk, and other dairy products are banned from PKU diets. Small amounts of protein foods (grains and potatoes) and low protein foods (some fruits and vegetables, low protein breads and pastas) are allowed. Sugar-free foods (diet soda, which contains the artificial sweetener aspartame) are also prohibited.

Blood tests should be done in the early morning when phenylalanine levels are the highest. All patients with PKU should adhere to a strictly controlled diet for life.

Kuvan (sapropterin dihydrocholoride) is the first drug which helps to manage PKU. The drug helps to reduce blood phenylalanine levels in individuals with PKU by increasing the activity of the PAH enzyme. Kuvan is effective only in individuals who have some PAH activity. Individuals who take this drug must continue to follow a phenylalanine-restricted diet and have blood tests to measure phenylalanine levels.

PKU patients who receive early and consistent dietary therapy can develop fairly normal mental capacity to within about five IQ points of their healthy peers. By comparison, untreated PKU patients generally have IQ scores below 50.

Prognosis

PKU is incurable, but early newborn screening, careful monitoring, and a life-long strict dietary management can

prevent the development of serious mental incapacity and help PKU patients to live normal, healthy, and long lives.

Homocystinuria

The term homocystinuria is actually a description of a biochemical abnormality, although many consider homocystinuria to be a disease. Homocystinuria refers to elevated levels of homocysteine in the urine. This can be caused by different biochemical abnormalities and there are eight different gene changes that are known to cause excretion of too much homocysteine in the urine. The best known and most common cause of homocystinuria is the lack of cystathionine b-synthase ("classical homocystinuria" that is caused by cystathionine b-synthase deficiency (CBS deficiency)).

Homocysteine is involved with the catabolism of methionine. Methionine is an essential amino acid. Methionine comes from dietary protein. Generally, the amount of methionine that is consumed is more than the body needs. Excess methionine is converted to homocysteine, which is then metabolized into cystathionine; cystathionine is then converted to cysteine. The cysteine is excreted in the urine. The conversion of homocysteine to cystathionine by cystathionine b-synthase requires vitamin B6 (pyridoxine). If cystathionine b-synthase is missing, then homocysteine cannot be broken down into cystathionine and cysteine, and instead, homocysteine is accumulated and the elevated levels of homocysteine and methionine can be found in the blood. Decreased levels of cysteine can be found in the blood. Elevated levels of homocysteine lead to a disease state that, if untreated, affects multiple systems, including the central nervous system, eyes, skeleton, and vascular system.

Classical homocystinuria is an autosomal recessive condition. The worldwide frequency of individuals with CBS deficiency is 1 in 350 000.

Signs and symptoms:

– tall and thin individuals with thinning and lengthening of the bones;

– long, narrow face;

– high arched palate (roof of the mouth);

– long and thin fingers (arachnodactyly);

– scoliosis;

– pectus excavatum or pectus carinatum;

– osteoporosis;

– stiff joints;

– dislocated lenses;

– nearsightedness (myopia);

– mental retardation or learning disabilities (untreated individuals);

– psychiatric problems (depression, chronic behavior problems, chronic obsessive-compulsive disorder, and personality disorders).

The most frequent cause of death associated with CBS deficiency is blood clots that are formed in veins and arteries (thromboembolisms), pulmonary embolus and strokes. Complication of CBS deficiency is severe premature arteriosclerosis.

Diagnosis:

– CBS deficiency is diagnosed during the newborn screening (elevated level of methionine in blood). The screening is done by collecting blood from a pinprick on the baby's heel prior to leaving the hospital, but at least 24 hours after birth. If the levels are elevated then follow-up testing to verify the diagnosis is performed. If not identified at newborn screening, the diagnosis is made by identifying low levels of cysteine in blood and urine;

– measurements of the amount of methionine and homocysteine produced by lymphoblasts or fibroblasts can confirm the diagnosis of CBS deficiency;

– DNA testing is available for families in which a gene alteration is identified. The prenatal diagnosis by chorionic villus sampling (CVS) and amniocentesis is made.

CBS deficiency has several features in common with Marfan syndrome. The dislocated lens in Marfan syndrome tends to be dislocated upward; the tendency for the lens dislocation is to be downward in CBS deficiency. Individuals who have Marfan syndrome tend to have congenital lens dislocation whereas individuals who have CBS deficiency have not been identified to have lens dislocation before 2 years of age.

Cystic fibrosis

Cystic fibrosis (also known as CF, mucoviscidosis) is an autosomal recessive inherited disease with high frequency in population (1:1000 – 1:3500 among newbornes). Frequency of heterozygote transmitters is 5 % and higher. Gene of mucoviscidosis is located in the 7^{th} chromosome and called the CFTR gene. There are over 500 known changes in the CFTR gene that can cause CF. However, 70 % of all people with an abnormal CFTR gene have the same defect, known as delta-F508. The CFTR protein helps to produce mucus. Mucus is a complex mixture of salts, water, sugars, and proteins that cleanses, lubricates, and protects many passageways in the body (lungs and pancreas). Cystic fibrosis affects the body's ability to move salt and water in and out of cells. This defect causes the lungs and pancreas to secrete thick mucus, blocking passageways and preventing function.

CF primarily affects people of white northern European descent; rates are much lower in non-white populations.

Signs and symptoms

The most severe effects of cystic fibrosis are observed in the gastrointestinal (digestive) system, the respiratory tract, the sweat glands and male fertility. Symptoms develop gradually.

Basic forms of the disease are:

– mixed, with affection of both gastrointestinal tract and respiratory system (75–80 %);

– mainly pulmonary (15–20 %);

– mainly intestinal (5 %).

Gastrointestinal system:

– meconium ileus. Babies who inherit CF have meconium ileus at birth (10–15 %). Meconium is the first dark stool that a baby passes after birth. The meconium of a newborn with meconium ileus is thickened, sticky. Meconium ileus causes abdominal swelling, vomiting and requires surgery immediately after birth;

– abdominal symptoms are caused by the inability of the pancreas to supply digestive enzymes to the intestine. Undigested food passes into the large intestine. Bacterial action can cause gas and abdominal swelling. The large amount of fat remaining in the feces makes it bulky, oily, and foul-smelling; The person is ravenously hungry, underweight, and shorter than expected for his age. When CF is not treated, a child may develop symptoms of malnutrition (anemia, bloating and appetite loss);

– diabetes (type I or insulin-dependent). Scarring of the pancreas destroys pancreatic cells which produce insulin;

– fat within the liver. Complications of liver enlargement include internal hemorrhaging, ascites, spleen enlargement and liver failure;

– other gastrointestinal symptoms can include a prolapsed rectum, intestinal obstruction and intussusception.

Somewhat fewer than 10 % of people with CF do not have gastrointestinal symptoms.

Respiratory tract

– pansinusitis (is observed in X-ray);

– nasal polyps, or growths (are not cancerous, do not require removal unless they become annoying);

– respiratory infection (bronchitis, bronchiolitis, pneumonia). The infecting organisms are the Staphylococcus aureus, Haemophilus influenzae and Pseudomonas aeruginosa. The fungus Aspergillus fumigatus may infect older children and adults. Lung infection is the major cause of death for people with CF;

– emphysema;

– recurrent respiratory infections lead to "digital clubbing," in which the last joint of the fingers and toes becomes slightly enlarged.

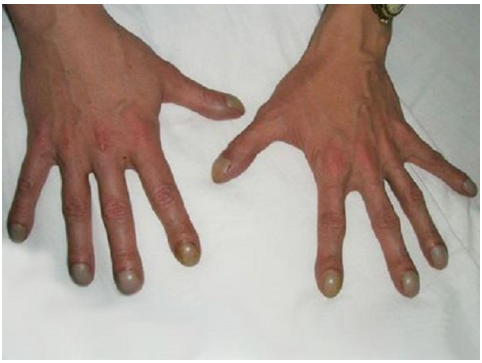

Figure 5 – Fingers of a patient with cystic fibrosis

Sweat glands

Sweat is much saltier than normal, and measuring the saltiness of a person's sweat is the most important diagnostic test for CF. Older children and adults with CF compensate for this extra salt loss by eating more salty foods. Infants and young children are in danger of suffering its effects (heat prostration). Heat prostration is marked by lethargy, weakness, loss of appetite and should be treated as an emergency condition.

Fertility

98 % of men with CF are sterile, due to complete obstruction or absence of the vas deferens, the tube carrying

sperm out of the testes. Most women with CF are fertile. In both boys and girls puberty is delayed due to the effects of poor nutrition or chronic lung infection.

Diagnosis:

– clinical pictures (gastrointestinal or respiratory symptoms, salty sweat);

– family history of CF;

– sweat test. It is both the easiest and the most accurate test for CF. A person with CF will have salt concentrations that are one-and-one-half to two times greater than normal. The test can be done for persons of any age, including newborns, and its results can be determined within an hour;

– genetic testing. Genes from a small blood or tissue sample are analyzed for specific mutations; presence of two copies of the mutated gene confirms the diagnosis of CF in all but a very few cases. A negative gene test cannot rule out the possibility of CF. Amniocentesis;

– newborn screening (IRT test). This is a blood test which measures the level of immunoreactive trypsinogen, which is generally higher in babies with CF than those without it. But there are many false positive results immediately after birth, that is why the second test is required in several weeks. The second positive result is usually followed by the sweat test.

Treatment and management

There is no cure for cystic fibrosis.

Nutrition:

– high-calorie diets;

– vitamin supplements;

– pancreatic enzymes to supplement or replace the inadequate secretions of the pancreas. Tablets containing pancreatic enzymes are taken with every meal. Because of incomplete absorption even with pancreatic enzymes, a person with CF needs to take in about 30 % more food;

– tube feeding for people with CF, who cannot absorb enough nutrients from the foods (nasogastric tube, gastrostomy tube or jejunostomy tube).

Respiratory health:

– lung function tests are done frequently (changes in functional lung volume and respiratory effort);

– chest X-rays are taken once a year;

– regular exercise;

– clearing mucus from the lungs helps to prevent infection, and mucus control is an important aspect of CF management;

– bronchial drainage (used to aid the mucociliary escalator). The person with CF lies on a tilted surface with head downward, alternately on the stomach, back, or side, depending on the section of lung to be drained. An assistant thumps the rib cage to help loosen the secretions;

– drugs (bronchodilators can open up the airways, steroids reduce inflammation, and mucolytics loosen secretions);

– antibiotics only during infection. Some antibiotics are given as aerosols directly into the lungs. Antibiotic treatment may be prolonged and aggressive;

– supplemental oxygen;

– lung transplantation. About 50 % of adults and more than 80 % of children who receive lung transplants live longer than two years.

Alternative treatment

Homeopathic medicine include:

• mucolytics to help thin mucous;

• supplementation of pancreatic enzymes to assist in digestion.

• respiratory symptoms can be addressed to open lung passages.

• hydrotherapy techniques to help ease the respiratory symptoms and help the body eliminate mucus;

• immune enhancements can help prevent the development of secondary infections;

• dietary enhancements and adjustments are used to treat digestive and nutritional problems.

Prognosis

Early diagnosis is important to prevent malnutrition and infection from weakening the young child. People with CF may lead relatively normal lives. Although most men with CF are functionally sterile, new procedures for removing sperm from the testes are being tried, and may offer the chance to become fathers. Approximately half of people with CF live till the age of 30.

Congenital hypothyroid syndrome

Congenital hypothyroid syndrome is a condition in which a child is born with a deficiency in thyroid gland activity or thyroid hormone levels. The thyroid gland secretes thyroid hormones (thyroxine (T4) and triiodothyronine (T3)) into the bloodstream. A deficiency in the level of these hormones can affect the brain, heart, muscles, skeleton, digestive tract, kidneys, reproductive function, blood cells, other hormone systems, heat production, and energy metabolism.

Other abnormalities can lead to congenital hypothyroidism including:

• abnormal synthesis of thyroid hormones;

• abnormal synthesis of thyroid-stimulating hormone (TSH) or thyrotropin-releasing hormone (TRH), which are regulatory hormones that affect the production of thyroid hormones;

• abnormal response to thyroid hormones, TSH or TRH;

• inadvertent administration of harmful drugs or substances to the pregnant mother, possibly resulting in temporary congenital hypothyroidism in the newborn;

• dietary deficiency of iodine, a raw component vital to the manufacture of thyroid hormones.

Some abnormalities in thyroid hormone synthesis (TSH synthesis), or the response to TSH, are inherited in autosomal recessive fashion.

Congenital hypothyroidism is twice as common in girls as in boys. The condition is less common in African Americans and more common in Hispanics and Native Americans.

Signs and symptoms

The signs and symptoms of congenital hypothyroidism are difficult to observe because the mother passes along some of her thyroid hormones to the fetus during pregnancy.

Features:

– jaundice (yellow skin);

– noisy breathing;

– enlarged tongue;

– feeding problems (constipation, sluggishness, sleepiness, cool hands and feet, failure to thrive);

– other signs (protruding abdomen, slow pulse, enlarged heart, dry skin, delayed teething, coarse hair, myxedema (swelling of the face, hands, feet, and genitals));

– retardation in physical growth, mental development, and sexual maturation.

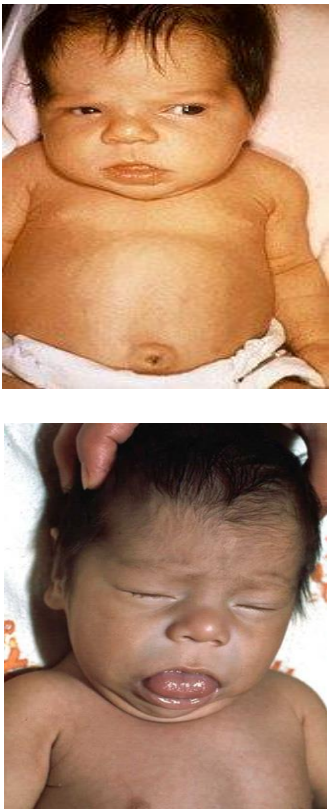

Figure 6 – Baby with congenital hypothyroidism

Diagnosis

– screening test is performed during a newborn's first few days of life. A sample of the child's blood is analyzed for levels of thyroxine (T4), thyroid-stimulating hormone (TSH), or both, also require a second round of screening performed one to four weeks later. Once the diagnosis of congenital hypothyroidism is made, other tests can pinpoint the nature of the abnormality;

– X-rays of the hip, shoulder, or skull often reveal characteristically abnormal patterns of bone development;

– scintigraphy is a method by which images of the thyroid gland and any ectopic thyroid tissue are obtained to determine if the thyroid is absent or ectopic.

Treatment and management

Early treatment offers a good probability of normal development. Treatment of congenital hypothyroidism requires replacement of deficient thyroid hormones with levothyroxine (oral tablet form of T4). There is no need to directly replace T3, since T4 is converted to T3 by the liver and kidney. Blood levels of T4 should be checked regularly to ensure appropriate replacement. The blood levels of TSH should also be monitored since TSH is an indicator of the effectiveness of T4 replacement.

Prognosis

If congenital hypothyroidism is detected and treated early in life, the prognosis is quite good. Most children will develop normally. The most severely affected infants may have mild mental retardation, speech difficulty, hearing deficit, short attention span, or coordination problems.

Neurofibromatosis

Neurofibromatosis (NF), or von Recklinghausen disease, is a dominant disorder which causes development of multiple soft tumors (neurofibromas). These tumors occur under the skin and throughout the nervous system. Both forms of neurofibromatosis are caused by a defective gene. Two types of NF exist, NF-1 (90 % of all cases), and NF-2 (10 % of all cases). NF-1 occurs due to a defect on chromosome 17; NF-2 results from a defect on chromosome 22.

Neurofibromatosis-I occurs in about 1 of every 4 000 births.

Signs and symptoms

Diagnostic criteria for NF1

There are two or more of the following criteria:

• six or more cafe-au-lait macules (patches of tan or light brown skin):

> 5 mm diameter before puberty;

> 15 mm diameter after puberty;

• two or more neurofibroma of any type (occur under the skin, located along nerves or within the gastrointestinal tract) or one plexiform neuroma. Neurofibromas are small and rubbery, and the skin overlying them may be somewhat purple in color;

• freckling in the axillary or inguinal regions;

• two or more Lisch nodules (tiny tumors) in the iris of the eye;

• optic glioma;

• skeletal deformities (scoliosis, pseudarthrosis, humpback or bowed legs);

• the presence of NF-1 in a patient's parent, child, or sibling.

Complications of NF1:

• plexiform neurofibromas;

• congenital bowing of tibia and fibula due to pseudarthrosis;

• optic glioma;

• scoliosis;

• hydrocephalus;

• epilepsy;

• hypertension;

• nerve root compression by spinal neurofibromas;

• malignancy (leukemia, rhabdomyosarcoma, pheochromocytoma, Wilms'tumor);

• learning disability.

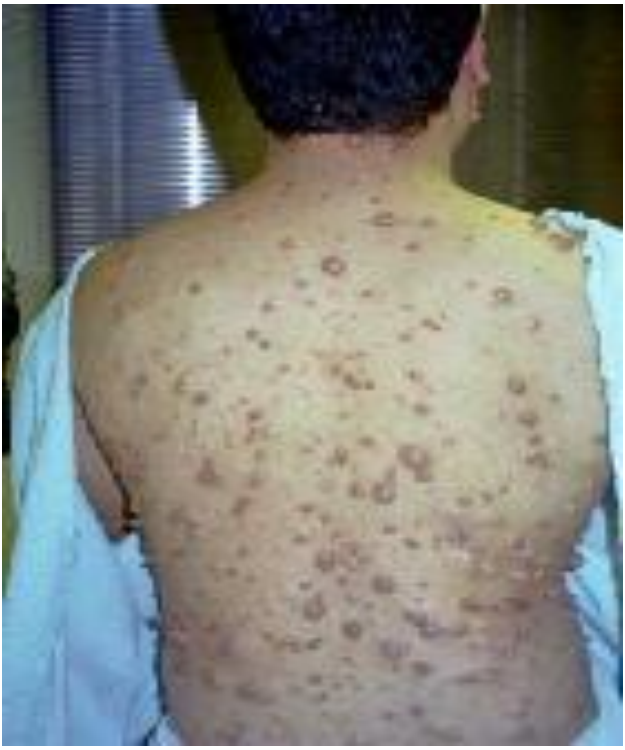

Figure 7 – Multiple neurofibromas

Multiple CLSs over the back. Note the dermal neurofibromas below the right scapula and right side of the lower back.

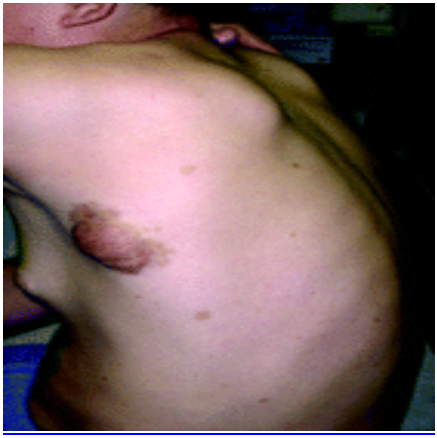

Figure 8 – Large plexiform neurofibroma adjacent to the left axilla

Diagnostic criteria for NF2:

• bilateral vestibular schwannomas – tumors along the acoustic nerve (loss of hearing, weakness of the muscles of the face, headache, dizziness, poor balance, and uncoordinated walking);

• first degree relative with NF2 and either:

a) unilateral vestibular schwannoma;

b) any two features listed below;

• unilateral vestibular schwannoma and two or more other features listed below;

• multiple meningiomas with one other feature listed below – meningioma, glioma, schwannoma, posterior sub-capsular lenticular opacities, cerebral calcification.

