PRIVOLZHSKY RESEARCH MEDICAL UNIVERSITY

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BASICS OF CLINICAL GENETICS

Training Manual

ИЗДАТЕЛЬСТВО
ПРИВОЛЖСКОГО ИССЛЕДОВАТЕЛЬСКОГО
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The article presents the introduction to clinical genetics, modern methods of diagnostics and treatment, information about chromosome disorders. Test questions and case studies are given to facilitate the acquisition of the material.

This manual can be used as a teaching and learning resource material for the third-year students of educational institutions of higher education studying in the specialty 31.05.01 «General Medicine» in English.

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ПРИВОЛЖСКИЙ ИССЛЕДОВАТЕЛЬСКИЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ

ОСНОВЫ КЛИНИЧЕСКОЙ ГЕНЕТИКИ

Учебное пособие

ИЗДАТЕЛЬСТВО
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Данное учебное пособие включает в себя введение в клиническую генетику, современные методы диагностики и лечения наследственных заболеваний, информацию о хромосомных нарушениях. Контрольные вопросы и примеры из практики даются, чтобы облегчить усвоение материала. Данное руководство может быть использовано в качестве учебно-методического материала для студентов третьего курса педиатрических и медицинских факультетов медицинских вузов.

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aCGH — Array comparative genomic hybridization
AFP — alpha-fetus protein
CGH — Comparative genomic hybridization
CVS — chorionic villus sampling
DNA — deoxyribonucleic acid
FISH — Fluorescence in situ hybridization
GWAS — genome-wide association studies
hCG — human Chorionic Gonadotropin
MS/MS — tandem mass spectrometry
NBS — Newborn screening
NIPT — noninvasive prenatal testing
PUBS — percutaneous umbilical blood sampling
UPD — uniparental disomy
WES — whole exome sequencing
WGS — whole genome sequencing

Clinical Genetics links research to the clinic, translating advances in our understanding of the molecular basis of genetic diseases for a practicing clinical geneticist.

Clinical genetics involves the study, counselling and treatment of individuals and families with heritable disorders and disease predisposition.

Diagnostic tools include standard ontologies for describing dysmorphology and traits, pedigree analysis, disease locus mapping by linkage or homozygosity, karyotyping, genome sequencing and genotyping.

A specialist in clinical genetics is called a clinical geneticist.

What does a clinical geneticist do?

1) appropriate management of rare disorders;

2) genetic testing;

3) reproductive options, e.g., the availability of prenatal testing for a specific disorder;

4) risk assessment of individuals and genetic counselling of family members.

A clinical geneticist manages such conditions as:

1) chromosomal abnormalities, which cause birth defects, mental retardation and/or reproductive problems;

2) single gene disorders such as cystic fibrosis, muscular dystrophy, Huntington's disease, and a sickle cell disease;

3) familial cancer and cancer-prone syndromes such as inherited breast or colorectal cancer and neurofibromatosis;

4) birth defects with a genetic component such as neural tube defects and cleft lip and palate;

5) inherited cardiac conditions associated with sudden death;

6) learning difficulties associated with other problems or a family history.

Clinical geneticists use the following tools in their work:
1) clinical history taking;
2) physical examination;
3) genetic testing;
4) genetic counselling;
5) performing skin biopsies;
6) taking clinical photographs;
7) using computerised databases.

Brief history. History of clinical genetics started in 1866, when Gregor Mendel published his theories of inheritance based on hybridization experiments in garden peas. In the late 19th century and early 20th century, a group of scientists (Francis Galdon, Karl Pearson and Raphael Weldon) did not agree with Mendelian inheritance, insisting that most phenotypic variation was continuous. The debate between supporters of Mendelian inheritance and the biometric school of inheritance lasted for two decades. But in 1918 R.A. Fisher in his publication of «The Correlation between Relatives on the Supposition of Mendelian Inheritance» finally resolved the disagreement. Fisher showed that the «continuous variation» described by biometricians could represent the cumulative result of many discrete genetic loci, and that the family resemblance of continuous traits could be explained by Mendelian inheritance. Hence, it became appreciated that most human complex traits and diseases (e.g., height, body mass index, serum lipid levels, blood pressure, psoriasis, dementia, numerous types of cancer) represent multifactorial traits –– i.e., the result of contributions from dozens or hundreds, perhaps thousands, of genes (polygenic), combined with additional modifying effects such as contribution of epigenetic effects and other environmental factors.

In the early 1900s, Sir Archibald Garrod described several human diseases that he termed «inborn errors of metabolism».

In 1909 Wilhelm Johannsen coined the term «gene», and in 1915, Thomas Morgan Hunt showed that genes, which exist on chromosomes, are the basic units of inheritance.

In 1920 Hans Winkler coined the term «genome» to refer to all the genes in an organism by combining the words gene and chromosome.

In 1953 James Watson, Francis Crick and Rosalind Franklin, discovered the double helix structure of DNA (deoxyribonucleic

acid), building on evidence that DNA was the primary component of chromosomes and genes.

In 1966 Marshall Nirenberg was «cracking the genetic code» and describing all RNA codones for all twenty amino acids. Based on this information, in 1978 genetically engineered bacteria were used to produce human insulin for the treatment of diabetes, and in 1983 scientists identified the gene responsible for Huntington's disease, which led to the first genetic test for a disease.

In 1984, Sir Alec John Jeffreys developed DNA profiling or «fingerprinting» to be used in paternity testing and forensics.

In 1989, Stephen Fodor developed the first DNA «microarray» and scanner, which would eventually lead to a method for testing hundreds of thousands of genetic variants simultaneously and thus foreshadowing the upcoming era of genome-wide association studies (GWAS).

Now we believed that genetic factors play a role in most human diseases, with gene variations contributing to their incidence or course.

Variations can be broadly categorized as (I) single gene, (II) chromosomal disorder and (III) complex.

I. Variations of a single gene may lead to rare diseases with predictable inheritance such as cystic fibrosis and Marfan syndrome. The identification of the specific DNA variations linked to them has opened up the possibilities for new treatments now.

II. Chromosome abnormalities arise from genomic variants. Because most chromosome abnormalities occur accidentally in the ovum or sperm, the abnormality is present in every cell of the body. Some abnormalities arise after birth, however, resulting in a condition in which a few cells have the abnormality and others do not. Chromosomes abnormalities can be either inherited from a parent (e.g., translocation) or develop spontaneously for the first time.

III. Variations involving multiple genes interacting with each other and with environmental factors (e.g., smoking, poor diet, lack of exercise) have been associated with 'complex common diseases'. These include a wide variety of conditions such as stroke, high blood pressure, high cholesterol level, heart disease, cancer, diabetes, and Parkinson's disease.

Topics of clinical genetics:

1. Linking of genetic variations to disease.
2. Genome rearrangements and disease.

3. Epigenetics and disease.
4. The translation of genotype to phenotype.
5. Genetics of complex diseases.
6. Management/intervention of genetic diseases.
7. Novel therapies for genetic diseases.
8. Developmental biology, as it relates to clinical genetics.
9. Cancer genetics.
10. Genome-wide analyses (genomics, transcriptomics, epigenomics) with an obvious significance for the clinic.
11. Studies of animal models that help understand the genetic basis of human diseases.
12. Bioinformatics and computational analysis of genetic/genomic disorders.
13. Ethical, legal, and social implications of medical genetics.
14. Social science research on the psychological and behavioural aspects of living with or being at risk of a genetic disease.

Red Flags for Genetic Disease:

1. The occurrence of the same condition in more than one family member (particularly first-degree relatives).
2. Multiple miscarriages.
3. Stillbirths.
4. Childhood deaths.
5. Family history of common adult conditions (heart disease, cancer, dementia) that occur in two or more relatives at relatively young ages.
6. Developmental delay/mental retardation.
7. Congenital abnormalities.
8. Multiply dysmorphologies (Table 1).

Table 1

Multiply dysmorphologies

Organ/system	Typical dysmorphologies
Skin	Telangiectasia, venous network, spots, depigmentation, dark brown freckles, hypertrichosis, hirsutism, fibroma
Subcutaneous adipose tissue	Excess or reduced deposition
Muscle	Hypertrophy, hypotrophy, aplasia

Hair	Dry, sparse, alopecia, low hair growth on the forehead or neck, gray hair above the forehead
Skull	Hydrocephalus, microcephaly, macrocephaly, brachycephaly, dolichocephaly, trigonocephaly, protruding forehead, occiput, flat occiput
Auricles	Anotia, macrotia, microtia, deformed, low-lying, protruding, deflected backward, preauricular fistulas and papillomas
Eyes	Anti-Mongoloid and Mongoloid eye incision, epicant, hypertelorism, Ptosis, strabismus, microphthalmos, exophthalmos, double and triple rows of eyelashes, iris coloboma, iris heterochromia, blue sclera, telangiectasia
Nose	Short, coracoid, saddle nose, flat wings of the nose
Jaws	Progeny, retrogenic, macrogenic, microgenic and macrognathic
Lips and mouth	Macrostomy and microstomy; lips tonic (thin) and thick; the palate is flat, high, gothic, the cleft of the palate; bifurcation of the uvula; macroglossia and microglossia
Teeth	Macrostomy and microstomy; lips tonic and thick; the sky is flat, high, gothic, the cleft of the sky; bifurcation of the uvula; macroglossia and microglossia
Neck	Short, long, pterygoid folds, low hairline, torticollis
Chest and trunk	Dolichostenomelia, funnel-shaped, keeled, accessory nipples, scoliosis, lordosis, kyphosis
Limbs	Dolichostenomelia, funnel-shaped, keeled, accessory nipples, scoliosis, lordosis, kyphosis
Nails	Wide, short, concave, hypoplasia

They are caused by DNA changes in a particular gene, and often have predictable inheritance patterns.

The mutated version of the gene responsible for the disorder is known as a mutant, or disease, allele.

Over 10,000 human disorders are caused by a mutation in a single gene.

Individually, single gene disorders are rather rare, but as a whole, they affect about one per cent of the population.

Since only a single gene is involved, these disorders can be easily tracked through families and the risk of them occurring in later generations can be predicted.

Single gene disorders can be divided into different categories: dominant, recessive and X-linked.

Dominant diseases are single gene disorders that occur in the heterozygous state — when an individual has one mutant copy of the relevant gene and one healthy copy.

The effects of the mutant version of the gene (allele) override the effects of the healthy version of the gene.

Dominant disorders tend to crop up in every generation of an affected family because everyone carrying a dominant mutant allele shows the symptoms of the disease.

Dominant disorders spread vertically down family trees, from parent to child.

In rare cases when an individual has two copies of the mutant gene (also known as being homozygous) the disorder symptoms are generally more severe.

Huntington's disease is a common example.

Recessive diseases are single gene disorders that only occur in the homozygous state — when an individual carries two mutant versions (alleles) of the relevant gene.

The effects of the healthy allele can compensate for the effects of the mutant allele.

Recessive diseases are more difficult to trace through family trees because carriers of a mutant allele do not show symptoms of the disease. It therefore appears that the disease has skipped a generation when it is seen in groups of children within a family.

The risk of an individual having a recessive disorder increases when two people who are closely related have a child together (consanguinity). Cystic fibrosis (CF) and Tay-Sachs disease are common examples.

Spinal muscular atrophy (SMA) is an autosomal recessive hereditary disease too. It is associated with severe muscle atrophy and weakness in the limbs and trunk. It is an important genetic cause of mortality in infants and progressive motor impairment in children and adults (Mercuri et al., 2012; Wadman et al., 2017). The disease presents with a wide spectrum of clinical severity and patients are classified into five types, depending on the age of onset and motor milestones achieved. SMA is caused by deficiency of the survival motor neuron (SMN) protein due to the homozygous loss of function of the SMN1 gene located on chromosome 5q13. This region has a complex structure with repeat sequences and many pseudo gene clusters, which make gene deletion and transformation easy. Several disease modifiers are known.