Cataracts frequently develop at an unusually early age.

Diagnosis:

– clinical signs, which can be detected by physical examination, ophthalmologic evaluation and audiogram;

– X-ray studies of the bones are frequently indicated to detect the development of deformities;

– CT scans and MRI scans are performed to track the development/progression of tumors in the brain and along the nerves;

– auditory evoked potentials (the electric response evoked in the cerebral cortex by stimulation of the acoustic nerve) may be helpful to determine acoustic nerve involvement;

– EEG (electroencephalogram) may be required for patients with suspected seizures;

– regular blood pressure monitoring.

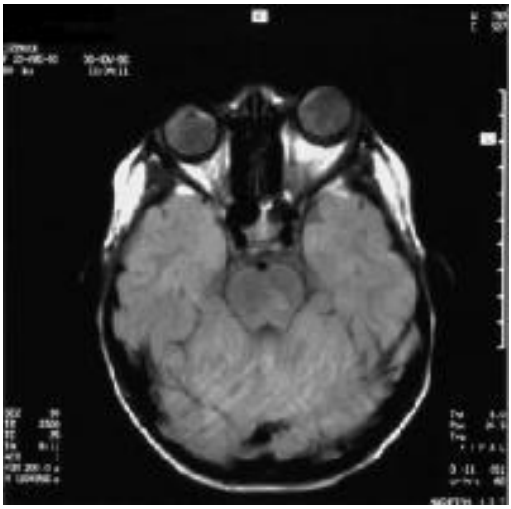

Figure 9 – MRI showing the left optic nerve glioma with thickening of the nerve and proptosis

Treatment

There are no available treatments for the disorders which underlie either type of neurofibromatosis. Skin tumors can be surgically removed or treated with drugs (chemotherapy) or X-ray treatments (radiation therapy). Twisting or curving of the spine and bowed legs may require surgical treatment, or the wearing of a special brace.

Prognosis

Prognosis varies depending on the tumor type which develops. As tumors grow, they begin to destroy surrounding nerves and structures. This destruction can result in blindness, deafness, increasingly poor balance, and increasing difficulty with the coordination. Deformities of the bones and spine can interfere with walking and movement. When cancers develop, prognosis worsens according to the specific type of cancer.

Ehlers – Danlos syndrome

The Ehlers – Danlos syndromes (EDS) is a group of genetic disorders that affect collagen structure and function.

Collagen is a strong, fibrous protein that lends strength and elasticity to connective tissues (skin, tendons, organ walls, cartilage, and blood vessels).

There are numerous types of EDS caused by changes in one of several genes. There are three patterns of inheritance for EDS: autosomal dominant, autosomal recessive, and X-linked (extremely rare).

EDS was described by Dr. Van Meekeren in 1682. Dr. Ehlers and Dr. Danlos further characterized the disease in 1901 and 1908, respectively. Today one in 5000 to one in 10000 people are affected by some form of EDS.

Signs and symptoms

Classification of EDS types was revised in 1997. The new classification involves categorizing the different forms of EDS into six major subtypes:

– classical;
– hypermobility;
– vascular;
– kyphoscoliosis;
– arthrochalasia;
– dermatosparaxis and collection of rare or poorly defined varieties.

Classical type

Classical type was divided into two separate types: type I and type II. EDS classical type is inherited in an autosomal dominant manner.

Symptoms:

– the skin has a smooth, velvety texture and bruises easily;

– patients typically have extensive scarring, particularly at the knees, elbows, forehead, and chin;

– joints are hyperextensible (tendency towards dislocation of the hip, shoulder, elbow, knee, or clavicle);

– decreased muscle tone (delay in reaching motor milestones);

– develop hernias or other organ shifts within the abdomen;

– sprains and partial or complete joint dislocations.

There are three major clinical diagnostic criteria for EDS classical type (skin hyperextensibility, unusually wide scars, joint hypermobility).

Figure 10 – Ehlers – Danlos syndrome.
Skin hyperextensibility

Hypermobility type (EDS type III):

– excessively loose joints (large joints: elbows and knees; small joints (toes and fingers));

– partial and total joint dislocations (jaw, knee, shoulder);

– many individuals experience chronic limb and joint pain (x rays of these joints appear normal);

– the skin may also bruise easily;

– osteoarthritis in adults;

– is inherited in an autosomal dominant manner;

– there are two major clinical diagnostic criteria: hyperextensible skin (or smooth and velvety skin) and generalized joint hypermobility.

Vascular type (type IV)

EDS vascular type is the most severe form and is inherited in an autosomal dominant manner. The connective tissue in the intestines, arteries, uterus, and other hollow organs may be unusually weak, leading to organ or blood vessels rupture (most likely between ages 20 and 40).

Classic features:

– individuals have large eyes, a thin pinched nose, thin lips, and a slim body;

– skin is thin and translucent, with veins dramatically visible, particularly across the chest;

– large joints have normal stability;

– small joints in the hands and feet are loose and hyperextensibile;

– other complications: skin bruises easily, collapsed lungs, premature aging of the skin on the hands and feet, ruptured arteries and veins; during and after pregnancy there is an increased risk of the arterial bleeding and rupture of uterus.

Death usually occurs before the age of 50 years.

EDS vascular type is caused by the change in the gene COL3A1, which codes for one of the collagen chains used to

build Collagen type III. Laboratory testing is available for this form of EDS. A skin biopsy may be used to demonstrate the structurally abnormal collagen. This type of biochemical test identifies more than 95 % of individuals with EDS vascular type. Laboratory testing is recommended for individuals with two or more of the major criteria. DNA analysis may be used to identify the change within the COL3A1 gene.

Kyphoscoliosis type (**type VI):**

– general joint looseness;

– muscle tone is poor;

– motor skill development is delayed;

– infants have scoliosis;

– eyes, skin and blood vessel are fragile and easily damaged.

Kyphoscoliosis type is inherited in an autosomal recessive manner.

This form of EDS is caused by the change in the PLOD gene on chromosome 1, which encodes the enzyme lysyl hydroxylase. A laboratory test is available in which urinary hydroxylysyl pryridinoline is measured. This test is extremely senstive and specific for EDS kyphoscolios type. Laboratory testing is recommended for infants with three or more of the major diagnostic criteria.

Arthrochalasia type (**type VIIB):**

– dislocation of the hip joint;

– other joints are of unusually loose, leading to recurrent partial and total dislocations;

– skin has a high degree of stretchability and bruises easily;

– individuals may experience mildly diminished bone mass, scoliosis, and poor muscle tone.

Arthrochalasia type is inherited in an autosomal dominant manner, and caused by the change in either of two components of Collagen type I, called proa1(I) type A and proa2 (I) type B. A skin biopsy may be performed to

demonstrate an abnormality in either component. Direct DNA testing is available.

Figure 11 – Ehlers – Danlos syndrome. Dorsiflexion of all fingers is easy and absolutely painless

Dermatosparaxis type (VIIC):

– extremely fragile skin that bruises easily but does not scar excessively;

– skin is soft and may sag, leading to an aged appearance even in young adults;

– individuals may experience hernias.

Dermatosparaxis type is inherited in an autosomal recessive manner, is caused by the change in the enzyme called procollagen I N-terminal peptidase. A skin biopsy may be preformed for a definitive diagnosis of dermatosparaxis type.

Other types

There are several other forms of EDS that have not been as clearly defined as the aforementioned types:

– soft, mildly stretchable skin;

– shortened bones;

– chronic diarrhea;

– joint hypermobility and dislocation;

– bladder rupture, or poor wound healing.

Inheritance patterns within this group include X-linked recessive, autosomal dominant, and autosomal recessive.

Diagnosis:

– clinical symptoms;

– family history;

– skin biopsies (for diagnosis of vascular, arthrochalasia, and dermatosparaxis types of EDS);

– urine test (for the kyphoscoliosis type).

Treatment and management

Medical therapy relies on managing symptoms and trying to prevent further complications. There is no cure for EDS. Braces may be prescribed to stabilize joints, but surgery is necessary to repair joint damage caused by repeated dislocations. Physical therapy teaches individuals how to strengthen muscles around joints and may help to prevent or limit damage.

Alternative treatment

Large daily doses (1–4 g) of vitamin C may help decrease bruising and aid in wound healing. Therapy involves protecting the skin with sunscreen and avoiding activities that place stress on the joints.

Prognosis

The outlook for individuals with EDS depends on the type of EDS. Most individuals will have a normal lifespan. People with EDS vascular type, have an increased risk of fatal complications. People with EDS live with fears of significant and painful skin ruptures, of becoming pregnant, of their condition worsening, of becoming unemployed due to physical and emotional burdens, and of social stigmatization in general.

3. HEREDITARY DISEASES OF METABOLISM. MAIN PRINCIPLES OF TREATMENT OF METABOLIC DISORDERS

Major categories of inherited metabolic diseases

Traditionally the inherited metabolic diseases were categorized as disorders of carbohydrate metabolism, amino acid metabolism, organic acid metabolism, or lysosomal storage diseases. In recent decades, hundreds of new inherited disorders of metabolism have been discovered and the categories have proliferated. Following are some of the major classes of congenital metabolic diseases with prominent examples of each class. Many others do not fall into these categories. ICD-10 codes are provided where available.

- 1. Disorders of carbohydrate metabolism:
  - glycogen storage disease.
- 2. Disorders of amino acid metabolism:
  - phenylketonuria, maple syrup urine disease, glutaric acidemia type 1.
- 3. Disorders of organic acid metabolism (organic acidurias):
  - alcaptonuria.
- 4. Disorders of fatty acid oxidation and mitochondrial metabolism:
  - medium chain acyl dehydrogenase deficiency (glutaric acidemia type 2).
- 5. Disorders of porphyrin metabolism:
  - acute intermittent porphyria.
- 6. Disorders of purine or pyrimidine metabolism:
  - Lesch – Nyhan syndrome.
- 7. Disorders of steroid metabolism:
  - congenital adrenal hyperplasia.
- 8. Disorders of mitochondrial function:
  - Kearns – Sayre syndrome.

- 9. Disorders of peroxisomal function:
  - Zellweger syndrome.
- 10. Lysosomal storage disorders:
  - Gaucher's disease, Niemann Pick disease.

Manifestations and presentations

Because of the enormous number of these diseases and wide range of systems affected, nearly every "presenting complaint" to a doctor may have a congenital metabolic disease as a possible cause, especially in childhood. The following are examples of potential manifestations affecting each of the major organ systems:

- growth failure, failure to thrive, weight loss;
- ambiguous genitalia, delayed puberty, precocious puberty;
- developmental delay, seizures, dementia, encephalopathy, stroke;
- deafness, blindness, pain agnosia;
- skin rash, abnormal pigmentation, lack of pigmentation, excessive hair growth, lumps and bumps;
- dental abnormalities;
- immunodeficiency, thrombocytopenia, anemia, enlarged spleen, enlarged lymph nodes;
- many forms of cancer;
- recurrent vomiting, diarrhea, abdominal pain;
- excessive urination, renal failure, dehydration, edema;
- hypotension, heart failure, enlarged heart, hypertension, myocardial infarction;
- hepatomegaly, jaundice, liver failure;
- unusual facial features, congenital malformations;
- excessive breathing (hyperventilation), respiratory failure;
- abnormal behavior, depression, psychosis;
- joint pain, muscle weakness, cramps;

- hypothyroidism, adrenal insufficiency, hypogonadism, diabetes mellitus.

Diagnostic techniques

Dozens of congenital metabolic diseases are now detectable by newborn screening tests, especially the expanded testing using mass spectrometry. This is an increasingly common way for the diagnosis to be made and sometimes results in earlier treatment and better outcome. There is a revolutionary GC/MS based technology with an integrated analytics system, which has now made it possible to test a newborn for over 100 genetic metabolic disorders.

Because of the multiplicity of conditions, many different diagnostic tests are used for screening. An abnormal result is often followed by a subsequent "definitive test" to confirm the suspected diagnosis.

Common screening tests used in the last sixty years:

- ferric chloride test (turned colors in reaction to various abnormal metabolites in urine);
- ninhydrin paper chromatography (detected abnormal amino acid patterns);
- guthrie bacterial inhibition assay (detected a few amino acids in excessive amounts in blood) The dried blood spot can be used for multi-analyte testing using Tandem Mass Spectroscopy (MS/MS). This points to a disorder. The same has to be further confirmed by enzyme assays, GC/MS or DNA Testing;
- quantitative measurement of amino acids in plasma and urine;
- urine organic acid analysis by gas chromatography-mass spectrometry;
- plasma acylcarnitines analysis by mass spectrometry;
- urine purines and pyrimidines analysis by gas chromatography-massspectrometry.

Specific diagnostic tests (or focused screening for a small set of disorders):

- tissue biopsy or necropsy: liver, muscle, brain, bone marrow;
- skin biopsy and fibroblast cultivation for specific enzyme testing;
- specific DNA testing.

Disorders of carbohydrate metabolism

Galactosemia is inherited by autosome-recessive type (classic and Duarte's and Negro type). Frequency of galactosemia is from 1:18000 to 1:187000. Last studies data testifies that galactosemia is observed not less than inherited disorders of aminoacids metabolism. At galactosemia the absence or considerable disease of enzyme galactose 1-phosphate-uridiltranspherase is observed, that leads to galactose and toxic galactose – 1 phosphate accumulation in blood and tissues. Increase of galactose amount is observed also in liquor and urine. Affection of liver, cerebrum, kidneys are observed, cataract, jaundice, hepatomegaly, dyspepsia develops, mental retardation is possible.

For diagnostics of galactosemia the study of blood glucose level by enzyme method using glucoseoxydase or with the help of ortholuidinic reaction or probe on reduced substances in urine, microbiological test with mutant E Coli (DG-73) are used to specify the diagnosis. On the second stage carbohydrates chromanography on thin layer of silicogel, studying of galactose – 1 phosphat – uridiltransferase (by U. E. Veltishev), determination of galactose utilization by child's erythrocytes (Tad's method) is conducted.

Hereditary ***fructose intolerance*** (HFI) or fructose poisoning is a hereditary condition caused by a deficiency of liver enzymes that metabolise fructose. It is also known as hereditary **fructosemia**.

At the core of the disease there is deficiency of fructose 1–phosphate aldolase, which leads to fructose accumulations in blood and excretion with urine.

In urine – albuminuria, hyperaminoaciduria, melituria. At older children hypoglycaemia conditions after eating fruits are possible (sharp paleness, weakness, sweating, arterial hypotension, vomiting, loss of consciousness, cramps, severe hypoglycaemia).

Lactose intolerance is inherited metabolic pathology, conditioned by lactase insufficiency, at which an organism is unable to uptake lactose at all. Degradation of lactose in the intestine causes increasing of the osmotic pressure and diarrhea development.

Disease symptoms show up from the first days of life because of milk eating. There develops: diarrhea, meteorism, hypotrophy and exicosis.

Hydrogen breath test

In a hydrogen breath test, overnight fasted, a dose of 50 grams of lactose (in a water solution) is swallowed. If the lactose cannot be digested, enteric bacteria metabolize it and produce hydrogen. This, along with methane, can be detected in the patient's breath by a clinical gas chromatograph or a compact solid state detector. The test takes about 2 to 3 hours. A medical condition with similar symptoms is fructose malabsorption.

In conjunction, measuring the blood glucose level every 10–15 minutes after ingestion will show a "flat curve" in individuals with lactose malabsorption, while the lactase persistent will have a significant "top", with an elevation of typically 50 to 100 % within 1–2 hours. However, given the need for frequent blood draws, this approach has been largely supplanted by breath testing.

Stool acidity test

This test can be used to diagnose lactose intolerance in infants, for whom other forms of testing are risky or impractical.

Intestinal biopsy

An intestinal biopsy can confirm lactase deficiency following discovery of elevated hydrogen in the hydrogen breath test. Modern techniques have enabled a test to be performed at the patient's bedside which identifies the presence/absence of the lactase enzyme in conjunction with upper gastrointestinal endoscopy. However, for research applications such as mRNA measurements a specialist laboratory is required.

Disorders of amino acid metabolism. Four types of amino acids metabolism disorders are distinguished:

1) disorders accompanied by increase of their concentration in blood and urine (phenylketonuria, histidinemia, tyrosinosis);

2) disorders accompanied by increase of their renal excretion without changes of blood level (homocystinuria);

3) inherited disorders of transport of aminoacids (cystinuria);

4) secondary hyperaminoacidurias as a result of tubular disorders.

Histidinemia, also referred to as ***histidinuria***, is a rare autosomal recessive metabolic disorder caused by a deficiency of the enzyme histidase. Histidase is needed for the metabolism of the amino acid histidine.

The type of inheritance is autosomal recessive. Accumulating of histidine (norm is 2.12 mg/100 ml) and its derivates (imidazole pyruvate, imidazole acetate and imidazole lactate) and decrease of concentration of urocanine, glutamine and other acids takes place as a result of blockade.

Histidinemia is characterized by increased levels of histidine, histamine and imidazole in blood, urine and

cerebrospinal fluid. This also results in decreased levels of the metabolite urocanic acid in blood, urine, and skin cells.

Tyrosinemia (or "Tyrosinaemia") is an error of metabolism, usually inborn, in which the body cannot effectively break down the amino acid tyrosine. Symptoms include liver and kidney disturbances and mental retardation. Untreated, tyrosinemia can be fatal.

Most inborn forms of tyrosinemia produce hypertyrosinemia (high levels of tyrosine).

There are three types of tyrosinemia, each with distinctive symptoms and is caused by the deficiency of a different enzyme.

At tyrosinosis, the change of parahydroxy-phenylpyruvate to homogentisine acid is disordered. Tyrosinemia and tyrosinuria develop.

Big quantity of parahydroxyphenyllactic acid, parahydroxyphenylpiruvate is excreted with urine.

Type of inheritance is autosome-recessive.

In blood the tyrosine level is increased and levels of other amino acids are a little decreased.

Disorders of organic acid metabolism (Organic acidurias)

Alkaptonuria (black urine disease or alcaptonuria) is a rare inherited genetic disorder of phenylalanine and tyrosine metabolism. This is an autosomal recessive condition that is due to a defect in the enzyme homogentisate 1.2-dioxygenase (EC 1.13.11.5) which participates in the degradation of tyrosine. As a result, a toxic tyrosine byproduct called homogentisic acid (or alkapton) accumulates in the blood and is excreted in urine in large amounts (hence – *uria*). Excessive homogentisic acid causes damage to cartilage (ochronosis, leading to osteoarthritis) and heart valves as well as precipitating kidney stones.

The diagnosis of alkaptonuria needs to be suspected before diagnostic testing can be performed using paper chromatography and thin layer chromatography. Both blood plasma and urine can be used for diagnosis. In healthy subjects, homogentisic acid is absent in both blood plasma and urine. In alkaptonuria, plasma levels are 6.6 micrograms/ml on average, and urine levels are on average 3.12 mmol/mmol of creatinine.

Niemann – Pick disease

Definition

Niemann – Pick disease (NPD) is a disorder of fat metabolism that causes abnormalities of the skin, eyes, musculoskeletal system, nervous system, liver, and lymphoid organs. It is named for German pediatricians Albert Niemann (1880–1921) and Ludwig Pick (1898–1935). Six types of the disease have been identified (A, B, C, D, E and F).

Description

Niemann – Pick disease is inherited through an autosomal recessive trait. The different types of NPD are characterized by an abnormal accumulation of sphingomyelin. A sphingomyelin is any group of sphingolipids (consists of a lipid and a sphingosine) containing phosphorus. It occurs primarily in the tissue of the nervous system.

Some characteristics of Niemann – Pick disease may be common for all types. Common symptoms include jaundice, hepatosplenomegaly (enlargement of the liver and spleen), physical and mental impairment, and feeding difficulties. Symptoms for most types of NPD (A, B, C and D) are seen in infancy or early childhood. Alternate names associated with the NPD disorder are lipid histiocytosis, sphingomyelin lipidosis, and sphingomyelinase deficiency.

Genetic profile

Niemann – Pick disease is caused by an autosomal recessive genetic trait, therefore the condition will not appear unless a person receives the same defective gene for fat

metabolism from each parent. This means that if a person is heterozygous for the trait then he/she will be a carrier and if they are homozygous then they will show the trait. There is a 25 % chance for each pregnancy that the disorder will be passed onto the child (ren) if both parents are heterozygous for the trait and a 100 % chance if both parents are homozygous for the trait.

The gene for Niemann – Pick disease types A and B has been located on the short arm (p) of chromosome 11. The gene for types C and D has been located on chromosome 18. NPD types C and D are believed to be allelic disorders. This term means that the two types are due to different mutations (a change in building block sequences) of the same gene. Type E is similar to type C and may be a variant form. It is possible that type F is a mild form of type B but as of 2000 there is no supportive research.

Demographics

Niemann – Pick disease affects males and females equally and has been identified in all races. Type A is the most common form of the disease and is responsible for about 80 % of NPD cases.

Types A and B occur mainly in families of eastern European Jewish descent (Ashkenazi). It is estimated that one in 75 may be a carrier. Type B is also common in individuals from Tunisia, Morocco, and Algeria. Type C is more common in Spanish-Americans in southern New Mexico and Colorado. As of 2000, it is believed that over 300 people in the United States are affected with type C and an estimated one million worldwide. Type D occurs in French-Canadian descendents from Nova Scotia. Type F has been found to affect people of Spanish descent. As of 2000, it is not clear as to which populations are affected by type E.

Signs and symptoms

Type A

This is the infantile or acute form of Niemann – Pick disease. Abnormal accumulation of sphingomyelin is seen in the developing fetus. Sphingomyelin accumulation could represent 2–5 % of the total body weight in individuals with type A. Symptoms may progress rapidly and include the following:

• **Hepatosplenomegaly**. Enlargement of the liver and spleen is due to the low levels of the enzyme sphingomyelinase. This enzyme is required to breakdown sphingomyelin in the body. The decreased levels of this enzyme cause sphingomyelin content of the liver and spleen to be abnormally high. This occurs between the ages of 6 and 12 months. Occurence of liver enlargement is seen more commonly than that of the spleen.

• **Musculoskeletal abnormalities**. Degenerative muscle weakness and floppiness may occur due to a decline in motor and intellectual functioning. This is caused by increased accumulation of sphingomyelin in the nervous system. Seizures and muscular spasms may also occur.

• **Macula.** Pigmentation in the tissue of the eyes may occur. Formation of cherry-red spots may be seen in approximately 50 % of patients diagnosed with NPD type A. This is not visible and can only be detected using special instrumentation.

• **Additional abnormalities**. These include jaundice, fever, and gastrointestinal (GI) problems such as vomiting, diarrhea, and abdominal distention.

Type B

This is the chronic form of Niemann – Pick disease. Symptoms progress slowly and begin during infancy or early childhood. Like type A, type B occurs due to a deficiency of the enzyme sphingomyelinase. Neurological involvement is minimal and usually absent. Symptoms are as follows:

• **Hepatosplenomegaly**. Abnormal enlargement of the liver and spleen occur due to the accumulation of sphingomyelin.

• **Macula**. The formation of cherry-red spots on the eyes may be seen in some affected individuals.

• **Additional abnormalities**. These include a slow growth rate and increased incidence of respiratory infections.

Type C

This type of Niemann – Pick disease occurs due to the inability to breakdown cholesterol. This may lead to a secondary deficiency of acid sphingomyelinase. Studies have shown that there may be two types of NPD type C, NPC1 and NPC2. NPC2 is believed to be caused by a deficiency of HE1 (human epididymis-1), which is a cholesterol-binding protein. NPD type C can occur at anytime between infancy and adulthood but is usually seen in children between the ages of 3 and 10. The progression of symptoms in NPD type C is slow and the loss of mental and motor function usually occur in early adulthood. Symptoms are as follows:

• **Hepatosplenomegaly**. The liver and spleen may be moderately enlarged due to the inability to breakdown cholesterol.

• **Musculoskeletal.** Psychomotor dysfunction, seizures, tremors, and spasticity of the muscles result due to excessive accumulation of cholesterol in the brain. An individual with NPD type C may also exhibit extreme muscle weakness due to emotional excitement and ataxia. Ataxia is the inability to coordinate voluntary muscle movements.

• **Eyes.** Type C is characterized by vertical gaze palsy.

This results in the difficulty or loss of up and down movement. Some individuals may experience opththalmoplegia (loss of muscle ability to move eyes). This is an impaired function of the muscles of the eyes and may cause the eyes to become stuck or fixed in an upward position.

• **Additional abnormalities**. These include dysarthria and jaundice. Dysarthria is the inability to form and speak words clearly. Jaundice is a yellow discoloration of the skin, eyes, and possibly the mucous membranes.

Type D

This is the Nova Scotia variant of Niemann – Pick disease. Like NPD type C, individuals with type D are unable to metabolize cholesterol properly. Individuals with type D do not suffer from a deficiency of acid sphingomyelinase. The symptoms of type D are very similar to type C but vary from case to case.

Type E

As of 2000, many researchers consider this to be a variant form of type C. NPD type E does not usually begin until adulthood and neurological impairment is rare. Symptoms include the following:

• **Hepatosplenomegaly**. Enlargement of the liver and spleen may occur due to the accumulation of cholesterol.

• **Dementia.** This is characterized by confusion, disorientation, deterioration of intellectual capacity and function, and impairment of the memory. Dementia is progressive and irreversible.

• **Ataxia.** Individuals may have an inability to coordinate voluntary muscle movements.

• **Opththalmoplegia.** Individuals with type E may have an inability to control the muscle movement of the eyes.This may cause the eyes to become stuck in a certain position.

Type F

This type of Niemann – Pick disease is characterized by finding of sea-coloured blue cells in the blood and/or bone marrow of individuals and therefore may be called sea-blue histocyte disease. It affects people of Spanish descent and may be a mild form of type B. Symptoms may include:

• **Hepatosplenomegaly**. Abnormal enlargement of the liver and spleen may occur in individuals with NPD type F.

• **Cirrhosis**. The lobes of the liver may become covered with fibrous tissue (thickened tissue). This fibrous tissue obstructs blood flow through the liver.

• **Mild thrombocytopenia**. Individuals with NPD type F may suffer from a decrease in the number of platelets found in the blood. Platelets are necessary for coagulation of the blood.

• **Macula.** Pigmentation in the tissue of the eyes may occur. Individuals may develop a white ring around the maculae of the eyes.

• **Hair.** Individuals may have an absence of hair in the axillary (armpit) area of the body.

Diagnosis

As of 2000, there is no objective diagnostic test for Niemann – Pick disease types D, E, and F. Types A and B are diagnosed through DNA testing or by a blood test.

Blood tests for individuals with types A and B will show low levels of the enzyme sphingomyelinase in white blood cells and elevated sphingomyelin and free cholesterol.

Type C can be diagnosed by prenatal testing of fibroclastic cells to determine their ability to process and store cholesterol. This is done by testing the amniotic fluid (liquid which bathes and cushions the fetus). Formation of foam cells occurs in all types of NPD and can be determined through a biopsy of bone marrow tissue.

Diagnosis of all types is made possible by taking a detailed family history and a thorough examination of the individual.

Symptoms of Niemann – Pick disease may be similar to those of Refsum syndrome (disorder of fat metabolism associated with abnormal accumulation of phytanic acid in the blood and other body tissues), Tay – Sachs disease (disorder found in Eastern European Jewish descendents that results in deterioration of the central nervous system), Sandhoff disease

(lipid storage disorder due to a deficiency of the enzyme hexosaminidase), Gaucher's disease (lipid storage disease), and Sialidosis (metabolic disorder due to a deficiency of the enzyme alpha-neuraminidase).

Treatment and management

As of 2000, there is no specific treatment available for any type of Niemann – Pick disease. Individuals are treated on a symptomatic basis. As of 2000, individuals with NPD types A and B have not benefited from enzyme replacement therapies or organ transplants. Cholesterol lowering drugs and low cholesterol diets are often used for individuals with NPD types C and D. As of 2000, these have not been effective in slowing the progress of types C and D.

Investigational therapies are being tested for types A, B, C and D. The possibility of treatment by bone marrow transplantation is being tested for types A and B. Studies have also been completed on the use of stem cell (a cell which produces usable tissues) transplantation as treatment for types A and B. Researchers at the National Institutes of Health are studying combinations of cholesterol lowering drugs for treatment of NPD types C and D.

Social and lifestyle issues

Individuals diagnosed with Niemann – Pick disease may want to seek counseling or attend support groups that focus on the psychological, physical, and social issues that may result due to the illness.

Parents may want to seek counseling or attend support groups that focus on the lifestyle changes associated with having a child diagnosed with Niemann – Pick disease.

Prognosis

The prognosis for all types of Niemann – Pick disease varies. In type A, death usually results in early childhood. In individuals with types C and D, death usually results in adolescence or early adulthood. Individuals with type B have a

prolonged survival due to the decrease of neurological involvement. As of 2000, the prognosis for types E and F has not been adequately researched. Affected individuals and their families may want to seek genetic counseling. Pregnant women can receive prenatal testing for NPD type C. Pregnant women that are carriers and have a partner that is a carrier should receive genetic counseling regarding the 25 percent chance of the child having Niemann – Pick disease. Early diagnosis is important. Due to advances in medicine an early diagnosis may increase life expectancy.

4. GENERAL CHARACTERISTICS OF CHROMOSOMAL ANOMALIES (NUMERICAL AND STRUCTURAL). CLINICAL PICTURES OF MOST COMMON CHROMOSOMAL DISORDERS

Chromosomal abnormalities are a group of conditions that are the result of a problem with one of the 23 pairs of chromosomes. Chromosomes are the structures within the body's cells that contain genes. Normally, people have 23 pairs of chromosomes. For each of these pairs, one chromosome comes from the mother and the other chromosome comes from the father. However, an abnormality can affect any chromosome, including the sex chromosomes.

A chromosomal abnormality can affect the number of chromosomes, the structure of certain chromosomes, or the composition of chromosomes. If the material found in chromosomes is balanced so that the expected amount is found in each cell, no abnormalities occur. If that balance is upset, chromosomal abnormalities occur and can cause a wide range of abnormalities, usually birth defects or death of the embryo or fetus before birth.

Chromosome abnormalities usually occur when there is an error in cell division. There are two kinds of cell division.

Mitosis results in two cells that are duplicates of the original cell. In other words, one cell with 46 chromosomes becomes two cells with 46 chromosomes each. This kind of cell division occurs throughout the body, except in the reproductive organs. This is how most of the cells that make up our body are made and replaced.

Meiosis results in cells with half the number of chromosomes, 23 instead of the normal 46. These are the eggs and sperm.

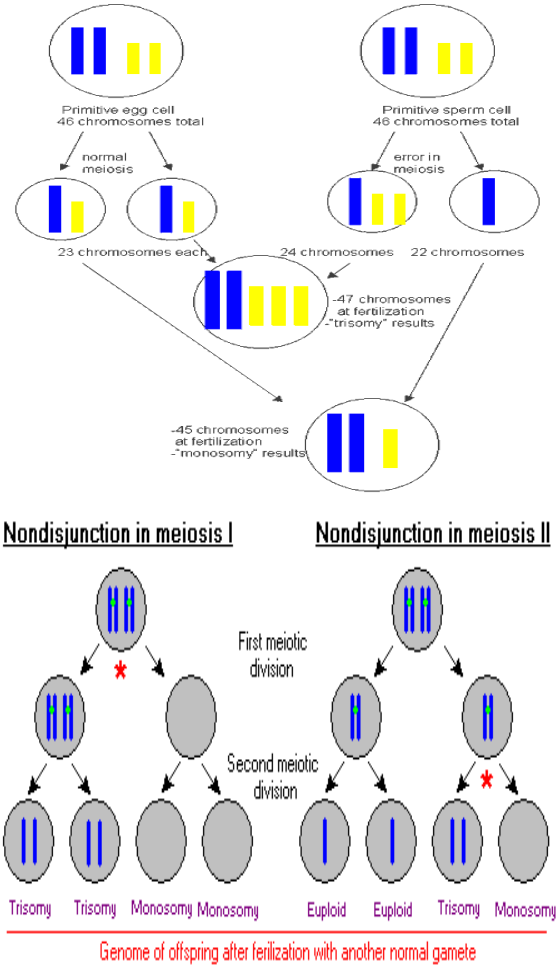

Figure 12 – Meiosis

In both processes, the correct number of chromosomes is supposed to end up in the resulting cells. However, errors in cell division can result in cells with too few or too many copies of a chromosome. Errors can also occur when the chromosomes are being duplicated.

Other factors that can increase the risk of chromosome abnormalities are:

Maternal Age. Women are born with all the eggs they will ever have. Therefore, when a woman is 30 years old, so are her eggs. Some researchers believe that errors can crop up in the eggs'genetic material as they age over time. Therefore, older women are more at risk of giving birth to babies with chromosome abnormalities than younger women. Since men produce new sperm throughout their life, paternal age does not increase risk of chromosome abnormalities.

Environment. Although there is no conclusive evidence that specific environmental factors cause chromosome abnormalities, it is still a possibility that the environment may play a role in the occurence of genetic errors.

- There are two types of chromosome abnormalities: abnormality of chromosome number or structure**.**

Abnormalities of Chromosome Structure

Deletions

Deletions occur when a piece of a chromosome is missing. They may occur as a simple deletion or as a deletion with duplication of another chromosome segment. The latter is usually caused by a crossover in meiosis in a translocation carrier, resulting in an unbalanced reciprocal chromosomal translocation. Deletions may be located at the chromosome ends or in interstitial segments of the chromosome and are usually associated with mental retardation and malformations. Small telomeric deletions may be relatively common in nonspecific mental retardation with minor anomalies. The most commonly observed deletions in humans are 4p-, 5p-, 9p-, 11p-, 13q-, 18p-, and 18q-, which are associated with well-described. Deletions may be observed in routine chromosome preparations, but microdeletions are detectable only under the microscope with prophase chromosome studies. In

submicroscopic deletions, the missing piece can be detected only by using molecular probes or DNA studies.

Microdeletions are defined as small chromosome deletions that are detectable only in high-quality (pro)metaphase preparations. These deletions often involve several genes so that the affected individual may be identified by an unusual phenotype associated with an apparent single gene mutation. Williams, Langer – Giedion, Prader – Willi, Angelman, Rubinstein – Taybi, Smith – Magenis, Miller – Dieker, Alagille, and velocardiofacial/DiGeorge syndromes have all been found to be associated with microdeletions. Submicroscopic deletions are not visible by microscopic examination and are detected only with specific probes for a DNA sequence or DNA studies. The deletion is recognized because of the absence of staining or fluorescence.

Duplications

Duplication is the presence of extra genetic material from the same chromosome. Duplications may result from the abnormal segregation in carriers of translocations or inversions.

Inversions

Inversions require the chromosome to break at two points. The broken piece is then inverted and joined into the same chromosome. Inversions have a frequency of 1/100 liveborns and may be pericentric or paracentric. In pericentric inversions, the breaks are in the two opposite arms of the chromosome so that the intervening portion that contains the centromere is reversed. They are usually discovered because they change the position of the centromere. In contrast, paracentric inversions involve only chromosomal material from one arm of a chromosome. Carriers of inversions are usually normal, but they may have an increased risk of miscarriages and chromosomally abnormal offspring.

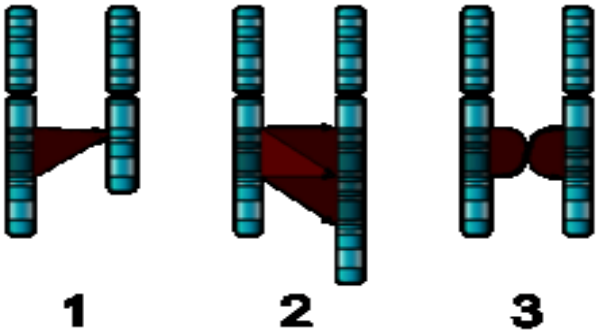

Figure 13 – The three major single chromosome mutations; deletion (1), duplication (2) and inversion (3)

Translocations

Translocations involve the transfer of chromosomal material from one chromosome to another. Translocations may be Robertsonian or reciprocal. They occur with a frequency of 1/500 liveborn human infants. They may be inherited from a parent or appear de novo, with no other affected family members.

Robertsonian translocations involve two acrocentric (centromere located at the end) chromosomes that fuse near the centromeric region with subsequent loss of the nonfunctional, very truncated short arms. The translocation chromosome is made up of the long arms of two fused chromosomes, hence the resulting count is only 45 chromosomes. The loss of the short arms of acrocentric chromosomes has no known deleterious effect. Although carriers of a Robertsonian translocation are usually phenotypically normal, they are at increased risk for miscarriages and abnormal offspring. Reciprocal translocations are the result of breaks in nonhomologous chromosomes with reciprocal exchange of the broken segments. Carriers of a reciprocal translocation are usually phenotypically normal but also have an increased risk

of having chromosomally abnormal offspring and miscarriages because of abnormalities in the segregation of the chromosomes in the germ cells.

Insertions

Insertions occur when a piece of chromosome breaks at two points and is incorporated into a break in another part of a chromosome. This requires three breakpoints and may occur between two chromosomes or within one.

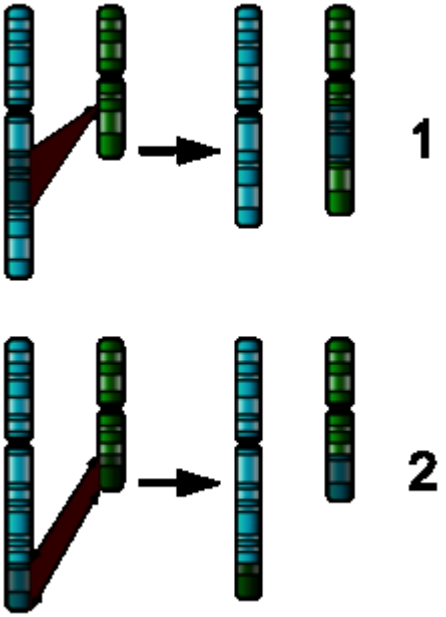

Figure 14 – The two major two chromosome mutations; insertion (1) and translocation (2)

Abnormalities of Chromosome Number

Aneuploidy and Polyploidy

When a human cell has 23 chromosomes, it is referred to as a haploid cell (the number of chromosomes in an ova or sperm). Any number of chromosomes that is an exact multiple

of the haploid number (e. g., 46, 69, 92 in humans) is referred to as euploid. Euploid cells with more than the normal diploid number of 46 chromosomes are called polyploid cells. Polyploid conceptions are usually not viable. However, they may be present in mosaic (more than one cell line) forms, which allow survival. Cells with three sets of chromosomes are called triploid and are frequently seen in abortus material and occasionally in viable humans, usually in mosaic form. Cells deviating from the multiples of the haploid number are called aneuploid (i. e., not euploid), indicating a missing or extra chromosome.

Trisomies

The most common abnormalities of chromosome number (aneuploidy) are trisomies. These occur when there are three representatives of a particular chromosome instead of the usual two. Trisomies are usually the result of meiotic nondisjunction (failure of a chromosome pair to separate). Trisomy may be present in all cells or may occur in mosaic form. Most individuals with trisomies exhibit a consistent and specific phenotype depending on the chromosome involved. The most frequent trisomy in humans is trisomy 21 or Down syndrome. Trisomies of chromosome 18 and chromosome 13 are also relatively common and are associated with a characteristic set of congenital anomalies and mental retardation.