The SMN1 and SMN2 genes are two highly homologous genes and are arranged in tandem on the chromosome.

There are a total of five nucleotide differences between the two genes. They are mainly distinguished by two loci on exon 7 and exon 8.

The SMN1 gene is the causative gene for SMA, and the SMN2 gene functions to modify the SMA phenotype (Figure 1).

SMA severity-modifying genes outside the SMN locus include plastin 3 (PLS3), neurocalcin delta (NCALD) and CORO1C.

X-linked dominant disorders are seen more commonly in females than in males, or in case of some diseases, they affect only females.

In the latter case, it is thought that the hemizygous males are so severely affected, they do not survive.

This may be reflected in the pedigree by multiple miscarriages or male infant deaths.

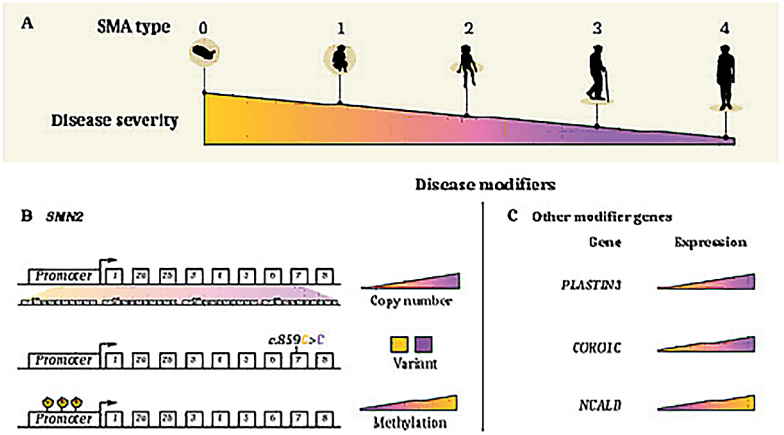


Figure 1. Known molecular disease modifiers. (A) Current classification of SMA as a discrete projection of a continuous spectrum of disease severity. This spectrum results from the combination of (B) disease modifiers such as SMN2copy number, SMN2 variant, and methylation state; (C) level of expression of CORO1C and PLASTIN3 was associated with milder phenotype, while NCALD overexpression was associated with more severe phenotype (Cit. Smeriglio, P. et al., J. Pers. Med. 2020, 10(3), 75; https://doi.org/10.3390/jpm10030075)

Examples of X-linked dominant disorders include Rett syndrome.

X-linked recessive disorders most often occur in males. A single recessive gene on the X chromosome will cause the disease.

Females can get an X-linked recessive disorder, but this is very rare.

• First: Female carriers can have a normal X chromosome that is abnormally inactivated. This is called «skewed X-inactivation». These females may have symptoms similar to those of males, or they may suffer only from mild symptoms.

• Second: in case of Turner syndrome when a woman has only one X chromosome she can suffer from X-linked recessive disease too.

Diseases such as hemophilia and Duchenne muscular dystrophy occur from a recessive gene on the X chromosome.

Y-linked disorders. The Y chromosome does not contain most of the genes of the X chromosome. Mutation can only be passed from father to son. Examples are Y chromosome infertility, an some cases of Swyer syndrome.

Codominant inheritance. Two different versions (alleles) of a gene are expressed, and each version makes a slightly different protein.

Both alleles influence the genetic trait or determine the characteristics of the genetic condition. Examples are ABO blood group, alpha-1 antitrypsin deficiency.

Mitochondrial inheritance also known as maternal inheritance applies to genes in mitochondrial DNA. Mitochondria contain a small amount of DNA. Because only egg cells contribute mitochondria to the developing embryo, only females can pass on mitochondrial mutations to their children. Conditions resulting from mutations in mitochondrial DNA can appear in every generation of a family and can affect both males and females, but fathers do not pass these disorders to their daughters or sons.

Nota bene! In some cases of recessive inheritance (autosomal or sex) you can see the signs of disease if patients have only one copy abnormal gene (heterozygous). It can be related with genomic imprinting. Usually both copies of each gene are active, or «turned on», in cells. In some cases, however, only one of the two copies is normally turned on. Which copy is active depends on the parent of origin: father or mother.

This phenomenon is known as genomic imprinting. In genes that undergo genomic imprinting, the parent of origin is often marked, or «stamped», on the gene during the formation of egg and sperm cells. This stamping process is called methylation of DNA. Small molecules called methyl groups identify which copy of a gene was inherited from the mother and which was inherited from the father. DNA methylation occurs mainly on the fifth carbon of the cytosine base, forming 5-methylcytosine or 5-methylcytidine (5-mC). It is found almost exclusively at (in)CpG dinucleotides and is a key epigenetic marker and regulator of gene expression. Methylated CpG clusters at gene promoters, or CpG islands, have been associated with gene inactivation.

DNA methylation is catalyzed by a family of enzymes called DNA methyltransferases and include DNMT1, DNMT3a and DNMT3b. DNMT3a and DNMT3b are de novo methyltransferases that are able to methylate previously unmethylated CpG dinucleotides. In contrast DNMT1 is a maintenance methyltransferase, and it modifies hemi-methylated DNA during replication. Only a small percentage of all human genes undergo genomic imprinting. Imprinted genes tend to cluster together in the same regions of chromosomes. Two major clusters of imprinted genes have been identified in humans, one on the short (p) arm of chromosome 11 (at position 11p15) and another on the long (q) arm of chromosome 15 (in the region 15q11 to 15q13).

You can find an additional information following to the next links:

• MIM (OMIM) — On-Line Mendelian Inheritance in Man https://www.ncbi.nlm.gov/omim.

• OMD — Oxford Medical Database https://dhmhd.mdx.ac.uk/LDDB/lddb.html.

• http://genome-euro.ucsc.edu/cgi-bin/hgGateway.

• https://www.rarechromo.org/.

• https://www.orpha.net/consor/cgi-bin/index.php — The portal for rare diseases and orphan drugs.