Trisomy 21 (Down syndrome)

Dr. John Langdon H. Down first described a cluster of mentally retarded patients in an England asylum in an essay, "Observations on an Ethnic Classification of Idiots" in 1866. It was not until the 1950s that an extra 21st chromosome was found to be responsible for what was to become known as Down syndrome. The incidence is 1 in 600–800 births. The sporadic form has a higher frequency with advanced maternal

age. In mothers less than 25 years of age, the risk is 1 in 2 000 births and climbs to 1 in 20 births for mothers over age 40. All patients with Down syndrome have three copies of chromosome 21. In 95 % of patients, there are 47 chromosomes with trisomy of chromosome 21. In about 5 %, there are 46 chromosomes, with an abnormally translocated 21st chromosome. Robertsonian translocations involve the transfer of chromosomal material from 21 to usual chromosome number 13, 14, or 15. The Down phenotype occurs when even a small, but critical piece of the long arm of chromosome 21 is trisomic. Carriers of a Robertsonian translocation are usually phenotypically normal, but are at increased risk for miscarriages and chromosomally abnormal children. Another cause is a 21q/21q translocation. This is rare, but significant because a carrier parent only has one 21st chromosome (the translocated chromosome with double genetic material). When this chromosome is passed on, all of the offsprings will have trisomy 21. An example of this is a mother who had this translocation and had four children with Down syndrome. Milder phenotypes may be seen when patients are mosaic for trisomy 21. This happens when nondisjunction occurs early in embryonic development as a mitotic error.

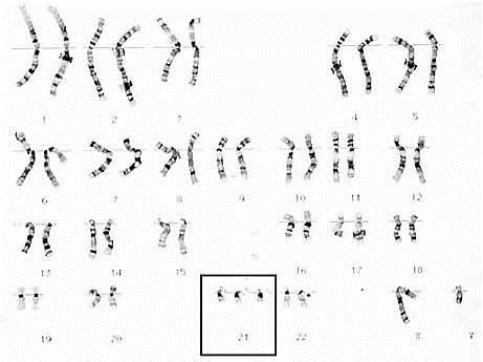

Figure 15 – Karyotype of a patient with Down syndrome

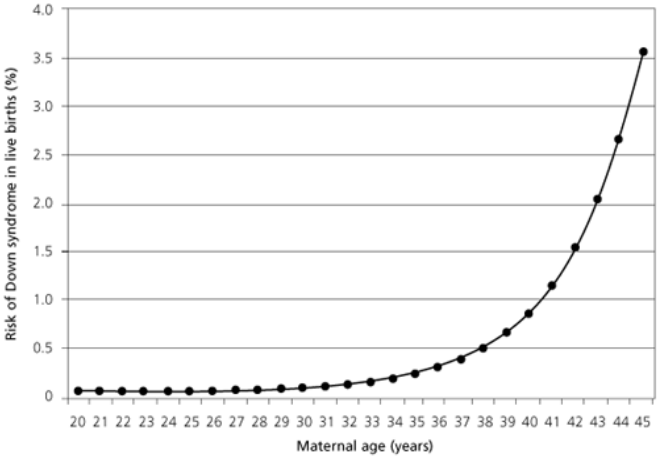

Figure 16 – Risk correlation between maternal age and having of babies with Down syndrome

Affected patients have a characteristic facies including epicanthal folds, a flat nasal bridge, small mouth, protruding tongue with microcephaly and a flat occiput. Other features may include a high arched palate, a single palmar crease (Simian crease). The pupils may have light smudgy opaque Brushfield spots.

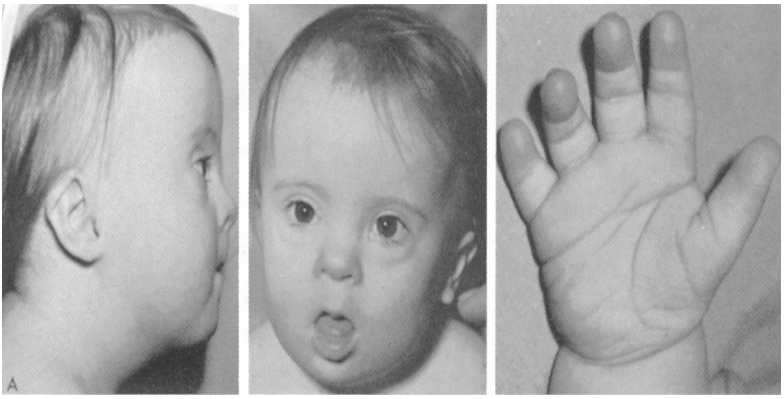

Figure 17 – Baby with Down syndrome

At birth, patients are often hypotonic and have a higher incidence of other types of malformations. Cardiac anomalies are present in 33–50 % and include endocardial cushion defects and ventricular septal defects. Gastrointestinal anomalies can include duodenal atresia and Hirschsprung's disease. Later in life, hypothyroidism and leukemia can occur, and there is an increased susceptibility to infections. Atlantooccipital instability may be present in a few patients and is a concern when intubating these patients. A pneumonic for remembering the major complications found in Down syndrome patients is using the word VALIDATE as follows: VSD, atlantooccipital instability, leukemia, immunodeficiency, duodenal atresia, Alzheimer's disease, thyroid dysfunction, and endocardial cushion defects.

Prenatal diagnosis

- The risk of trisomy 21 is directly related to maternal age.
- Patients who will be 35 years or older on their due date should be offered chorionic villus sampling or second-trimester amniocentesis.
- Women younger than 35 years should be offered maternal serum screening at 16 to 18 weeks of gestation.
- The maternal serum markers used to screen for trisomy 21 are alpha-fetoprotein, unconjugated estriol and human chorionic gonadotropin.
- The use of ultrasound to estimate gestational age improves the sensitivity and specificity of maternal serum screening. During the first trimester of the majority of pregnancies, it is possible to measure the size of the fluid area at the back of the fetus's neck, known as the nuchal translucency or NT. The increasing size of the NT indicates a greater risk of the fetus having Down syndrome.

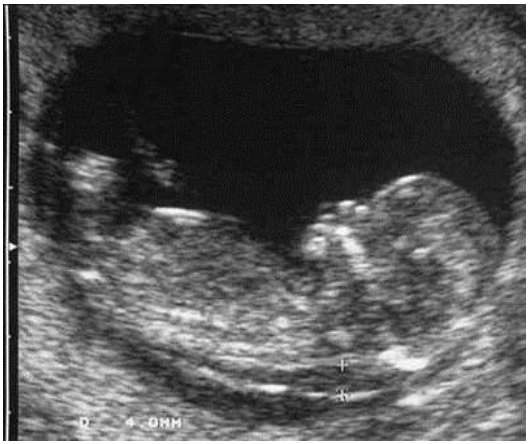

Figure 18 – Ultrasound examination of a fetus with Down syndrome

There is no treatment for the trisomy itself, so therapy is directed towards other complications present, such as cardiac and gastrointestinal anomalies, thyroid dysfunction, and infections. Their IQ is usually about 50–75, at best they function at a sixth grade level, and few are severely retarded. These children are placed in infant stimulation programs, enrolled in special education classes, and later given occupational training to help them become more independent and a functioning part of society. It is very important to counsel parents who have one child with Down syndrome about the risk of having a second affected child. The risk of recurrence is 1 % in otherwise low risk moms and if the parent is not a translocation carrier. Obstetric screening tests can identify some pregnancies at risk, so that fetal chromosome testing can be offered. Involvement in compassionate support groups should be encouraged to the parents.

Trisomy 18 (Edwards Syndrome or Trisomy E)

Infants with trisomy 18 are severely affected and usually die in the first week of life. Less than 10 % of patients

survive beyond twelve months and are profoundly mentally retarded. The cause of this syndrome is usually full trisomy for chromosome 18. The incidence is 1 in 4000–8000 births, with a 3:1 predominance of affected females to males. The risk increases with maternal age. The recurrence risk is very low based on the observation that most affected children die in utero. Patients with trisomy 18 mosaicism have a less severe clinical expression and longer survival depending on the degree of mosaicism. Partial trisomy 18 varies in its clinical picture from mild mental deficiency and improved survival to being indistinguishable from full trisomy 18. This depends on the extent and which part of the chromosome is affected.

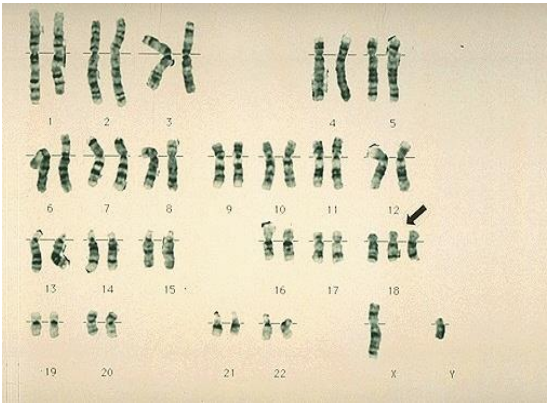

Figure 19 – Karyorgam of a patient with trisomy 18 (Edwards syndrome)

Numerous malformations have been reported in trisomy 18. These infants have a characteristic head shape and facial features, such as a prominent occiput, low-set ears, and micrognathia. The hands are often clenched with the index finger overlapping the third finger. Dysmorphic joints produce this typical finger position. Deformities of the lower extremities include hypoplastic nails of the feet, malaligned

toes and “rocker-bottom feet”, where the calcaneus is prominent. Heart defects, such as ventricular septal defect, patent ductus arteriosus or atrial septal defect, are found in at least 50 % of these patients. They also have a short sternum with small nipples.

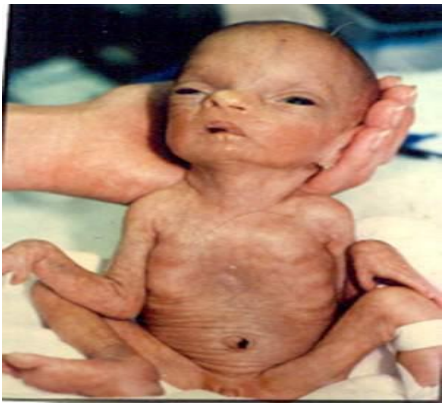

Figure 20 –Trisomy 18 (Edwards Syndrome)

Supportive care is the treatment for this syndrome. Genetic counseling is indicated. Chromosomal studies should be done to determine translocation cases. The recurrence risk for full 18 trisomy cases is less than 1 % because most die in embryonic or fetal life. Some children with trisomy 18 have reached adulthood. Heart failure or pneumonia are some of the complications that often cause death.

Trisomy 13 (Patau Syndrome or Trisomy D)

The constellation of findings in this condition has been described as far back as the 1600s. Klaus Patau and his colleagues were the first to attribute the syndrome of trisomy for chromosome 13 by cytogenetic analysis in 1960. The cause of this syndrome is usually trisomy for chromosome 13. Its overall incidence is 1 in 12 000 births, but the risk increases

with maternal age. The recurrence rate is very low, except for the parent who has a balanced translocation. Patients with trisomy 13 mosaicism have been described and usually have a milder phenotype depending on the degree of mosaicism.

Patients are severely affected and often die in infancy. The median survival has been reported as two and a half days. Surviving infants have severe mental retardation and may have midline CNS defects, incomplete development of the forebrain, apneic episodes and EEG abnormalities. Bilateral cleft lip and palate are common. Microcephaly, microphthalmia, and deafness may also be found. Lethal cardiac anomalies are found in 80 % of patients, with VSD being the most common. PDA, ASD, dextroposition, and valvular abnormalities can also occur. Disorders of the extremities may include a single palmar crease (Simian crease). Polydactyly, particularly of all extremities, strongly suggests trisomy 13. Overlap of the middle and ring fingers are seen.

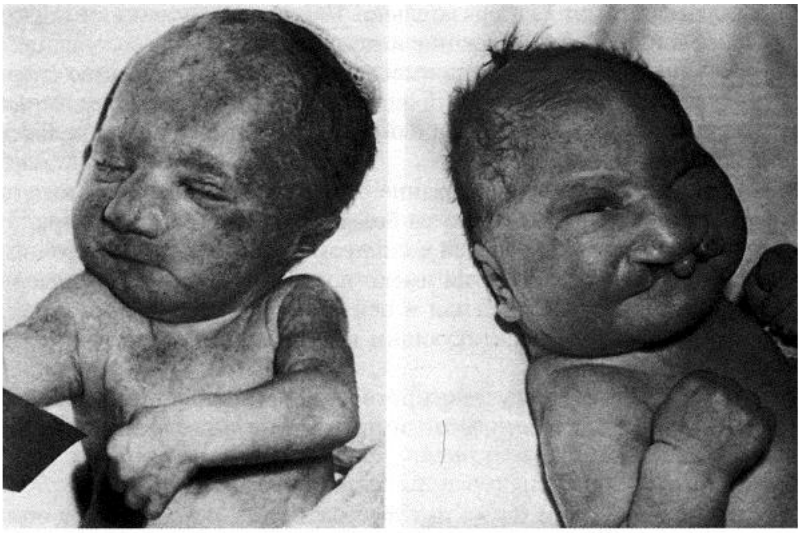

Figure 21 – Trisomy 13 (Patau syndrome)

Treatment is supportive. Prevention by genetic counseling is indicated. Critical decisions in regards to extensive therapy and resuscitation measures in a severely affected infant must be decided at birth. Those affected usually die in infancy; very few live to become adults. Children who survive are severely retarded and are rarely able to suck. They fail to thrive and have seizures.

Klinefelter Syndrome (47 XXY)

Harry Fitch Klinefelter worked with Dr. Fuller Albright in clinical endocrinology when he saw his first patient, who was a young man with small testes and gynecomastia. During his fellowship, he encountered 8 other patients with similar findings and described Klinefelter syndrome in 1942. Klinefelter syndrome affects 1 in 1000 newborn boys and is caused by an extra X chromosome from meiotic nondisjunction. The incidence is 1 % among the mentally retarded and 3 % among males seen at infertility clinics. In 54 % of the patients, the extra X chromosome comes from the mother and in the other 46 %, the extra X chromosome comes from the father. Maternal age, but not paternal age is often advanced. The most common chromosomal pattern is 47,XXY. Others have mosaic patterns: 46,XY/47,XXY; 46,XY/48,XXYY; or 46,XX/47,XXY.

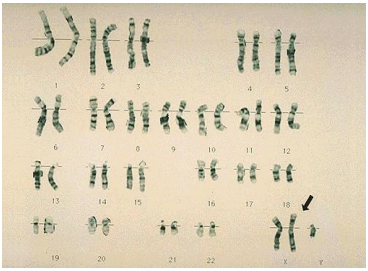

Figure 22 – Karyorgam of a patient
with Klinefelter syndrome (47 XXY)

These patients tend to have milder phenotypes. Children with mosaicism have a better prognosis for fertility and virilization. In chromosome variants with multiple X chromosomes (XXXY and XXXXY), the clinical manifestations are much more severe. In general, the mental and physical abnormalities associated with Klinefelter syndrome worsen as the number of X chromosomes increase.

The characteristic findings of Klinefelter syndrome usually do not become apparent until after puberty. They have a eunuchoid habitus; usually tall, slim and underweight, with long legs. One third have gynecomastia and they have less facial hair. Their gonads are small and soft, and the phallus tends to be smaller than average. The seminiferous tubules are atrophied because of excess gonadotropin. There is hyperplasia of the Leydig cells, producing azoospermia and infertility. Cryptorchidism may occur in some patients. Hypogonadism becomes recognized after puberty when the testicles fail to grow and develop normally. This is usually the time when the diagnosis is suspected. Pubertal development may be delayed. These patients tend to have learning and psychosocial problems. They have normal to borderline IQ, with a mean IQ of 90. In boys with mental retardation, learning disabilities or adjustment problems at school, Klinefelter syndrome should be a consideration. Later in life, these individuals are at higher risk to develop diabetes mellitus. There is also an increased incidence of cancer of the breast, varicose veins, and pulmonary disease.

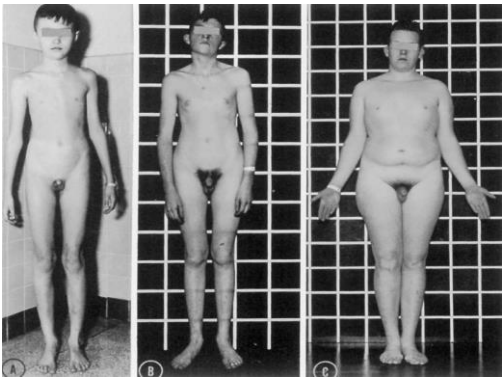

Figure 23 – Patients with Klinefelter syndrome (47 XXY)

Chromosomal analysis should be done to confirm the diagnosis of Klinefelter syndrome. In males younger than 10 years of age, they have normal levels of FSH and LH and respond appropriately to GnRH and hCG. In late adolescence, testosterone levels decrease, and gonadotropins increase. In adults, urinary excretion of gonadotropins is high, with levels comparable to those seen in post menopausal women. Gynecomastia is a result of an elevated estradiol to testosterone ratio. In the management of Klinefelter syndrome, testosterone replacement therapy should start at 11 to 12 years of age, if testosterone levels are deficient and gonadotropin levels become elevated. With early recognition and diagnosis, treatment can be initiated to allow a more normal maturation for the affected male, but infertility cannot be reversed.

Turner Syndrome (45X)

In 1938, a series of young women with failure of sexual maturation, short stature, and neck webbing were reported by Henry Turner. He believed this was due to a defect in the anterior pituitary gland. It was not until 1959, when the absence of the X chromosome was first described by Charles Ford. The incidence is 1 in 2 500 live female births, 95 % of

45X fetuses die in utero. About 50 % of patients apparently have the full monosomy 45,X, the others all have detectable mosaicism. About 2/3 retain the maternal X and 1/3 have the paternal X. Advanced maternal age is not a factor in this syndrome. Only one X is normal and functioning; the other X is not present or is missing a part of its chromosome by structural abnormality, deletion or translocation. Mosaicism 45,X/46,XX or 45,X/46,XX/47,XXX may also be present. 10 % of Turner mosaics have a Y chromosome, 45,X/46,XY is seen. The presence of the Y chromosome increases the risk of gonadoblastoma. Milder phenotypes are usually seen in those without the full monosomic karyotype.

The characteristic features include a triangular face, small mandible, prominent ears, webbed neck, low posterior hair line, shield chest with wide set nipples, cubitus valgus (increased carrying angle of the elbow), and short stature. Sometimes short stature may be the only clinical finding present in a young girl. Their average adult height is about 143 cm (4 ft., 8 in.). In most of these girls, secondary sexual characteristics are absent.

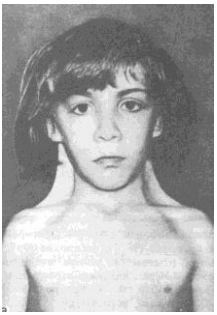

Figure 24 – Webbed neck of a patient with Turner syndrome (45X)

They have amenorrhea and are infertile due to ovarian dysgenesis. Intelligence is usually normal, but they may have a learning disability. These patients may have cardiac defects;

30–50 % have bicuspid aortic valve, and 10–20 % have coarctation of the aorta. Other cardiac complications include aortic stenosis, aortic dissection and idiopathic hypertension. Urinary tract malformations are found with a higher frequency in these patients. Most common presentations include a horseshoe kidney, kidney located in the pelvis, double collecting system, or absence of a kidney.

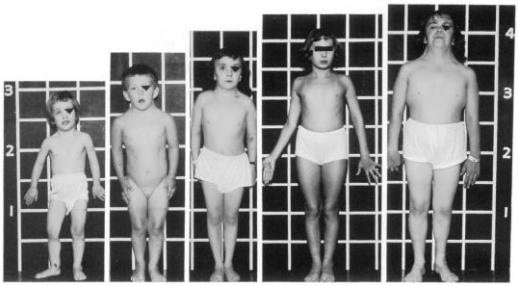

Figure 25 – Short stature. Tendency to become obese

Growth hormone alone or in combination with anabolic steroids has been successful in managing these patients. This is still very controversial. Opponents claim that growth hormone accelerates growth, but does not increase adult height. Therapy should begin when the height of the patient drops below the 5th percentile on the growth curve. For those who are deficient in estrogen and progestin, long term replacement therapy is required for development of secondary sexual characteristics and initiation of the menstrual cycle. As for all post-menopausal women, these women especially need hormonal therapy, in combination with calcium supplementation and exercise, to help prevent osteoporosis. Rarely, spontaneous puberty and menses can occur and pregnancy is possible. There is an increased risk of giving birth to a child with chromosomal or congenital anomalies. Genetic counseling and a medical work up are required. Support groups can be of much benefit. These women can become independent

and lead productive lives. Most die in utero, but for those who survive, the average age of death is 69. Increased mortality can primarily be attributed to cardiovascular disease.

Noonan Syndrome

This syndrome resembles Turner syndrome and occurs in males and females. The occurrence is often sporadic, but an autosomal dominant inheritance has been reported. A gene for this disorder has been mapped to chromosome 12q; although not in every family, suggesting that other loci may be involved.

The clinical manifestations are short stature, short or webbed neck, shield chest and pectus excavatum or carinatum. They have a characteristic facies; epicanthal folds, ptosis, hypertelorism, downslanting palpebral fissures, and low or abnormal ears. Interestingly, their facies can normalize with age. The most common cardiac abnormality is pulmonary valve stenosis, but they can also have atrial septal defects, left ventricular hypertrophy, or patent ductus arteriosus. Males may have cryptorchidism, small penis, and hypogonadism. However, in males without cryptorchidism, fertility is normal. Fertility is also normal in females. About 25 % of affected patients have some degree of mental deficiency.

There is no specific treatment. Genetic counselling should be offered to parents regarding the recurrence risk.

XYY Syndrome

In random newborn males, the incidence of the 47,XYY karyotype is 1 in 1000. In some prison populations, the incidence is 1–2 %. In institutionalized men or juvenile delinquents taller than 6 feet, 10 % have XYY syndrome. Most 47XYY males are phenotypically normal, however variable, non-specific characteristics may be seen. Stature tends to be tall, and patients may have large teeth and severe nodulocystic acne. They are not well coordinated or strong relative to their large size. Some males have anti-social

behavior. They may be impulsive, hyperactive, have poor social skills, and low self esteem. They were once stereotyped as being violent and aggressive. However, longitudinal studies suggest that aggressive behavior is usually not a problem, and they learn to control their anger by the time they become young adults. These males have a normal to low IQ, and 50 % have a learning disability. The majority of patients are fertile. Their sperm may have 23X, 23Y, 24XY or 24YY, so the syndrome can recur. However, they usually have children with normal chromosomes.

XYY syndrome is diagnosed by karyotype. There are no consistent clinical findings. There is no treatment for this syndrome. In general, affected children are normal. However, behavioral modification is necessary in dealing with the hyperactivity and aggressiveness that may be seen during childhood. The majority are well-adapted citizens.

Fragile X Syndrome

The syndrome was first described by Martin and Bell in 1943, though the fragile site on the X chromosome was reported in 1969 by Lubs. It occurs in 1 in 1 000 males. This disorder is caused by an expanded trinucleotide repeat (CGG) in the FMR-1 gene on the X chromosome with X-linked inheritance. When cells, from patients with this disorder, are cultured in a certain medium deficient in folate or thymidine, the fragile site can be visualized. The normal allele repeat range is 6–40. The intermediate allele repeat range is 41–60 repeats (most are stably inherited), and the premutation allele repeat range is 61–200 repeats. Carriers of the premutation are usually phenotypically normal and not at increased risk for retardation. Females who carry this premutation may pass down an expanded version resulting in full expression of the phenotype in males and variable phenotypic expression in females. The severity correlates with the increase in size of the repeat sequence. The full mutation (disease causing) repeat

range is greater than 200 repeats. Other disorders that are caused by expanded trinucleotide repeats include Huntington disease, myotonic dystrophy and Friedreich ataxia.

Physical abnormalities in males with fragile X syndrome include large ears, a large jaw and large, soft testicles. Connective tissue dysfunction, mitral valve prolapse and dental crowding can also be found. Cluttered speech, autism, hyperactivity and mild to severe mental retardation are common. Females with fragile X syndrome (usually heterozygous) tend to have a less severe clinical expression and only 1/3 have mental retardation.

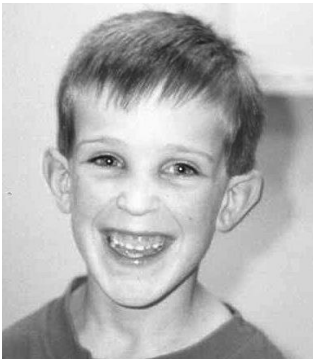

Figure 26 – Patient with Fragile X Syndrome

Treatment and management includes supportive care. Genetic counselling is important in prenatal and postnatal diagnosis of fragile X syndrome. Clinical expression is different depending on which parent transmits the gene. DNA analysis helps to identify full mutations as well as premutation carriers. The life expectancy is normal.

5. GENERAL CHARACTERISTICS OF MULTIFACTORIAL DISORDERS. PREVENTIVE METHODS

Multifactorial inheritance

The concept of multifactorial inheritance implies that a disease is caused by the interaction of several adverse genetic and environmental factors. The liability of a population to a particular disease follows a normal distribution curve, most people showing only moderate susceptibility and remaining unaffected. Only when a certain threshold of liability is exceeded the disorder is manifested. Relatives of an affected person will show a shift in liability, with a greater proportion of them being beyond the threshold. Familial clustering of a particular disorder may therefore occur. Genetic susceptibility to common disorders is likely to be due to sequence variation in a number of genes, each of which has a small effect, unlike the pathogenic mutations seen in Mendelian disorders. These variations will also be seen in the general population and it is only in combination with other genetic variations that disease susceptibility becomes manifested. Unravelling the molecular genetics of the complex multifactorial diseases is much more dif ficult than for single gene disorders. Nevertheless, this is an important task as these diseases account for the great majority of morbidity and mortality in developed countries. Approaches to multifactorial disorders include the identification of disease associations in the general population, linkage analysis in affected families, and the study of animal models. Identification of genes causing the familial cases of diseases that are usually sporadic, such as Alzheimer disease and motor neurone disease, may give insights into the pathogenesis of the more common sporadic forms of the disease. In the future, understanding genetic susceptibility may enable screening for and prevention of common diseases as well as identifying people likely to respond to particular drug regimes. Several

common disorders thought to follow polygenic inheritance (such as diabetes, hypertension, congenital heart disease and Hirschsprung disease) have been found in some individuals and families to be due to single gene defects. In Hirschprung disease (aganglionic megacolon) family data on recurrence risks support the concept of sex-modified polygenic inheritance, although autosomal dominant inheritance with reduced penetrance has been suggested in some families with several affected members. Mutations in the ret protooncogene on chromosome 10q11.2 or in the endothelin-B receptor gene on chromosome 13q22 have been detected in both familial and sporadic cases, indicating that a proportion of cases are due to a single gene defect.

Risk of recurrence

The risk of recurrence for a multifactorial disorder within a family is generally low and mainly affects first degree relatives. In many conditions family studies have reported the rate with which relatives of the index case have been affected. This allows empirical values for risk of recurrence to be calculated, which can be used in genetic counselling. Risks are mainly increased for first degree relatives. Second degree relatives have a slight increase in risk only and third degree relatives usually have the same risk as the general population. The severity of the disorder and the number of affected individuals in the family also affect recurrence risk. The recurrence risk for bilateral cleft lip and palate is higher than the recurrence risk for cleft lip alone, and the recurrence risk for neural tube defect is 4 % after one affected child, but 12 % after two. Some conditions are more common in one sex than the other. In these disorders the risk of recurrence is higher if the disorder has affected the less frequently affected sex. As with the other examples, the greater genetic susceptibility in the index case confers a higher risk to relatives. A rational approach to preventing multifactorial disease is to modify

known environmental triggers in genetically susceptible subjects. Folic acid supplementation in pregnancies at increased risk of neural tube defects and modifying diet and smoking habits in coronary heart disease are examples of effective intervention, but this approach is not currently possible for many disorders.

Heritability

The heritability of a variable trait or disorder reflects the proportion of the variation that is due to genetic factors. The level of this genetic contribution to the aetiology of a disorder can be calculated from the disease incidence in the general population and that in relatives of an affected person. Disorders with a greater genetic contribution have higher heritability, and hence, higher risks of recurrence.

Factors increasing risk to relatives in multifactorial disorders:

- high heritability of disorder;
- close relationship to index case;
- multiple affected family members;
- severe disease in index case;
- index case being of sex not usually affected.

Twins

Twins share a common intrauterine environment, but though monozygous twins are genetically identical with respect to their inherited nuclear DNA, dizygous twins are no more alike than any other pair of siblings, sharing, on average, half their genes. This provides the basis for studying twins to determine the genetic contribution in various disorders, by comparing the rates of concordance or discordance for a particular trait between pairs of monozygous and dizygous twins. The rate of concordance in monozygous twins is high

for disorders in which genetic predisposition plays a major part in the aetiology of the disease.

The phenotypic variability of genetic traits can be studied in monozygous twins, and the effect of a shared intrauterine environment may be studied in dizygous twins. Twins may be derived from a single egg (monozygous, identical) or two separate eggs (dizygous, fraternal). Examination of the placenta and membranes may help to distinguish between monozygous and dizygous twins but is not completely reliable. Monozygosity, resulting in twins of the same sex who look alike, can be confirmed by investigating inherited characteristics such as blood group markers or DNA polymorphisms (fingerprinting).

Twinning

Dizygous twins:

• may be familial;

• more common in black people than white Europeans.

Monozygous twins:

• seldom familial;

• occur in 0.4 % of all pregnancies;

• associated with twice the risk of congenital malformations as singleton or dizygous twin pregnancies.

Diabetes

A genetic predisposition is well recognised in both type I insulin dependent diabetes (IDDM) and type II non-insulin dependent diabetes (NIDDM). Maturity onset diabetes of the young (MODY) is a specific form of non-insulin dependent diabetes that follows autosomal dominant inheritance and has been shown to be due to mutations in a number of different genes. Clinical diabetes or impaired glucose tolerance also occurs in several genetic syndromes, for example, haemochromatosis, Friedreich ataxia, and Wolfram syndrome (diabetes mellitus, optic atrophy, diabetes insipidus and deafness). Rarely, diabetes is caused by the secretion of an

abnormal insulin molecule. IDDM affects about 3 per 1 000 of the population in the UK and is a T cell dependent autoimmune disease. Genetic predisposition is important, but only 30 % of monozygous twins are concordant for the disease and this indicates that environmental factors (such as triggering viral infections) are also involved. About 60 % of the genetic susceptibility to IDDM is likely to be due to genes in the HLA region. The overall risk to siblings is about 6 %. This figure rises to 16 % for HLA identical siblings and falls to 1 % if they have no shared haplotype. An association with DR3 and DR4 class II antigens is well documented, with 95 % of insulin dependent diabetics having one or both antigens, compared to 50–60 % of the normal population. As most people with DR3 or DR4 class II antigens do not develop diabetes, these antigens are unlikely to be the primary susceptibility determinants. Better definition of susceptible genotypes is becoming possible as subgroups of DR3 and DR4 serotypes are defined by molecular analysis.

For example, low risk HLA haplotypes that confer protection always have aspartic acid at position 57 of the DQB1 allele. High risk haplotypes have a different amino acid at this position and homozygosity for non-aspartic acid residues is found much more often in diabetics than in non-diabetics. The second locus identified for IDDM was found to be close to the insulin gene on chromosome 11. Susceptibility is dependent on the length of a 14bp minisatellite repeat unit. Short repeats (26–63 repeat units) confer susceptibility, perhaps by influencing the expression of the insulin gene in the developing thymus. Subsequent mapping studies have identified a number of other possible IDDM susceptability loci throughout the genome, whose modes of action are not yet known. NIDDM is due to relative insulin deficiency and insulin resistance. There is a strong genetic predisposition although other factors such as obesity are important. Concordance in monozygotic twins is 40–100 % and the risk to

siblings may approach 40 % by the age of 80. Although the biochemical mechanisms underlying NIDDM are becoming better understood, the genetic causes remain obscure. In rare cases, insulin receptor gene mutations, mitochondrial DNA mutations or mild mutations in some of the MODY genes are thought to confer susceptability to NIDDM.

Coronary heart disease

Environmental factors play a very important role in the aetiology of the coronary heart disease, and many risk factors have been identified, including high dietary fat intake, impaired glucose tolerance, raised blood pressure, obesity, smoking, lack of exercise and stress. A positive family history is also important. The risk to first degree relatives is increased to six times above that of the general population, indicating a considerable underlying genetic predisposition. Lipids play a key role, and coronary heart disease is associated with high LDL cholesterol, high ApoB (the major protein fraction of LDL), low HDL cholesterol and elevated Lp(a) lipoprotein levels. High circulating Lp(a) lipoprotein concentration has been suggested to have a population attributable risk of 28 % for myocardial infarction in men aged under 60. Other risk factors may include low activity of paraoxonase and increased levels of homocysteine and plasma fibrinogen. Lipoprotein abnormalities that increase the risk of heart disease may be secondary to dietary factors, but often follow multifactorial inheritance. About 60 % of the variability of plasma cholesterol is genetic in origin, influenced by allelic variation in many genes including those for ApoE, ApoB, ApoA1 and hepatic lipase that individually have a small effect. Familial hypercholesterolaemia (type II hyperlipoproteinaemia), on the other hand, is dominantly inherited and may account for 10–20 % of all early coronary heart disease. One in 500 of the general population is estimated to be heterozygous for the mutant LDLR gene. The risk of coronary heart disease

increases with age in heterozygous subjects, who may also have xanthomas. Severe disease, often presenting in childhood, is seen in homozygous subjects. Familial aggregations of early coronary heart disease also occur in people without any detectable abnormality in lipid metabolism. Risks to other relatives will be high, and known environmental triggers should be avoided. Future molecular genetic studies may lead to more precise identification of subjects at high risk as potential candidate genes are identified.

Schizophrenia and affective psychoses

A strong familial tendency is found in both schizophrenia and affective disorders. The importance of genetic rather than environmental factors has been shown by reports of a high incidence of schizophrenia in children of affected parents and concordance in monozygotic twins, even when they are adopted and reared apart from their natural relatives. The same is true of manic depression. Empirical values for lifetime risk of recurrence are available for counselling, and the burden of the disorders needs to be taken into account. Both polygenic and single major gene models have been proposed to explain genetic susceptibility. A search for linked biochemical or molecular markers in large families with many affected members has so far failed to identify any major susceptibility genes.

Congenital malformations

Syndromes of multiple congenital abnormalities often have Mendelian, chromosomal or teratogenic causes, many of which can be identified by modern cytogenetic and DNA techniques. Some malformations are non-genetic, such as the amputations caused by amniotic bands after early rupture of the amnion. Most isolated congenital malformations, however, follow multifactorial inheritance and the risk of recurrence depends on the specific malformation, its severity and the

number of affected people in the family. Decisions to have further children will be influenced by the fact that the risk of recurrence is generally low and that surgery for many isolated congenital malformations is successful. Prenatal ultrasonography may identify abnormalities requiring emergency neonatal surgery or severe malformations that have a poor prognosis, but it usually gives reassurance about the normality of a subsequent pregnancy.

Mental retardation or learning disability

Intelligence is a polygenic trait. Mild learning disability (intelligence quotient 50–70) represents the lower end of the normal distribution of intelligence and has a prevalence of about 3 %. The intelligence quotient of offspring is likely to lie around the mid-parental mean. One or both parents of a child with mild learning disability often have similar disability themselves and may have other learning-disabled children. Intelligent parents who have one child with mild learning disability are less likely to have another similarly affected child. By contrast, the parents of a child with moderate or severe learning disability (intelligence quotient 50) are usually of normal intelligence. A specific cause is more likely when the retardation is severe and may include chromosomal abnormalities and genetic disorders. The risk of recurrence depends on the diagnosis but in severe non-specific retardation is about 3 % for siblings. A higher recurrence risk is observed after the birth of an affected male because some of these cases represent X linked disorders. Recurrence risks are also higher (about 15 %) if the parents are consanguineous, because of the increased likelihood of an autosomal recessive aetiology. The recurrence risk for any couple increases to 25 % after the birth of two affected children.

6. GENETIC COUNSELLING

Definition and aims of genetic counselling

Genetic counselling is a communication process that deals with the human problems associated with the occurrence or risk of occurrence, of a genetic disorder in a family. Genetic counsellors are health care professionals with specialized training and experience in the areas of medical genetics and counselling. The process aims to help the individual or family to:

understand:

• the diagnosis, prognosis and available management;

• the genetic basis and chance of recurrence;

• the options available (including genetic testing);

choose:

• the course of action appropriate to their personal and family situation;

adjust:

• to the psychosocial impact of the genetic condition in the family.

Types of genetic counselling

Genetic counsellors work with people concerned about the risk of an inherited disease. These patients represent several different patient populations.

Prenatal genetic counselling is provided to couples that have an increased risk for birth defects or inherited conditions and are expecting a child or planning a pregnancy.

Pediatric genetic counselling is provided to families with children suspected of having a genetic disorder or with children previously diagnosed with a genetic disorder.

Adult genetic counselling is provided to adults with clinical features of an inherited disease or a family history of an inherited disease.

Cancer genetic counselling is provided to those with a strong family history of certain types of cancer.

Genetic diagnosis

The role of clinical geneticists is to establish an accurate diagnosis on which to base counselling and then to provide information about prognosis and follow up, the risk of developing or transmitting the disorder, and the ways in which this may be prevented or ameliorated.

When a child is born with birth defects, for example, the information needs to be gathered concerning parental age, maternal health, pregnancy complications, exposure to potential teratogens, fetal growth and movement, prenatal ultrasound scan findings, mode of delivery and previous pregnancy outcomes.

Examination

Thorough physical examination is required, but emphasis will be focused on relevant anatomical regions or body systems. A careful search should be made for both minor and major congenital abnormalities. Measurements of height, weight and head circumference are important and standard growth charts and tables are available for a number of specific conditions. In some cases, clinical geneticists will need to rely on the clinical findings of other specialists (ophthalmologists, neurologists and cardiologists) to complete the clinical evaluation of the patient.

Investigations

Investigation of affected individuals and family members may include conventional tests (x-rays, biochemical analysis) as well as cytogenetic and molecular genetic tests. A search for associated anomalies in children with chromosomal disorders often includes cranial, cardiac and renal imaging along with tests for other specific components of the particular syndrome (immune deficiency).

Genetic specialists

Clinical geneticists are medical practitioners who undertake specialist training in this discipline. Their primary training is usually in paediatrics or adult internal medicine but can be in other areas (obstetrics and gynaecology).

Areas that are covered by genetic specialist practice include:

- dysmorphology;
- prenatal counselling and testing;
- neurogenetics;
- cancer genetics;
- bone dysplasias;
- metabolic diseases.