Chromosomes are the structures that hold our genes. Each of our chromosomes has a characteristic structure. Historically, scientists have used a staining technique that colours the chromosomes into a banding pattern. These banding patterns make each of our individual chromosomes easier to identify, like a map. A set of chromosomes, as seen under a microscope, is known as a karyotype.

Any deviation from the normal karyotype (chromosomes (or parts of chromosomes) are missing or changed or extra) is known as a chromosome abnormality and is characterized by malformations or malfunctions in any of the body's systems.

Most chromosome abnormalities originate in the egg or sperm (gametes) but some happen during embryo development or are inherited from a parent.

Cytogenetic abnormalities are present in 50% of fetal deaths before 20 weeks of the gestational age and are present in 6 to 13% of stillbirths.

Most autosomal disorders cause death before the age of 5 years or multi-domain disability (exclude Down's syndrome).

Disorders of the sex chromosomes have a lesser effect on survival but can cause infertility, congenital malformations such as congenital heart disease in Turner syndrome, as well as having neurodevelopmental and psychological impacts.

Risk factors:

1) maternal age (the frequency of meiotic nondisjunction increases with maternal age);

2) environmental factors such as exposure to certain drugs.

Classifications of chromosome disorder:

1. Numerical — whole chromosomes either missing from or extra to the normal pair.

2. Structural — part of an individual chromosome is missing, extra, switched to another chromosome, or turned upside down.

Numerical disorders.The most severe chromosome disorders are caused by the loss or gain of whole chromosomes, which can affect hundreds, or even thousands, of genes and are usually fatal. A few numerical abnormalities support development to term, either because the chromosome is small and/or contains relatively few genes or because there is a natural mechanism present to help adjust gene dosage.

The major numerical abnormalities (Table 2).

Table 2

The major numerical abnormalities

Syndrome	Abnormality	Incidence
Down's	Trisomy 21	15 in 10,000
Edwards'	Trisomy 18	3 in 10,000
Patau's	Trisomy 13	2 in 10,000
Turner	Monosomy X	2 in 10,000 (female births)
Klinefelter's	XXY	10 in 10,000 (male births)
XXX	XXX	10 in 10,000 (female births)
XYY	XYY	10 in 10,000 (male births)

Robertsonian translocations are named for the America insect geneticist W.R.B. Robertson who first described this form of translocation (in grasshoppers) in 1916 and are also known as whole-arm or centric-fusion translocations or rearrangements.

A common and significant type of chromosome rearrangement that is formed by fusion of the whole long arms of two acrocentric chromosomes (chromosomes with the centromere near the very end).

One in about 900 babies is born with a Robertsonian translocation making it the most common kind of chromosome rearrangement known in people.

All five of the acrocentric chromosomes in people — chromosome numbers 13, 14, 15, 21 and 22 — have been found to engage in Robertsonian translocations (Figure 2).

Specifically, rob (13q14q) and rob (14q21q) are the most common, constituting approximately 85% of all Robertsonian.

In balanced form, a Robertsonian translocation takes the place of two acrocentric chromosomes and results in no problems for the person carrying it.

плохое качество!!!

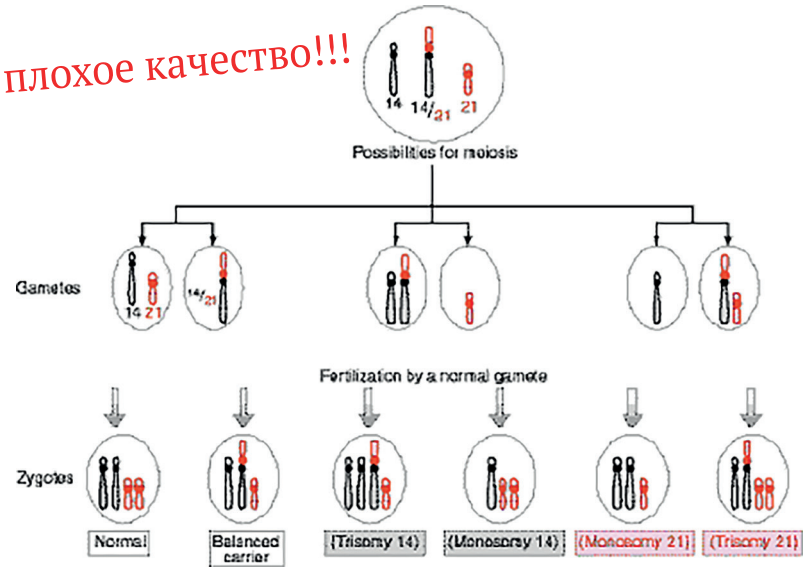


Figure 2. The possibilities of Robertsonian translocations

But in unbalanced form, Robertsonian translocations between chromosomes 13 and 14 lead to the trisomy 13 (Patau syndrome). And the Robertsonian translocations

The number of live born babies with Down syndrome is 10% if the mother is the translocation carrier, and 2.5% if the father is the translocation carrier.

Rob (21; 21) (q10, q10) is the rarest of all translocations causing Down syndrome (Figure 3).

These patients cannot be clinically differentiated from Down syndrome caused by other genetic causes. Robertsonian translocations may either be de novo or inherited from a balanced carrier parent. In ~50% of cases of Robertsonian, the rearrangements occur de novo and ~95% of the de novo cases originate during maternal meiosis.

For the de novo mutation of rob (21q; 21q) with genetically normal parents, the risk of recurrence is 2–3%. Balanced Robertsonian translocation carriers (21;21) (q10, q10) are phenotypically normal but will always have the offspring with Down syndrome.