Indications for formal genetic counselling

Anybody who suspects that there might be an increased risk of a genetic condition or producing a child with a genetic condition or birth defect may wish to receive formal genetic counselling. This includes:

- individuals who themselves have a genetic disorder (e. g. myotonic dystrophy);
- couples who have had a stillbirth;
- couples who have had a child with a birth defect;
- couples who have had a child with mental retardation;
- family history of any of the above;
- family history of known genetic disorders (e. g. Huntington disease, muscular dystrophy);
- multiple miscarriages;
- exposure to radiation or drugs during pregnancy;
- advanced maternal age;
- consanguinity;
- chromosome translocations;
- cancers.

For appropriate genetic counselling, the following are essential:

- diagnostic precision;

• knowledge of risk;
• knowledge of burden;
• knowledge of reproductive options;
• knowledge of scientific advances;
• counselling skills.

Process of genetic counselling

The genetic specialist needs to devote at least 1 hour in quiet surroundings to each family.

Medical records and doctors' reports are best obtained before undertaking counselling as this will make the consultation more efficient.

Detailed histories or records of probands (the affected individual through whom a family with a genetic disorder is ascertained) need to be obtained. A pedigree is drawn with a minimum of three generations. This should include information about stillbirths, deaths and health problems.

Probands and other members of the family are examined carefully and investigated as necessary.

Collection of samples for DNA

With advances in DNA technology it has become essential to store samples of DNA from family members with the condition in question who are likely to die, as well as relatives such as grandparents. This enables subsequent family members to benefit from advancing knowledge. Such samples can also be obtained at the time of postmortem if not collected earlier.

Testing to confirm a clinical diagnosis

When a genetic test is requested to confirm a clinical diagnosis in a child or adult, specialist genetic counselling may not be requested until after the test result. It is therefore the responsibility of the clinician offering the test to inform the patient (or the parents, if a child is being tested) before the test is undertaken, that the results may have genetic as well as clinical implications. For late onset conditions, it is crucial that samples sent for diagnostic testing are from patients already

symptomatic, as there are stringent counselling protocols for presymptomatic testing.

Predictive testing

Predictive (presymptomatic) testing is a test on an asymptomatic person that allows an individual to know whether or not he or she continues developing the condition in question. Not all genetic diseases show their effect immediately at birth or in early childhood. Although the gene mutation is present at birth, some diseases do not appear until adulthood. If a specific mutated gene responsible for a late-onset disease has been identified, a person from an affected family can be tested before the symptoms appear. Predictive testing is mainly available for neurodegenerative diseases (Huntington disease, autosomal dominant spinocerebellar ataxia) and some cancers (familial breast/ovarian cancer, familial bowel cancer).

7. METHODS OF PRENATAL DIAGNOSIS

Prenatal diagnosis

Prenatal diagnosis or prenatal screening (note that prenatal diagnosis and prenatal screening refer to two different types of tests) is testing for diseases or conditions in a fetus or embryo before it is born. The aim is to detect birth defects such as neural tube defects, Down syndrome, chromosome abnormalities, genetic diseases and other conditions, such as spina bifida, cleft palate, Tay – Sachs disease, sickle cell anemia, thalassemia, cystic fibrosis, muscular dystrophy, and fragile X syndrome. Screening can also be used for prenatal sex discernment. Common testing procedures include amniocentesis, ultrasonography including nuchal translucency ultrasound, serum marker testing, or genetic screening. In some cases, the tests are administered to determine if the fetus should be aborted, though physicians and patients also find it useful to diagnose high-risk pregnancies early so that delivery can be scheduled in a tertiary care hospital where the baby can receive appropriate care.

Fetal screening is also done to determine characteristics of generally not considered birth defects, and avail for sex selection, for example. The rise of designer babies and parental selection for specific traits raises a host of bioethical and legal issues that are expected to dominate reproductive rights debates in the 21st century.

Some screening tests performed on the woman are intended to detect traits or characteristics of the fetus. Others detect conditions in the woman that may have an adverse effect on the fetus, or that threaten the pregnancy. For example, abnormally low levels of the serum marker PAPP-A have been shown to correspond to an increased risk of pre-eclampsia, in which the mother’s high blood pressure can threaten the pregnancy, though many physicians find regular blood-pressure monitoring to be more reliable.

Reasons for prenatal diagnosis

There are three purposes of prenatal diagnosis:

1. To enable timely medical or surgical treatment of a condition before or after birth.

2. To give parents the chance to abort a fetus with the diagnosed condition.

3. To give parents the chance to "prepare" psychologically, socially, financially, and medically for a baby with a health problem or disability, or for the likelihood of a stillbirth.

Having this information in advance of the birth means that healthcare staff as well as parents can better prepare themselves for the delivery of a child with a health problem. For example, Down syndrome is associated with cardiac defects that may need intervention immediately upon birth.

Many expectant parents would like to know the sex of their baby before birth. Methods include amniocentesis with karyotyping, and prenatal ultrasound. In some countries, health care providers are expected to withhold this information from parents, while in other countries they are expected to give this information.

Qualifying risk factors

Because of the miscarriage and fetal damage risks associated with amniocentesis and CVS procedures, many women prefer to first undergo screening so they can find out if the fetal risk of birth defects is high enough to justify the risks of invasive testing. Since screening tests yield a risk score which represents the chance that the baby has the birth defect, the most common threshold for high-risk is 1:270. A risk score of 1:300 would therefore be considered low-risk by many physicians. However, the trade-off between risk of birth defect and risk of complications from invasive testing is relative and subjective; some parents may decide that even a 1:1000 risk of birth defects warrants an invasive test while others wouldn't opt for an invasive test even if they had a 1:10 risk score.

Indications for prenatal diagnosis

- *Women over the age of 35.* Due to the well-known association of advanced maternal age with increased risk of having a baby with Down syndrome and other autosomal trisomy syndromes, advanced maternal age has been the most common indication to be considered for prenatal diagnosis. The age of 35 years and onward at the expected date of delivery is usually considered as advanced maternal age to offer prenatal diagnosis by invasive methods (like amniocentesis and chorionic villus sampling). The reason to consider this age is probably the risk, at 35 years, of having a fetus with a chromosome abnormality is thought to be equal to the risk of miscarriage associated with amniocentesis (approx. 1 in 250).
- *Previous child with a chromosome abnormality.* Despite having the normal chromosomes themselves, the parents of a child with chromosomal aneuploidy are at increased risk of having chromosomal abnormality in a subsequent child. For example, a woman at 30 years of age with a previous child having Down syndrome has a recurrence risk for any chromosomal abnormality of about 1/100, compared to the age-related population risk of about 1/390.
- *Family history of a chromosome abnormality.* Prenatal diagnosis for a couple is often indicated if there is a family history of chromosomal abnormality, most commonly Down syndrome. For most couples the risk is no greater than that for the general population. This is because most cases of trisomy 21 or other chromosomal abnormality will have arisen as a result of non-disjunction rather than as a result of the familial chromosomal translocation or other rearrangements.
- *Presence of a structural chromosome abnormality in one of the parents.* In such case, the type of chromosomal abnormality and sometimes the parent of origin determine the risk of having anomaly in a child. The greatest risk, 100 % for

Down syndrome, occurs only if either parent has a 21q21q Robertsonian translocation or isochromosome.

- *Family history of a single-gene disorder.* For a positive family history for a single gene disorder (or if prospective parents have already had an affected child or if one of the parents is affected) that bears a significant risk to an offspring (recurrence risk 25 % to 50 %), the prenatal diagnosis is strongly indicated. Prenatal diagnosis is generally carried out by either biochemical or DNA analysis for a large number of single-gene disorders. In case of a family history of an X-linked disorder for which there is no specific prenatal diagnostic test, the parents of a boy affected with an X-linked disorder may use fetal sex determination to help them decide whether to continue or to terminate a subsequent pregnancy because of the high recurrent risk (25 %) associated with it.
- *Family history of a neural tube defect.* Because of the high risk of having a child with a neural tube defect (NTD), the prenatal diagnosis is indicated for first-degree relatives (and often second-degree relatives) of patients with NTDs. Ultrasonographic examination of the fetus along with assay of maternal serum alpha fetoprotein (AFP) can be used as non-invasive method (instead of invasive amniocentesis) of prenatal diagnosis to detect NTD.
- *Family history of other congenital structural abnormalities.* In such cases, a careful evaluation of the pedigree is helpful to determine the risk associated with each pregnancy. If the risk to a pregnancy is increased, a detailed ultrasonographic examination looking for the specific structural abnormality can be offered at around 16–18 weeks' gestation.
- *Abnormalities identified in pregnancy.* Any abnormalities identified during pregnancy by prenatal diagnostic screening procedures, such as triple testing and fetal anomaly scanning, require invasive prenatal diagnostic methods like amniocentesis and chorionic villus sampling for further confirmation.

- *Other high-risk factors.* These include: parental consanguinity, as it is associated with an increased risk that a child will have a hereditary disorder or congenital anomaly.
- *A history of recurrent miscarriages or a previous unexplained stillbirth* is also associated with an increased risk of the problem during future pregnancy. A history of three or more unexplained miscarriages requires parental chromosome analysis to exclude a chromosomal rearrangement such as a translocation or inversion.
- *Women who are pregnant with multiples* (twins or more).

There are multiple ways of classifying the methods available, including the invasiveness and the time performed.

Table 1 – Methods of prenatal screening and diagnosis

Invasiveness	Test	Comments	Time
1	2	3	4
Non-invasive	Fetal cells in maternal blood (FCMB)	Based on enrichment of fetal cells which circulate in maternal blood. Since fetal cells hold all the genetic information of the developing fetus they can be used to perform prenatal diagnosis	First trimester
Non-invasive	Cell-free fetal DNA in maternal blood	Based on DNA of fetal origin circulating in the maternal blood. Testing can potentially identify fetal aneuploidy and gender of a fetus as early as six weeks of pregnancy. Fetal DNA ranges from about 2–10 % of the total DNA in maternal blood	First trimester
Non-invasive	Preimplanta-tion genetic diagnosis (PGD)	During in vitro fertilization (IVF) procedures, it is possible to sample cells from human embryos prior the implantation. PGD is non-invasive itself, but IVF usually involves invasive procedures such as transvaginal oocyte retrieval	Prior to implanta-tion
Non-invasive	External examination	Examination of the woman's uterus from outside the body	First or second trimester

Continuation of the table 1

1	2	3	4
Non-invasive	Ultrasound detection	Commonly dating scans (sometimes known as booking scans) from 7 weeks to confirm pregnancy dates and look for twins. The specialised nuchal scan at 11–13 weeks may be used to identify higher risks of Down syndrome. Later morphology scans from 18 weeks may check for any abnormal development	First or second trimester
Non-invasive	Fetal heartbeat	Listening to the fetal heartbeat	First or second trimester
Non-invasive	Non-stress test	Use of cardiotocography during the third trimester to monitor fetal wellbeing	Third trimester
Less invasive	Transcervical retrieval of trophoblast cells	Cervical mucus aspiration, cervical swabbing, and cervical or intrauterine lavage can be used to retrieve trophoblast cells for diagnostic purposes, including prenatal genetic analysis. Success rates for retrieving fetal trophoblast cells vary from 40 % to 90 %. It can be used for fetal sex determination and identify aneuploidies. Antibody markers have proven useful to select trophoblast cells for genetic analysis and to demonstrate that the abundance of recoverable trophoblast cells diminishes in abnormal gestations, such as in ectopic pregnancy or anembryonic gestation	First trimester

Continuation of the table 1

1	2	3	4
Less invasive	Maternal serum screening	Including β-hCG, PAPP-A, alpha fetoprotein, inhibin-A	First or second trimester
More invasive	Chorionic villus sampling	Involves getting a sample of the chorionic villus and testing it. This can be done earlier than amniocentesis, but may have a higher risk of miscarriage, estimated at 1 %.	After 10 weeks
More invasive	Amniocentesis	This can be done once enough amniotic fluid has developed to sample. Cells from the fetus will be floating in this fluid, and can be separated and tested. Miscarriage risk of amniocentesis is commonly quoted as 0.06 % (1:1600). By amniocentesis, it is also possible to cryopreserve amniotic stem cells	After 15 weeks
More invasive	Embryoscopy and fetoscopy	Though rarely done, these involve putting a probe into a woman's uterus to observe (with a video camera), or to sample blood or tissue from the embryo or fetus	
More invasive	Percutaneous umbilical cord blood sampling	PUBS is a diagnostic genetic test that examines blood from the fetal umbilical cord to detect fetal abnormalities	24–34 weeks

Maternal serum screening

Maternal serum alpha-fetoprotein (MSAFP)

Maternal Serum Alpha Fetoprotein (MSAFP) screening is non-invasive procedure in which the mother's blood is taken and alpha-fetoprotein levels are measured. It is usually carried out during the 2nd trimester and is used to detect abnormalities such as neural tube defects, more specifically anencephaly spina bifida, encephalocele, open ventral wall defects such as gastroschisis as well as Down's syndrome.

Alpha-Fetoprotein (AFP) is an embryo specific glycoprotein which is produced during the early stages of development by the liver, yolk sac as well as a small amount being produced by the gastrointestinal tract. AFP in adults is functionless as levels decrease drastically after birth with very low traces of AFP found in the average older adult with the only women experiencing spikes occurring in AFP levels during the onset of pregnancy, and it is in fact through the testing of the blood of pregnant women, that AFP levels can be measured. The function of AFP itself is unknown but due to its similarity to albumin it has been hypothesized that AFP could be a carrier protein or may even play a role in the metabolism of bilirubin or even may play a role in the control of female fertility through its anti-estrogenic actions. Furthermore, it has been observed that it does play a role in the embryonic and early fetal stages of development as fluctuating levels of AFP indicate the presence of abnormalities within a fetus.

Ranges and Levels

First Trimester:

200–400 mg/dL.

Second Trimester:

14 weeks of gestation: 25.6 ng/ml;
15 weeks of gestation: 29.9 ng/ml;
16 weeks of gestation: 34.8 ng/ml;
17 weeks of gestation: 40.6 ng/ml;
18 weeks of gestation: 47.3 ng/ml;

19 weeks of gestation: 55.1 ng/ml;
20 weeks of gestation: 64.3 ng/ml;
21 weeks of gestation: 74.9 ng/ml.

It should also be noted that “normal” values are around 200 % higher in women with twin pregnancies.

AFP in Pregnancy

The highest maternal AFP concentration occurs in the mid third trimester of the pregnancy where the mean level is 150–250 ng/ml. The concentration of AFP in maternal serum at any moment of gestation development seems to be related to the AFP level in the fetal circulation as well as in the placental size.

Instances of abnormal AFP values can partly been explained by physiological deviations from the expected normal pregnancy e. g. in cases of under – or overestimated gestational age and multiple pregnancies. In other instances it has been found to indicate the presence of various fetal morphogenetic defects, such as open NTD (neural tube defect), hereditary congenital nephrosis (Finnish type), omphalocele, pilonidal sinus, esophageal atresia, and others.

The maternal AFP level has often reported to be increased in pregnancies where the fetus has a neural tube defect.

Figure 27 – Encephalocele

The optimal practical time for detecting open spinabifida by measuring materal serum AFP is at 16–18 comepleted weeks of pregnancy. Because there is a certain degree of overlapping between the maternal AFP levels in pregnancies with and without fetal NTD, the AFP estimation in materal serum cannot per se serve as a specific diagnostic test, but it seems to be a useful screening test so as to select certain symptom-free women for further diagnostic procedures such as ultrasonography, amniocentesis, and amniography.

Maternal Serum Alpha Protein as a Screening Test

It should be made clear that MAFP is not a diagnostic test and is used only for screening purposes to determine the likelihood of a disease being present, with further testing always necessary for any sort of accurate diagnosis to take place. Furthermore, MAFP is a screening test that is carried out during the second trimester whereas other tests may be carried out during the first trimester and are more accurate. It is part of two tests, one called the Triple Screen Test which is a battery of tests that measure AFP levels as well as human chorionic gonadotropin (HCG) and unconjugated estriol uE3 and a second series of tests known as the Quadruple Screen Test that tests AFP, HCG, uE3 as well as Inhibin A which is a hormone that is released by the placenta. These tests also take into account age, ethnic background, weight as well as the baby's gestational age. Currently, there are no known risks or side effects that have been associated with the MSAFP screening test except for any discomfort involved with the drawing of blood from the patient.

When the maternal blood serum is being tested and has been collected the alpha fetoprotein undergoes an enzyme immunoassay procedure in order to determine the concentration of the protein in the blood. Firstly AFP is marked, usually with a colour or florescence marker, then the assay is placed in a spectrometer for a result on the concentration. Other less common procedures which are used

to determine the concentration of AFP are radio-immuno assay, bioluminescence and chemiluminescence methods.

Maternal serum beta-HCG

This test is most commonly used as a test for pregnancy. Beginning at about a week following conception and implantation of the developing embryo into the uterus, the trophoblast will produce enough detectable beta-HCG (the beta subunit of human chorionic gonadotropin) to diagnose pregnancy. Thus, by the time the first menstrual period is missed, the beta-HCG will virtually always be elevated enough to provide a positive pregnancy test. The beta-HCG can also be quantified in serum from maternal blood, and this can be useful early in pregnancy when threatened abortion or ectopic pregnancy is suspected, because the amount of beta-HCG will be lower than expected.

Later in pregnancy, in the middle to late second trimester, the beta-HCG can be used in conjunction with the MSAFP to screen for chromosomal abnormalities, and Down syndrome in particular. An elevated beta-HCG coupled with a decreased MSAFP suggests Down syndrome.

Very high levels of HCG suggest trophoblastic disease (molar pregnancy). The absence of a fetus on ultrasonography along with an elevated HCG suggests a hydatidiform mole. The HCG level can be used to follow up treatment for molar pregnancy to make sure that no trophoblastic disease, such as a choriocarcinoma, persists.

Maternal serum estriol

The amount of estriol in maternal serum is dependent upon a viable fetus, a properly functioning placenta, and maternal well-being. The substrate for estriol begins as dehydroepiandrosterone (DHEA) made by the fetal adrenal glands. This is further metabolized in the placenta to estriol. The estriol crosses to the maternal circulation and is excreted by the maternal kidney in urine or by the maternal liver in the bile. The measurement of serial estriol levels in the third

trimester will give an indication of general well-being of the fetus. If the estriol level drops, then the fetus is threatened and delivery may be necessary emergently. Estriol tends to be lower when Down syndrome is present and when there is adrenal hypoplasia with anencephaly.

Inhibin-A

Inhibin is secreted by the placenta and the corpus luteum. Inhibin-A can be measured in maternal serum. An increased level of inhibin-A is associated with an increased risk for trisomy 21. A high level of inhibin-A may be associated with a risk for preterm delivery.

Pregnancy-associated plasma protein A (PAPP-A)

Low levels of PAPP-A as measured in maternal serum during the first trimester may be associated with fetal chromosomal anomalies including trisomies 13, 18, and 21. In addition, low PAPP-A levels in the first trimester may predict an adverse pregnancy outcome, including a small for gestational age (SGA) baby or stillbirth. A high PAPP-A level may predict a large for gestational age (LGA) baby.