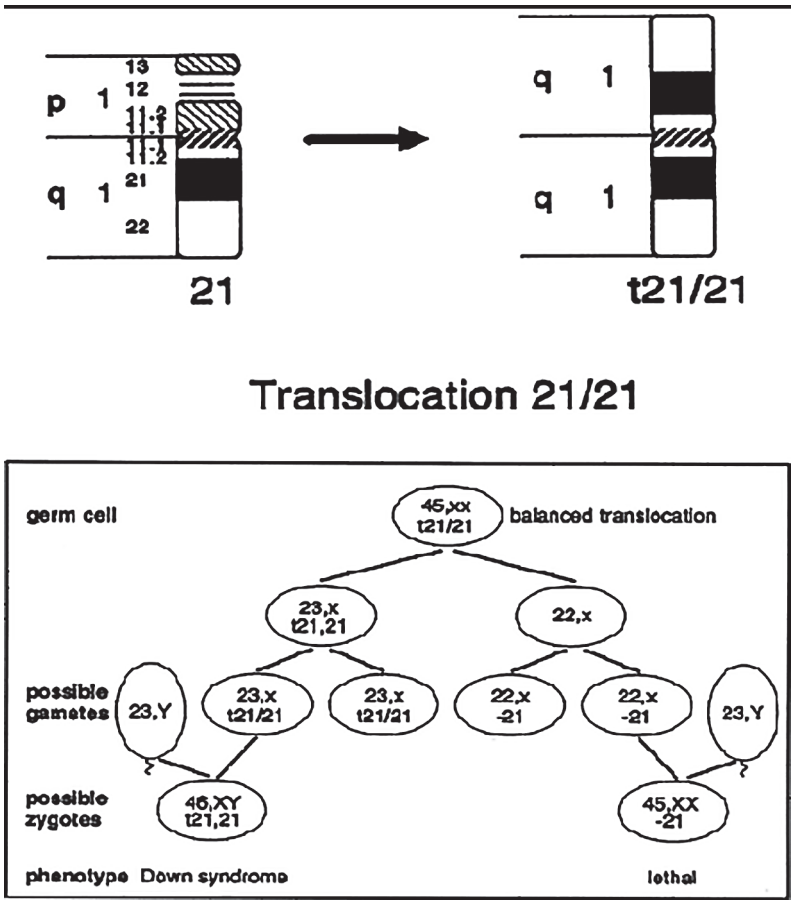


Figure 3. The process of Rob (21q; 21q) formation

If a t(21q; 21q) balanced translocation carrier person marries someone who is also a t(21q; 21q) balanced translocation carrier, the possibilities are:

1) 21 tetrasomy;
2) null chromosome 21;
3) t(21q;21q) balanced translocation carrier.

Because the first two types of fetuses are not compatible with viability, probably they will be aborted, and all viable children will be

balanced translocation carriers and will have normal phenotypes, as do their parents. However, the effect of uniparental disomy may be seen in these children. The second problem is repeat miscarriages.

Structural chromosome disorders. Result from breakages within a chromosome.

In these types of disorders there may be more or less than two copies of any gene.

This difference in number of copies of genes may lead to clinical differences in affected individuals.

The loss of a segment of a chromosome is usually more serious than having an extra copy of the same segment.

Knowing the gene(s) involved can sometimes, but not always, help to predict signs and symptoms.

Balanced structural abnormalities involve the rearrangement of genetic material but with no overall gain or loss. For example, inversions and translocations.

Balanced structural abnormalities can have an immediate effect, but the major consequence is the production of eggs or sperm with incomplete or partially duplicated sets of chromosomes.

Unbalanced structural abnormalities involve genetic material being gained or lost.

Even tiny unbalanced structural abnormalities can affect many genes and, consequently, have severe effects on the individual.

Chromosomal deletions. A piece or section of chromosomal material is missing.

Deletions can occur in any part of any chromosome.

When there is just one break in the chromosome, the deletion is called a terminal deletion because the end (or terminus) of the chromosome is missing. When there are two breaks in the chromosome, the deletion is called an interstitial deletion because a piece of chromosome material is lost within the chromosome. Deletions that are too small to be detected under a microscope are called microdeletions.

Cri du chat syndrome — caused by a missing piece (deletion) of the short (p) arm of chromosome 5. This chromosomal change is written as 5p-. The size and location of the deletion varies among affected individuals, but studies suggest that larger deletions tend to result in more severe symptoms than smaller deletions.

The incidence ranges from 1 : 15,000 to 1 : 50,000 liveborn infants.

The most characteristic finding is a high-pitched, monotonous cry (cat-like cry).

Other features include microcephaly, low birth weight, hypotonia, psychomotor retardation, and craniofacial malformations.

Microdeletion syndromes involve chromosomal deletions that include several genes but are too small to be detected by karyotype and require the application of molecular cytogenetic techniques such as Fluorescence In Situ Hybridisation (FISH) or chromosomal microarray (CMA).

They are usually de novo and tend to recur in the same regions due to homologous recombination of flanking low-copy repeat gene clusters. These low-copy repeats (duplicons) are prone to deletion, duplication, and inversion.

The classical microdeletion syndromes were first described based on common clinical and phenotypic features, and the underlying associated chromosomal defect was later identified. But a lot of recurrent pathogenic microdeletions are not, and thus they can only be diagnosed by screening.

Classic:

1. Angelman syndrome (15qll-l)1 in 15,000.
2. Prader-Willi syndrome(15qll-13) 1 in 15,000.
3. DiGeorge (22qll) 1 in 4,000.
4. Wilms tumour-aniridia syndrome -microdeletion from short arm of chromosome 11, 1 in 500,000 to 1 million.

Even if chromosomes had not numerical and structural abnormalities, the normal work of genes can be interrupted by epigenetic influences. So, uniparental disomy (UPD) occurs when a person receives two copies of a chromosome, or part of a chromosome, from one parent and no copies from the other parent. UPD can occur as a random event during the formation of egg or sperm cells or may happen in early fetal development. In many cases, UPD likely has no effect on health or development. In some cases, however, it does make a difference whether a gene is inherited from a person's mother or father. A person with UPD may lack any active copies of essential genes that undergo genomic imprinting. Angelman syndrome can result when a baby inherits both copies of a section of chromosome 15 from the father. It can also occur, even when chromosome 15 is inherited normally — 1 chromosome coming from each parent, but section of the mother's chromosome 15 is deleted. This deletion

of a section of the maternally inherited chromosome is the most common cause of AS and it is called as microdeletion syndrome.

Prader-Willi syndrome (PWS), on the other hand, can result when a baby inherits both copies of a section of chromosome 15 from the mother.

Chromosomal duplications An extra copy of a segment of a chromosome. A person with a duplication has three copies of a particular chromosome segment instead of the usual two copies. Like deletions, duplications can happen anywhere along the chromosome.

MECP2 duplication syndrome occurs almost exclusively in males and is characterized by moderate to severe intellectual disability.

Most people also have weak muscle tone in infancy, feeding difficulties, poor or absent speech, or muscle stiffness (rigidity). Individuals with MECP2 duplication syndrome have delayed development of motor skills such as sitting and walking and the loss of previously acquired skills (developmental regression).

Many individuals with MECP2 duplication syndrome have recurrent respiratory tract infections.

These respiratory infections are a major cause of death in affected individuals, with only half surviving over the age 25.

Translocations. Balanced translocations occur when a chromosome segment is moved from one chromosome to another, there is no detectable net gain or loss of DNA.

Unbalanced translocations occur when a chromosome segment is moved from one chromosome to another. The overall amount of DNA has been altered (some genetic material has been gained or lost).

Inversions. Chromosome breaks in two places and the resulting piece of DNA is reversed and re-inserted into the chromosome. Inversions that involve the centromere are called pericentric inversions; inversions that do not involve the centromere are called paracentric inversions.

Isochromosomes. Abnormal chromosomes with identical arms — either two short (p) arms or two long (q) arms. Both arms are from the same side of the centromere, are of equal length, and possess identical genes.

Pallister-Killian mosaic syndrome is a developmental disorder that affects many parts of the body. This condition is characterized

Figure 4. Tetrasomy 12p karyotype using a basic staining technique. The extra 12p isochromosome is indicated by an arrow and labelled i12p (Cited by https://www.rarechromo.org/)

by extremely weak muscle tone (hypotonia) in infancy and early childhood, intellectual disability, distinctive facial features, sparse hair, areas of unusual skin coloring (pigmentation), and other birth defects (extra fingers and/or toes, large big toes (halluces), and unusually short arms and legs, ets). An error in cell division called nondisjunction likely results in a reproductive cell containing an isochromosome 12p (Figure 4, 5). If this atypical reproductive cell contributes to the genetic makeup of a child, the child will have two normal copies of chromosome 12 along with an isochromosome 12p. As cells divide during early development, some cells lose the isochromosome 12p, while other cells retain the abnormal chromosome. This situation is called mosaicism. Almost all cases of Pallister-Killian mosaic syndrome are caused by mosaicism for an isochromosome 12p. If all body's cells contained the isochromosome, the resulting syndrome would probably not be compatible with life.

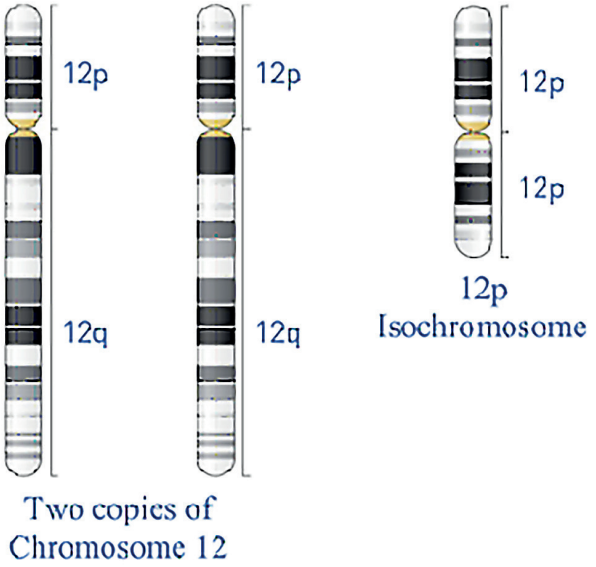


Figure 5. Cells with tetrasomy 12p have two copies of chromosome 12 and an extra isochromosome made from two opposite facing p arms of chromosome 12 (Cited by https://www.rarechromo.org/)

Mosaicism is when a person has a chromosome abnormality in some, but not all, cells (Figure 6). It is often difficult to predict the effects of mosaicism because the signs and symptoms depend on which cells of the body have the chromosome abnormality.

Dicentric chromosomes result from the abnormal fusion of two chromosome pieces, each of which includes a centromere.

Ring chromosomes -the ends of both arms of the same chromosome are deleted, which causes the remaining broken ends of the chromosome to be «sticky». These sticky ends then join to make a ring shape. The deletion at the end of both arms of the chromosome results in missing DNA, which may cause a chromosome disorder.

Ring chromosome 14 syndrome is a condition characterized by seizures and intellectual disability. Recurrent seizures (epilepsy) develop in infancy or early childhood. In many cases, the seizures are resistant to treatment with anti-epileptic drugs. Slow growth