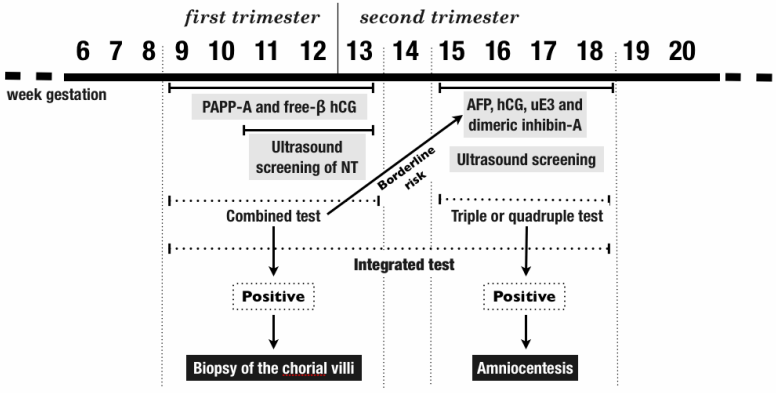

Figure 28 – Screening strategies in the first and second trimester of pregnancy: “Triple” or “Quadruple” screen

The triple test is one of a range of screening tests that are used to identify a pregnant woman whose fetus is likely to be affected by trisomy 21 (Down syndrome) and who should then be offered a diagnostic test. All of the tests similar to the triple test are based on the same mathematical principle (Bayes theorem) and work by combining a prior probability derived from maternal age at expected date of delivery with a likelihood ratio usually based on two multivariate Gaussian distribution functions.

This combination results in a reasonably accurate risk estimate of the probability that the fetus has Down syndrome. Women whose risk exceeds a specified cutoff are then offered a diagnostic test (i. e., amniocentesis or chorionic villus biopsy), which allows a cytogenetic diagnosis to be determined.

The triple test is used only in the second trimester of pregnancy and now has a range of competitors. Combining the maternal serum assays may aid in increasing the sensitivity and specificity of detection for fetal abnormalities. The classic test is the Ttriple screen for alpha-fetoprotein (MSAFP), beta-HCG, and estriol (uE3). The "quadruple screen" adds inhibin-A.

Table 2 – Screening tests

Test name	Used in	Analytes
Double test	Second trimester	AFP + HCG (total or free-β)
Triple test	Second trimester	As double test + unconjugated estriol
Quadruple test	Second trimester	As triple test (using free-β HCG) + inhibin-A
Combined test	First trimester	Ultrasound measurement of NT+ PAPP-A + free-β HCG
Serum integrated test	Both first and second trimester	PAPP-A (first trimester) + quadruple test (or triple test)
Integrated test	Both first and second trimester	As serum integrated test + NT in first trimester
Contingent test	Both first and second trimester	Dependent on structure of contingent screen chosen
Abbreviations: AFP – α-fetoprotein; HCG – human chorionic gonadotropin; NT – nuchal translucency; PAPP-A – pregnancy-associated plasma protein A		

Ultrasound in prenatal diagnosis

This is a non-invasive procedure that is harmless to both the fetus and the mother. High frequency sound waves are utilized to produce visible images from the pattern of the echos made by different tissues and organs, including the baby in the amniotic cavity. The developing embryo can first be visualized at about 6 weeks of gestation. Recognition of the major internal organs and extremities to determine if any are abnormal can best be accomplished between 16 to 20 weeks of gestation.

Although an ultrasound examination can be quite useful to determine the size and position of the fetus, the size and position of the placenta, the amount of amniotic fluid, and the appearance of fetal anatomy, there are limitations to this procedure. Subtle abnormalities may not be detected until later in pregnancy, or may not be detected at all. A good example of this is Down syndrome (trisomy 21) where the morphologic abnormalities are often not marked, but only subtle, such as nuchal thickening.

First trimester

In the first trimester, ultrasound can be used to confirm the presence of an intra-uterine pregnancy, and determine its viability.

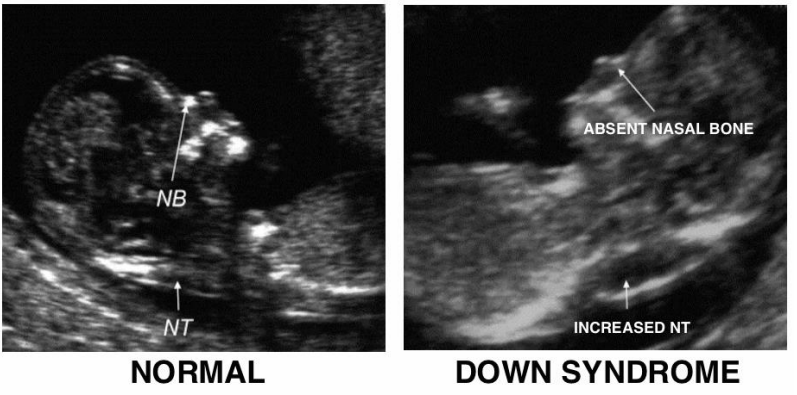

Figure 30 – First trimester, ultrasound

The crown-rump length measurement is used in dating until about 14 weeks, after which the biparietal diameter, head and abdominal circumferences, and femur length are used. Many of the major structural abnormalities can now be diagnosed at this stage in pregnancy. If the pregnancy is multiple, the number and chorionicity can be noted. Between 10 and 14 weeks, the fluid at the back of the neck of the fetus can be measured (nuchal translucency). This is a useful screening test for predicting the likely risk of the fetus being affected by a chromosomal anomaly. If this test is unavailable, reliable dating by ultrasound is essential for biochemical screening for chromosomal abnormality, as the hormone levels vary with gestational age.

Second trimester

The second trimester ultrasound scan is usually done between 18 and 22 weeks of gestation. This involves a search for morphological abnormalities, including anomalies associated with chromosomal disorders (markers), and an assessment of fetal growth. Doppler ultrasound of the uterine arteries is performed between 20 and 24 weeks of gestation. Abnormal uterine artery Doppler waveform patterns are predictive of uteroplacental complications that may occur later in the pregnancy.

Third trimester

In the third trimester, or when the fetus becomes potentially viable, ultrasound can be used to monitor growth, or help in the diagnosis of fetal growth restriction. In addition to the growth of the fetus, ultrasound allows us to investigate its environment in utero, and determine the ability of the fetus to cope with adverse situations. Biophysical assessment, including liquor estimation and assessment of body and breathing movement, fetal heart rate analysis and umbilical artery with Doppler are accepted tests of wellbeing, performed throughout the world. Doppler assessment of the fetal

circulation provides more specific information regarding the fetal condition in-utero, but is not routinely available.

The safety of ultrasound examinations during pregnancy has naturally been questioned. The consensus of opinion is that no detrimental effects of ultrasound use have been reported during pregnancy. At the levels of power and intensity currently used, there are numerous benefits derived but no known complications.

Doppler fetal monitor

Doppler fetal monitors provide information about the fetus similar to that provided by a fetal stethoscope. One advantage of the Doppler fetal monitor over a (purely acoustic) fetal stethoscope is the electronic audio output, which allows people other than the user to hear the heartbeat. One disadvantage is the greater complexity and cost and the lower reliability of an electronic device.

Originally intended for use by health care professionals, this device is becoming popular for personal use. However, the FDA recommends against their home use, citing possible harm to a developing fetus and that these should only be used under the supervision of a healthcare professional when medically indicated for the benefit of the health of mother and child.

Fetal heart rates

Starting at week 5 the fetal heart rate accelerates by 3.3 bpm per day for the next month.

The fetal heart begins to beat at approximately the same rate as the mother's, which is typically 80 to 85 bpm. The approximate fetal heart rate for weeks 5 to 9 (assuming a starting rate of 80) is as follows:

– week 5 starts at 80 and ends at 103 bpm;

– week 6 starts at 103 and ends at 126 bpm;

– week 7 starts at 126 and ends at 149 bpm;

– week 8 starts at 149 and ends at 172 bpm;

– at week 9 the fetal heartbeat tends to beat within a range of 155 to 195 bpm.

At this point, the fetal heart rate begins to decrease, and generally falls within the range of 120 to 160 bpm by week 12.

Nonstress test

A nonstress test (NST) is a screening test used in pregnancy. The premise of the NST is that a well-oxygenated, non-acidemic fetus will spontaneously have temporary increases in the fetal heart rate (FHR).

Interpretation

- Reactive (normal) presence of two or more fetal heart rate accelerations within a 20-minute period, with or without fetal movement discernible by the woman. Accelerations are defined as 15 bpm above baselines for at least 15 seconds if beyond 32 weeks of gestation, or 10 bpm for at least 10 seconds if at or below 32 weeks.
- Nonreactive presence of less than two fetal heart rate accelerations within a 20-minute period over a 40-minute testing period.

Vibroacoustic stimulation can wake the fetus, and is sometimes used to speed up the test or to facilitate further evaluation of a nonreactive nonstress test.

Biophysical profile

A biophysical profile is a prenatal ultrasound evaluation of fetal well-being involving a scoring system, with the score being termed Manning's score. It is often done when a non-stress test is non reactive, or for other obstetrical indications.

Table 3 – Biophysical profile

Parameter	Normal (2 points)	Abnormal (0 points)
NST/Reactive FHR	At least two accelerations in 20 minutes	Less than two accelerations to satisfy the test in 20 minutes
US: Fetal breathing movements	At least one episode of > 30 s or > 20 s in 30 minutes	None or less than 30 s or 20 s
US: Fetal activity/ gross body movements	At least three or two movements of the torso or limbs	Less than three or two movements
US: Fetal muscle tone	At least one episode of active bending and straightening of the limb or trunk	No movements or movements are slow and incomplete
US: Qualitative AFV/AFI	At least one vertical pocket > 2 cm or more in the vertical axis	Largest vertical pocket ≤ 2 cm

Procedure

The biophysical profile (BPP) has 5 components: 4 ultrasound (US) assessments and a nonstress test (NST). The nonstress test evaluates fetal heart rate and response to fetal movement. The five discrete biophysical variables are:

- fetal movement;
- fetal tone;
- fetal breathing;
- amniotic fluid volume;
- fetal heart rate.

The "modified biophysical profile" consists of the nonstress test and amniotic fluid index only.

Interpretation

Each assessment is graded either 0 or 2 points, and then added up to yield a number between 0 and 10. A BPP of 8 or 10 is generally considered reassuring. A BPP normally is not performed before the second half of a pregnancy, since fetal breathing movements do not occur in the first half.

The presence of these biophysical variables implies absence of significant central nervous system hypoxemia/acidemia at the time of testing. By comparison, a compromised fetus typically exhibits loss of accelerations of the fetal heart rate (FHR), decreased body movement and breathing, hypotonia, and, less acutely, decreased amniotic fluid volume.

Table 4 – Recommended management based on the biophysical profile

BPP	Recommended management
≤ 2	Labour induction
4	• Labour induction if gestational age > 32 weeks. • Repeating test same day if < 32 weeks, then delivery if BPP < 6. • Labor induction if > 36 weeks if favourable cervix and normal AFI. • Repeating test in 24 hours if < 36 weeks and cervix unfavourable; then delivery if BPP < 6, and follow-up if > 6
8	Labour induction if presence of oligohydramnios

Cardiotocography

In medicine (obstetrics), cardiotocography (CTG) is a technical means of recording *(-graphy)* the fetal heartbeat *(cardio-)* and the uterine contractions *(-toco)* during pregnancy, typically in the third trimester. The machine used to perform the monitoring is called a cardiotocograph, more commonly known as an electronic fetal monitor (EFM).

External cardiotocography is used for continuous or intermittent monitoring. The fetal heart rate and the activity of the uterine muscle are detected by two transducers placed on the mother's abdomen (one above the fetal heart and the other at the fundus). Doppler ultrasound provides the information which is recorded on a paper strip known as a cardiotocograph (CTG).

Internal cardiotocography uses an electronic transducer connected directly to the fetal scalp. A wire electrode is attached to the fetal scalp through the cervical opening and is connected to the monitor. This type of electrode is sometimes called a spiral or scalp electrode. Internal monitoring provides a more accurate and consistent transmission of the fetal heart rate than external monitoring because factors such as movement do not affect it. Internal monitoring may be used when external monitoring of the fetal heart rate is inadequate, or closer surveillance is needed.

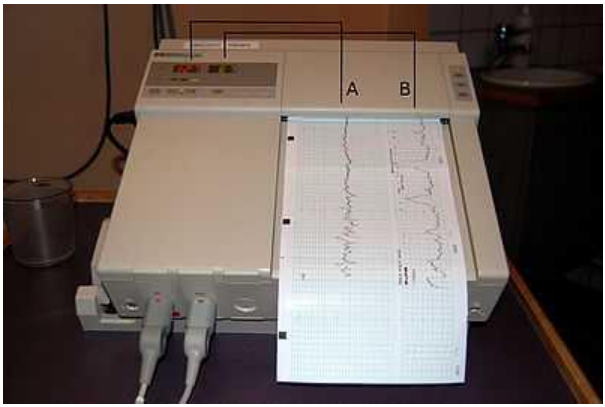

Figure 31 – Schematic explanation of cardiotocography: heart rate (A) is calculated from fetal heart motion determined by ultrasound, and uterine contractions are measured by a tocodynamometer (B). These numbers are represented on a time scale with the help of a running piece of paper, producing a graphical representation

Interpretation of a CTG tracing requires both qualitative and quantitative description of:

- uterine activity (contractions);
- baseline fetal heart rate (FHR);
- baseline FHR variability;
- presence of accelerations;
- periodic or episodic decelerations;
- changes or trends of FHR patterns over time.

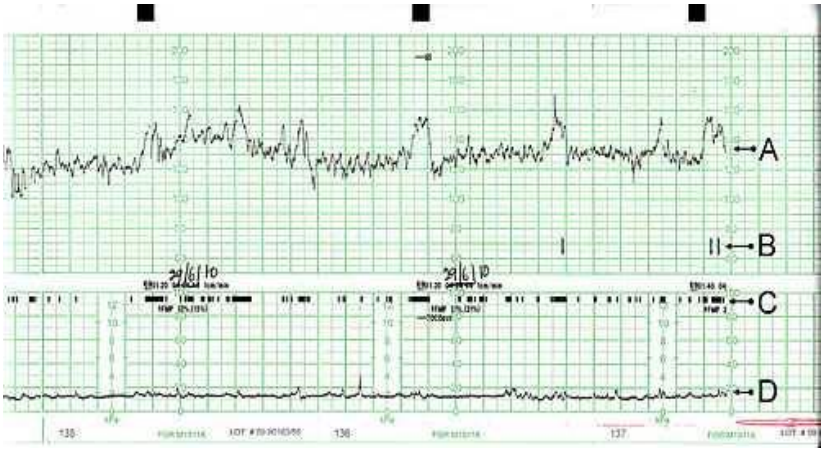

Figure 32 – A typical CTG output for a woman not in labour. A – fetal heartbeat; B – indicator showing movements felt by mother (caused by pressing a button); C – fetal movement; D – uterine contractions

Invasive methods of prenatal diagnosis

There is a variety of invasive techniques available for prenatal diagnosis. Each of them can be applied only at specific time periods during pregnancy for the greatest utility. The invasive techniques employed for prenatal diagnosis include:

- amniocentesis;
- chorionic villus sampling;
- cordocentesis;
- fetoscopy.

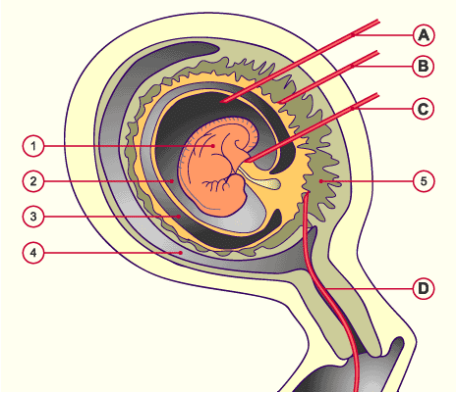

Figure 33 – Schema of pregnant uterus

1. Embryo.
2. Amniotic cavity.
3. Chorion cavity.
4. Uterine cavity.
5. Chorion frondosum.

A. Amniocentesis.
B. Chorion biopsy through the abdominal wall.
C. Umbilical blood sampling (V. umbilicalis).
D. Transvaginal chorion biopsy

Chorionic Villus Sampling (CVS)

In this procedure, a catheter is passed via the vagina through the cervix and into the uterus to the developing

placenta under ultrasound guidance. Alternative approaches are transvaginal and transabdominal. The introduction of the catheter allows sampling of cells from the placental chorionic villi. These cells can then be analyzed by a variety of techniques. The most common test employed on cells, obtained by CVS, is chromosome analysis to determine the karyotype of the fetus. The cells can also be grown in culture for biochemical or molecular biologic analysis. CVS can be safely performed between 9.5 and 12.5 weeks of gestation.

Indications

Possible reasons for having a CVS can include:

- abnormal first trimester screen results;
- increased nuchal translucency or other abnormal ultrasound findings;
- family history of a chromosomal abnormality or other genetic disorders;
- parents are known carriers for a genetic disorder;
- advanced maternal age (maternal age above 35). AMA is associated with an increased risk of Down's syndrome and at age 35, the risk is 1:400. Screening tests are usually carried out first before deciding if CVS should be done.

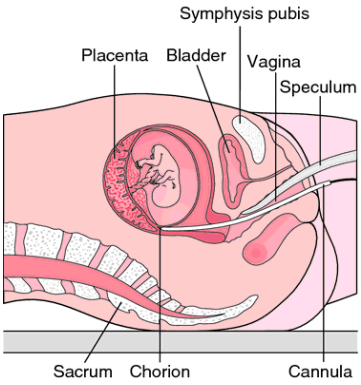

Figure 34 – A diagram of the technique of transvaginal chorionic villus sampling

CVS has the disadvantage of being an invasive procedure, and it has a small but significant rate of morbidity for the fetus; this loss rate is about 0.5 to 1 % higher than for women undergoing amniocentesis. Rarely, CVS can be associated with limb defects in the fetus. The possibility of maternal Rh sensitization is present. There is also the possibility that maternal blood cells in the developing placenta will be sampled instead of fetal cells and confound chromosome analysis.

Amniocentesis

Amniocentesis is a procedure used to diagnose fetal defects in the early second trimester of pregnancy. A sample of the amniotic fluid, which surrounds a fetus in the womb, is collected through a pregnant woman's abdomen using a needle and syringe. Tests performed on fetal cells found in the sample can reveal the presence of many types of genetic disorders, thus allowing doctors and prospective parents to make important decisions about early treatment and intervention.

Since the mid-1970s, amniocentesis has been used routinely to test for Down syndrome, by far the most common, nonhereditary, genetic birth defect, afflicting about one in every 1 000 babies. By 1997, approximately 800 different diagnostic tests were available, most of them for hereditary genetic disorders such as Tay – Sachs disease, sickle cell anemia, hemophilia, muscular dystrophy and cystic fibrosis.

Amniocentesis, often called amnio, is recommended for women who will be older than 35 on their due-date. It is also recommended for women who have already borne children with birth defects, or when either of the parents has a family history of a birth defect for which a diagnostic test is available. Another reason for the procedure is to confirm indications of Down syndrome and certain other defects which may have shown up previously during routine maternal blood screening.

One of the most common reasons for performing amniocentesis is an abnormal alpha-fetoprotein (AFP) test. Alpha-fetoprotein is a protein produced by the fetus and present in the mother's blood. A simple blood screening, usually conducted around the 15th week of pregnancy, can determine the AFP levels in the mother's blood. Levels that are too high or too low may signal possible fetal defects. Because this test has a high false-positive rate, another test such as amnio is recommended whenever the AFP levels fall outside the normal range.

Amniocentesis is generally performed during the 16th week of pregnancy, with results usually available within three weeks. It is possible to perform an amnio as early as the 11th week but this is not usually recommended because there appears to be an increased risk of miscarriage when done at this time. The advantage of early amnio and speedy results lies in the extra time for decision making if a problem is detected. Potential treatment of the fetus can begin earlier. Important, also, is the fact that elective abortions are safer and less controversial the earlier they are performed.