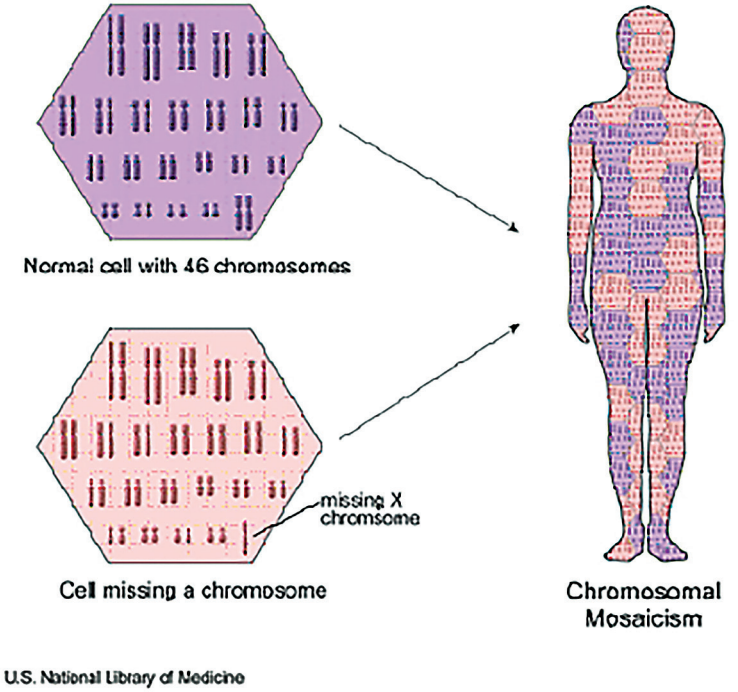


Figure 6. The illustration of chromosomal mosaicism

and short stature, a small head (microcephaly), puffy hands and/or feet caused by a buildup of fluid (lymphedema), and subtle differences in facial features. Some affected individuals have problems with their immune system that lead to recurrent infections, especially involving the respiratory system. Its prevalence is unknown.

Although complex disorders often cluster in families, they do not have a clear-cut pattern of inheritance. This makes it difficult to determine a person's risk of inheriting or passing on these disorders.

Complex disorders are also difficult to study and treat because the specific factors that cause most of these disorders have not yet been identified. Researchers continue to look for major contributing genes for many common complex disorders. For example, researchers have found about 60 genomic variants to be present more frequently in people with coronary artery disease. Most of these variants are dispersed across the genome and do not cluster on one specific chromosome. Polygenic risk score is an estimate of an individual's genetic liability to a trait or disease, calculated according to their genotype profile and relevant genome-wide association study (GWAS) data. (Figure 7).

Each red «v» represents variants in an individual's genome that is associated with coronary artery disease. Each smaller gray «v» is a variant that is also present in the person's genome but is not implicated in disease (Cited from https://www.genome.gov/sites/default/files/inline-images/prs-complex.jpg)

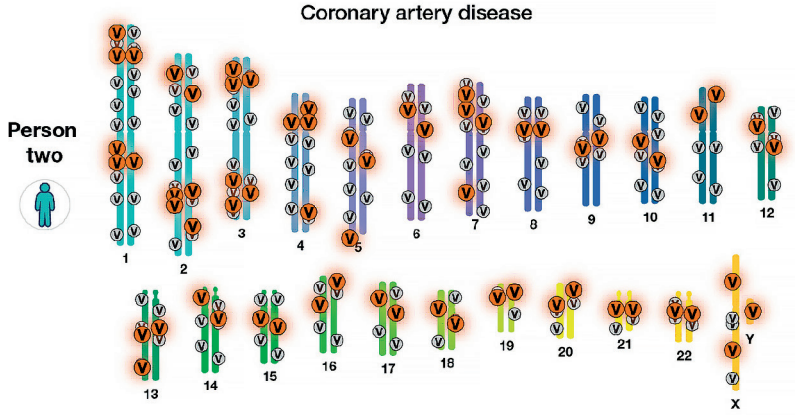


Figure 7. Chromosomal loci responsible for the formation of coronary artery disease.

The diagnostics of a genetic disease requires three major elements:

1) a physical examination (see Table 1);

2) a detailed medical family history;

3) clinical and laboratory testing if available:

– cytogenetic,

– biochemical (inherited metabolic diseases),

– immunological (immunohystochemistry),

– molecular genetic.

The diagnosis of a genetic disease can be made by using of:

A) Screening program:

– public health program of screening;

– selective screening and targeted screening.

B) Diagnostic testing.

Confirmation of a diagnosis when a particular condition is suspected based on physical signs and symptoms.

Public health program of screening is defined as:

«Screening is the process of identifying healthy people who may be at increased risk of disease or condition. The screening provider offers information, further tests and gives treatment. This is to reduce associated risks or complications» (UK National Screening Committee https://www.gov.uk/guidance/nhs-population-screening-explained).

Or screening refers to the use of simple tests across an apparently healthy population to identify individuals who have risk factors or early stages of a disease, but do not yet have symptoms (WHO).

The different categories of genetic tests are:

1. Predictive genetic testing: can inform you about the chances that a healthy person with or without a family history of a certain disease might develop this disease. These diseases can include some types of cancer and heart disease.

2. Presymptomatic genetic testing: can reveal if a person with a family history for a genetic disease but without any symptoms, has a genetic alteration associated with the disease.

3. Carrier testing: can determine if a person carries one copy of a gene linked to a certain disease. An «autosomal recessive» disease means that the disease will appear only if two copies of the altered gene are inherited. Couples who both carry the same autosomal recessive gene have a one in four, or 25 percent, chance with each pregnancy to have a child with the disease.

In many countries there are population-wide screening programs for unborn (antenatal) or newborn babies. These screening programs are used to detect whether the baby is at a higher risk of certain conditions and therefore lead to an earlier diagnosis and inform timely of decisions about their care and/or treatment.

If family members are at high risk for having a child with a severe or fatal defect, options might include:

1. Pre-implantation diagnosis. It is used only in case of in vitro fertilization combined with a blastomere/trophectoderm biopsy to diagnose a genetic disease in an embryo before it is implanted into the mother's uterus. The cell or cells obtained by biopsy are tested for the monogenic disorder (PGT-M) and/or chromosomal anomalies (PGT-SR). This technique raised many ethical discussions in various countries and is not allowed everywhere. Studies showed a wide approval of PGD for medical purposes, such as hereditary cancer, and a general denial for other purposes, such as sex-selection.

2. Using donor sperm or donor eggs (used only in case of in vitro fertilization) or donor embryo.

3. Adoption

4. Taking the risk and having a child.

5. Becoming pregnant and having specific prenatal testing to diagnose a genetic disease or condition in the developing fetus. It includes blood screening, ultrasound examination, invasive procedures (amniocentesis, chorionic villus sampling (CVS), and percutaneous umbilical blood sampling (PUBS)). Possible option, if fetus has a severe or fatal defect or chromosomal disorder, it includes fetal surgery and treatment (if possible) or ending the pregnancy.