As an invasive surgical procedure, amnio poses a real, although small, risk to the health of a fetus. Parents must weigh the potential value of the knowledge gained, or indeed the reassurance that all is well, against the small risk of damaging what is in all probability a normal fetus. The serious emotional and ethical dilemmas that adverse test results can bring must also be considered. The decision to undergo amnio is always a matter of personal choice.

Procedure

Before the start of the procedure, a local anesthetic can be given to the mother in order to relieve the pain felt during the insertion of the needle used to withdraw the fluid. After the local anesthetic is in effect, a needle is usually inserted through the mother's abdominal wall, then through the wall of the uterus, and finally into the amniotic sac. With the aid of

ultrasound-guidance, a physician punctures the sac in an area away from the fetus and extracts approximately 20 ml of amniotic fluid. If used for prenatal genetic diagnosis, fetal cells are separated from the extracted sample. The cells are grown in a culture medium, then fixed and stained. Under a microscope the chromosomes are examined for abnormalities. The most common abnormalities detected are Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Turner syndrome (monosomy X). In regard to the fetus, the puncture heals and the amniotic sac replenishes the liquid over the next 24–48 hours.

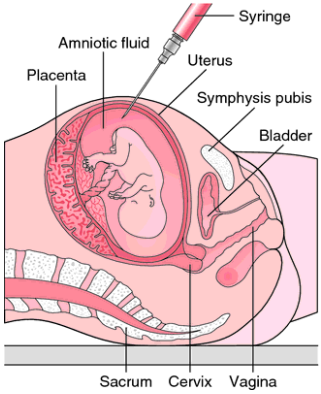

Figure 35 – A diagram of the technique of amniocentesis

Indications and results

1. Early in pregnancy, amniocentesis is used for diagnosis of chromosomal and other fetal problems such as:

- Down syndrome (trisomy 21);
- trisomy 13;
- trisomy 18;
- fragile X;
- rare, inherited metabolic disorders;
- neural tube defects (anencephaly and spina bifida) by alpha-fetoprotein levels.

2. Lung maturity. Amniocentesis can predict fetal lung maturity, which is inversely correlated to the risk of infant respiratory distress syndrome. In pregnancies of greater than 30 weeks, the fetal lung maturity may be tested by sampling the amount of surfactant in the amniotic fluid. Several tests are available that correlate with the production of surfactant. These include the lecithin-sphingomyelin ratio ("L/S ratio"), the presence of phosphatidylglycerol (PG), and more recently, the surfactant/albumin (S/A) ratio. For the L/S ratio, if the result is less than 2:1, the fetal lungs may be surfactant deficient. The presence of PG usually indicates fetal lung maturity. For the S/A ratio, the result is given as mg of surfactant per gram of protein. An S/A ratio < 35 indicates immature lungs, between 35–55 is indeterminate, and > 55 indicates mature surfactant production (correlates with an L/S ratio of 2.2 or greater).

3. Infection, in which amniocentesis can detect a decreased glucose level, a Gram stain showing bacteria or an abnormal differential count of white blood cells.

4. Rh incompatibility.

5. Decompression of polyhydramnios.

An emerging indication for amniocentesis is in the management of preterm rupture of membranes where measurement of certain amniotic fluid inflammatory markers may be helpful. If amniotic fluid IL-6, a marker of inflammation, is elevated, the fetus is at high risk and delivery should be considered.

Risks and drawbacks

Amniocentesis is performed between the 15^{th} and 20^{th} week of pregnancy; performing this test earlier may result in fetal injury. The term "early amniocentesis" is sometimes used to describe use of the process between weeks 11 and 13.

Complications of amniocentesis include preterm labour and delivery, respiratory distress, postural deformities, chorioamnionitis, fetal trauma and alloimmunisation of the mother (rhesus disease). Studies from the 1970s originally

estimated the risk of amniocentesis-related miscarriage at around 1 in 200 (0.5 %).

Social implications

The prenatal diagnosis of chromosomal abnormalities can have social drawbacks as technology changes the way people think about disability and kinship. There is potential for intensification of attitudes of discrimination towards those with disability, whose births could have been prevented through technology such as amniocentesis. When reproduction becomes stratified, groups of people become disempowered to reproduce, and the standard of entry into human community is questioned. In one sense, amniocentesis offers a window of control, and in another – an anxiety-provoking responsibility to make rational decisions about complex, emotional and culturally contingent issues.

Cordocentesis

Percutaneous umbilical cord blood sampling (PUBS), also called **cordocentesis**, **fetal blood sampling**, or **umbilical vein sampling** is a diagnostic genetic test that examines blood from the fetal umbilical cord to detect fetal abnormalities. Fetal and maternal blood supply is typically connected in utero with one vein and two arteries to the fetus. The umbilical vein is responsible for delivering oxygen rich blood to the fetus from the mother; the umbilical arteries are responsible for removing oxygen poor blood from the fetus. This allows for the fetus' tissues to properly perfuse. PUBS provides a means of rapid chromosome analysis and is useful when information cannot be obtained through amniocentesis, chorionic villus sampling, or ultrasound (or if the results of these tests were inconclusive); this test carries a significant risk of complication and is typically reserved for pregnancies determined to be at high risk for genetic defect. It has been used with mothers with immune thrombocytopenic purpura.

Procedure

If the fetus is viable, the procedure is performed close to an operating room in case an emergency cesarean section is necessary due to complications caused by the procedure. Currently, there is no definite age of viability because this depends on the fetus' ability to survive outside the womb, which in cases of premature births, can depend on access to medical care and technology needed to keep the fetus alive through the neonatal stage. Fetal viability typically occurs at about 24 to 25 weeks of gestation. When the fetus is in between the ages of 24–34 weeks, a glucocorticoid is given to the patient about 24 hours before the procedure to stimulate lung maturity. An ultrasound is performed before the procedure to view the position of the fetus and may be used during the procedure to help guide the needle. The mother's blood is drawn for comparison against fetal blood, and intravenous access is established in the mother in order to supply medications as needed. To reduce the risk of intraamniotic infection, antibiotics are supplied through the intravenous access about 30–60 minutes before the procedure. If movement of the fetus is a risk to the success of the procedure, the fetus may be paralyzed using a fetal paralytic drug.

A 20 or 22 gauge spinal needle is typically used in PUBS and may be prepared with an anticoagulant, which helps to reduce the risk of clot formation. During the procedure, the first step is to locate a relatively stable segment of the umbilical cord. A typical sampling site would be where the segment of the umbilical cord is closest to the placenta. However, there is a risk of maternal blood contamination at this site. Blood sampling may be achieved with more ease if the placenta is in the anterior position. However, if the placenta is in the posterior position, the fetus might block direct access to the umbilical cord. Once the umbilical cord is reached and the correct position of the needle is confirmed, the fetal blood is drawn. The needle is removed after all necessary samples are

taken. The site of puncture is monitored after the procedure for bleeding. Also, if the fetus is viable, fetal heart rate is monitored post-procedure for one to two hours.

After the blood samples are obtained, they are placed into tubes containing anticoagulants in order to stop the blood from clotting. If the blood sample was obtained at the site close to the placenta, a fetal blood confirmation test should be done to ensure no mixing of fetal and maternal blood occurred before the diagnostic tests are done on the blood. Fetal red blood cells (RBC) are usually bigger than maternal RBCs, and the average volume of RBCs, the mean corpuscular volume (MCV), is one of the methods used to determine whether or not the fetal blood has been contaminated. Another method, human chorionic gonadotropin (HCG) determination, can detect maternal blood because maternal blood has high levels of HCG. The hemoglobin alkaline denaturation test (Apt test) can detect the presence of maternal blood, which is indicated by a colour change from red to brown when the sample is added to alkali reagent. Blood typing would also detect maternal blood, as the I antigen only occurs in adults. The Kleihauer – Betke test can detect very small amounts of maternal blood before the third trimester of pregnancy by monitoring hemoglobin elution in acid because adult and fetal hemoglobin elute differently in acid. Finally, a white blood cell count can detect maternal blood in the sample, as fetal white blood cells are primarily leukocytes, while maternal white blood cells are mostly neutrophils. If amniotic fluid infiltrated the sample, then there would be a reduction in the volume of RBCs, white blood cells, and platelets in the sample. Also, patterns consistent with amniotic fluid would be visible in the sample.

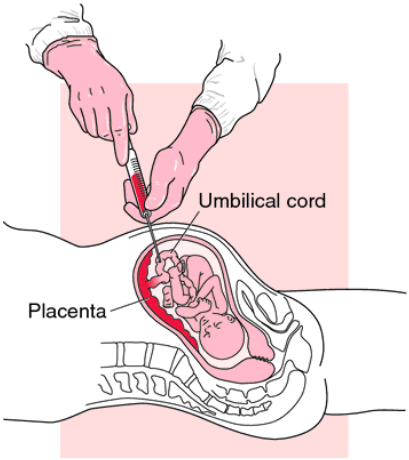

Figure 35 – Percutaneous umbilical cord sampling, also known as cordocentesis

Associated risks

The most common complication is a hemorrhage, or bleeding, of the puncture site and can be especially dangerous when the fetus is younger than 21 weeks. The risk of hemorrhage is greater if the fetus has a defect that affects its platelets. A transfusion of donor platelets is usually done in such cases to reduce the risk of bleeding. If the bleeding is severe, immediate delivery is an option as long as the fetus is old enough to survive, or fetal blood volume restoration may be considered. Another possible complication is cord hematoma, which doesn't have any characteristic symptoms but can be indicated by sudden bradycardia. If the hematoma is under control, the fetus is monitored until stabilized. If the fetus remains unstable, a delivery may be done. Fetomaternal hemorrhage is another complication that occurs when the fetal blood mixes into the maternal blood. A small fetomaternal hemorrhage could cause an increase in maternal antigens, while a large fetomaternal hemorrhage could cause fetal anemia and death. Fetal bradycardia, low heart rate, is another complication that may occur. Most cases of fetal bradycardia

are self-resolved within five minutes. The complication of infection has a low incidence rate, and preventative measures are implemented against the risk of infection, such as antibiotic usage and the aseptic technique. However, vertical transmission of a virus such as HIV may occur. Fetal loss may also occur, especially in the presence of several risk factors, including fetal abnormalities, operator errors, placental penetration, and viability of the fetus.

Intrahepatic vein fetal blood sampling may be done as an alternative to PUBS. It involves the needle being inserted into the intrahepatic part of the umbilical cord in the fetal abdomen.The benefits of this alternative, compared to PUBS, are that chances of contamination of the fetal blood are very low, the risk of fetomaternal hemorrhage is reduced, the risk of bleeding from the sampling site is reduced, and access to the sampling site is easy regardless of the position of the placenta. In pregnancies with high risk of fetal thrombocytopenia, this is the preferred method of blood samples due to the very low risk of site bleeding.

Indications and contraindications

PUBS is not a diagnostic test that is indicated in every pregnancy. It is, however, suggested in pregnancy cases in which the blood gas levels and pH would aid in diagnosis of a condition, such as anemia, or delivery plan, if termination of the pregnancy is being considered or special plans must be made. Severe fetal growth issues in conjunction with low oxygen in the fetus' blood and high levels in the mother's blood also indicate the use of PUBS. With more detailed observations and information on fetal tissue perfusion and metabolism, better predictions on development can be made. For pregnancies in which genetic abnormalities may be present, PUBS can be used to construct a karyotype, usually within 48 hours, and detect irregular chromosomal patterns. Karyotypes are able to confirm or detect monosomies, trisomies, or missing portions of chromosomes to give a

detailed picture of the severity of the genetic defect as well as predicting developmental future. PUBS is also indicated in the cases of twins with accumulation of amniotic fluid and substantially different growth rates (at least 10 %), if the fetus is expected to be breaking down red blood cells improperly, and in the alleviation of hydrops fetalis, a build-up of fluid in at least 2 parts of the fetus. Suspicion of fetal infections, such as rubella and toxoplasmosis, as well as the need to supply medicine or blood transfusions to the fetus are indications for the use of PUBS.

Due to its invasive nature, the contraindications of PUBS, reasons to not undergo the procedure, must be taken into account in order to ensure the safety of the fetus and the mother. During the first 18 weeks of pregnancy, the umbilical vein from which the blood sample is taken is not very stable, which could lead to excessive bleeding; therefore, PUBS is contraindicated in any fetus under the age of 18 weeks old. While blood gas levels and pH values are able to give parents and medical professionals a snapshot of fetal status, these fetuses can be monitored with less invasive procedures and equipment, such as ultrasounds, cardiotocography, or maternal blood tests. Mothers affected by hepatitis B are not advised to undergo PUBS. In these cases, the fetus would be put at an increased risk of contracting the hepatitis virus from the mother. However, the necessity of the procedure should be considered along with this risk. PUBS should not be performed in mothers testing positive for the human immunodeficiency virus (HIV) due to increased risk of fetal contraction. If PUBS is being used to determine if the fetus has been infected with HIV it may not be contraindicated

Fetoscopy

Fetoscopy is a procedure that utilizes an instrument called a fetoscope to evaluate or treat the fetus during pregnancy.

There are two different types of fetoscopy: external and endoscopic.

External fetoscopy

An external fetoscope resembles a stethoscope, but with a headpiece. It is used externally on the mother's abdomen to auscultate (listen to) the fetal heart tones after about 18 weeks gestation. It also allows a birth attendant to monitor the fetus intermittently and ensure that the baby is tolerating labour without the mother having to be attached to a continuous fetal monitor.

Endoscopic fetoscopy

The second type of fetoscope is a fiber-optic endoscope. It is inserted into the uterus either transabdominally (through the abdomen) or transcervically (through the cervix) to visualize the fetus, to obtain fetal tissue samples, or to perform fetal surgery.

Approximately 3 % of babies born each year have a complex birth defect. Certain birth defects are complicated by the labour and delivery process, while others may progress quickly after birth to cause significant disability or death. Fetal surgical techniques utilizing the endoscopic fetoscope offer early intervention in order to treat such defects before they become serious.

Some of the fetal abnormalities that may be treated by endoscopic fetoscopy are:

- Congenital diaphragmatic hernia (CDH). In babies with CDH, the diaphragm (the thin muscle that separates the chest from the abdomen) doesn't develop properly. The abdominal organs may enter the chest cavity through a hole (hernia) and cause pulmonary hyperplasia (underdeveloped lungs). CDH occurs in about one out of every 2 000 births.
- Urinary tract obstruction. The urethra (the tube that carries urine from the bladder to the outside of the body) may become obstructed in utero or fail to develop normally. When this happens, urine can back up into the kidneys and destroy

tissue or cause the bladder to become enlarged. The amount of amniotic fluid also decreases because fetal urine is its major component.

- Pulmonary hypoplasia usually results because the lungs rely on amniotic fluid in their development.
- Twin/twin transfusion syndrome (TTTS). In some twin pregnancies, the two fetuses will share a placenta (called a monochorionic pregnancy). TTTS occurs in approximately 15 % of these twins when blood volume between the fetuses is unequal, causing abnormally low blood volume in the donor twin and abnormally high blood volume in the recipient twin. There is often a large difference in size between the twins. Approximately 70–80 % of fetuses suffering from TTTS will die without intervention.
- Acardiac twin. This condition also occurs in monochorionic pregnancies, but one twin develops normally while the other develops without a heart. The acardiac twin receives its blood supply from the normal twin, whose heart must now work harder to pump blood through both fetuses. Approximately 50–75 % of acardiac twins will die as a result. An acardiac twin occurs in 1 % of monochorionic pregnancies and one out of 35 000 overall pregnancies.

The external fetoscope is used to listen to fetal heart tones for rate and rhythm. The earpieces and the headpiece allow auscultation (listening) via both air and bone conduction. External fetoscopy is inexpensive, noninvasive, and does not require electricity. It is difficult, however, to clearly hear the fetal heart tones prior to 18 to 20 weeks gestation. Doppler ultrasound can detect fetal heart tones around weeks 10 to 12.

Endoscopic fetoscopy uses a thin (1 mm) fiberoptic scope. Developed in the 1970s, the endoscope was originally inserted transabdominally to visualize the fetus for gross abnormalities suspected by ultrasound or to obtain tissue and blood samples. It was performed after about 18 weeks of gestation. Even with practitioner expertise, associated fetal loss

was 3–7 %. During the 1980s, ultra-sound-guided needle sampling of cord blood replaced fetoscopy when samples of fetal blood were required.

As laparoscopic and microsurgical techniques have become more common and the instrumentation has become more advanced technologically, fetoscopy has improved for fetal diagnostic and therapeutic purposes. Fetal surgery performed through an open maternal abdomen has a higher risk of such complications as infection, premature rupture of membranes, preterm labour, or fetal death. If surgery is performed via fetoscopy, which requires a very small transabdominal incision, the risks are much smaller. Techniques have advanced enough to allow some fetoscopy to be performed in the first trimester via the mother's cervix. The term "obstetrical endoscopy" may be used for surgery on the placenta, umbilical cord, or on the fetal membranes. The term "endoscopic fetal surgery" is used for such procedures as the repair of a fetal congenital diaphragmatic hernia or obstructed bladder.

Diagnosis/Preparation

The use of external fetoscopy requires access to the maternal abdomen, with the mother lying supine or in a semi-seated position. Afterwards, the mother is able to get up and resume a normal activity level.

Preparation for endoscopic fetoscopy will depend on the extent of the procedure, and whether it is performed transcervically or transabdominally. Obtaining a small fetal tissue sample is a smaller procedure by comparison to fetal surgery. Other factors include outpatient versus inpatient stay and anesthesia (both maternal and fetal). For some procedures medication may be administered to temporarily decrease fetal movement to lower the risk of fetal injury. Maternal anesthesia may be local, regional, or general.

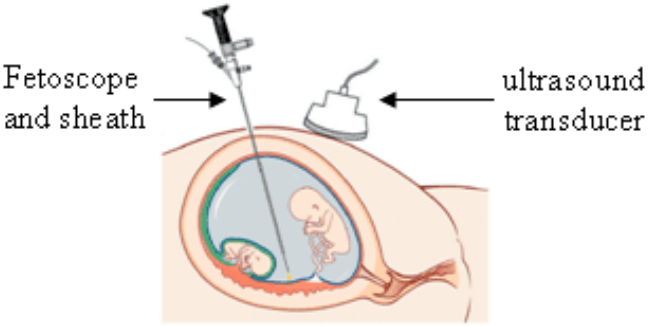

Figure 36 – Fetoscopy

Aftercare

External fetoscopy does not require aftercare. The care following fetal endoscopic use will depend on the extent of the procedure and the type of anesthesia used. If the procedure is done on an outpatient basis, the mother and fetus will be monitored for a period of time prior to discharge. More extensive surgery will require inpatient hospital postoperative care.

Risks

The only potential complication with external fetoscopy is the possibility of missing an abnormal heart rate or rhythm. Its usefulness and accuracy depend on the skill of the practitioner.

Endoscopic fetoscopy has the potential for causing infection in the fetus and/or mother; premature rupture of the amniotic membranes; premature labour; and fetal death. When endoscopic fetal surgery is done instead of open-uterus fetal surgery, the risks to the mother and fetus are decreased. The risks are because the incision is significantly smaller, with less potential blood loss, decreased uterine irritability, and decreased risk of early miscarriage.

Normal results

The normal fetal heart rate is 120 to 160 beats per minute, regardless of the method used for auscultation (external fetoscopy or Doppler ultrasound). Some variability of fetal heart rate is expected, as the heart rate increases with fetal activity and slows with fetal rest.

Results expected using endoscopic fetoscopy will vary depending on the procedure undertaken. The goal is for the maximum benefit with the minimum of risk or complication to both the mother and fetus.

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