In some countries there may be law restrictions on ending the pregnancy or fetus treatment (usually for religious reason).

First trimester screening is performed between 8–12 weeks and is a two-step prenatal test that offers early information about a baby's risk of certain chromosomal conditions, including Down syndrome (Trisomy 21) and Edwards syndrome (Trisomy 18):

1. Maternal blood testing: pregnancy-associated plasma protein-A (PAPP-A), free beta human chorionic gonadotropin (free beta-hCG).

2. Fetal ultrasound — measures the size of the clear space in the tissue at the back of the baby's neck (nuchal translucency).

A computer program combines these test results plus maternal age, weight and baby's correct gestational age, to identify the risk of Down syndrome, Trisomy 13 and Trisomy 18. First trimester screening correctly identifies about 85 percent of women who are carrying a baby with Down syndrome. About 5 percent of women have a false-positive result, meaning that the test result is positive, but the baby does not actually have Down syndrome.

Second trimester screening includes the triple and quadruple screens.

It is performed between 15 and 20 weeks of pregnancy and screening is for neural tube defects such as spina bifida and for the same chromosomal disorders tested for in the first trimester. At this trimester the specialists using the ultrasound examination for testing.

In cases of positive screening for chromosomal disorder the invasive procedures will be done, such as amniocentesis, chorionic villus sampling (CVS) and percutaneous umbilical blood sampling (PUBS).

You need to remember that invasive procedures pose a small risk of miscarriage.

An noninvasive prenatal testing (NIPT). During pregnancy, the mother's bloodstream contains a mix of cfDNA that comes from her cells and cells from the placenta (usually identical to the DNA of the fetus).

The test analyzes small fragments (fewer than 200 DNA building blocks) of DNA that are circulating in the pregnant woman's blood. These small fragments arise when cells die off and get broken down and their contents, including DNA, are released into the bloodstream.

NIPT is most often used to look for chromosomal disorders that are caused by the presence of an extra or missing copy (aneuploidy) of a chromosome (Down syndrome, trisomy 18, trisomy 13, and extra or missing copies of the X chromosome and Y chromosome.

NIPT may include screening for additional chromosomal disorders that are caused by missing (deleted) or copied (duplicated) sections of a chromosome. NIPT is beginning to be used to test for single-gene disorders.

The Family Genetic Counseling is the important part of clinical geneticists' work.

Family Genetic Counseling is needed for parents to be provided with the relevant information about the methods, associated risks, and the available options if the test results are positive. Furthermore, some tests can give an indicative but inconclusive result leaving the parents uncertain or facing further risky tests for a definitive result to confirm the presence of an abnormality. Risk information should be tailored to the social, religious, and cultural background of the user(s). The final decision about prolongation, termination of pregnancy or fetus treatment will be made by the family, not by a specialist.

It is especially important to consider genetic counseling if:

1) a standard prenatal screening test has an abnormal result;

2) an amniocentesis test yields an unexpected result;

3) either parent or a close relative has an inherited disease or birth defect;

4) either parent already has children with birth defects, intellectual disabilities, or genetic disorders;

5) the mother-to-be has had two or more miscarriages or babies that died in infancy;

6) the mother-to-be will be 35 or older when the baby is born. Chances of having a child with Down syndrome increase with the mother's age;

7) there is concern about genetic defects that happen often in an ethnic or racial group;

8) either parent is concerned about the effects of exposure to radiation, medicines, illegal drugs, infections, or chemicals.

It is a public health program of screening in infants shortly after birth for conditions that are treatable, but not clinically evident in the newborn period.

NBS started with the discovery that the amino acid disorder phenylketonuria (PKU) could be treated by dietary adjustment, and that early intervention was required for the best outcome.

Screening programs are often run by state or national governing bodies with the goal of screening all infants born in the jurisdiction for a defined panel of treatable disorders. The number of diseases screened for is set by each jurisdiction and can vary greatly.

Newborn screening varies from country to country and can include:

A. Blood cell disorders:

1. Sickle cell anemia (Hb SS) >1 in 5,000; among African Americans 1 in 400.
2. Sickle-cell disease (Hb S/C) >1 in 25,000.
3. Hb S/Beta-Thalassemia (Hb S/Th) >1 in 50,000.

B. Inborn errors of amino acid metabolism:

1. Tyrosinemia I (TYR I) <1 in 100,000.
2. Argininosuccinic aciduria (ASA) <1 in 100,000.
3. Citrullinemia (CIT) <1 in 100,000.
4. Phenylketonuria (PKU) >1 in 25,000.
5. Maple syrup urine disease (MSUD) <1 in 100,000.
6. Homocystinuria (HCY) <1 in 100,000.

C. Inborn errors of organic acid metabolism:

1. Glutaric acidemia type I (GA I) >1 in 75,000.
2. Hydroxymethylglutaryl lyase deficiency (HMG) <1 in 100,000.
3. Isovaleric acidemia (IVA) <1 in 100,000.
4. 3-Methylcrotonyl-CoA carboxylase deficiency (3MCC) >1 in 75,000.
5. Methylmalonyl-CoA mutase deficiency (MUT) >1 in 75,000.
6. Methylmalonic aciduria, cblA and cblB forms (MMA, Cbl A,B) <1 in 100,000.
7. Beta-ketothiolase deficiency (BKT) <1 in 100,000.
8. Propionic acidemia (PROP) >1 in 75,000.
9. Multiple-CoA carboxylase deficiency (MCD) <1 in 100,000.

D. Inborn errors of fatty acid metabolism:

1. Long-chain hydroxyacyl-CoA dehydrogenase deficiency (LCHAD) >1 in 75,000.
2. Medium-chain acyl-CoA dehydrogenase deficiency (MCAD) >1 in 25,000.
3. Very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD) >1 in 75,000.
4. Trifunctional protein deficiency (TFP) <1 in 100,000.
5. Carnitine uptake defect (CUD) <1 in 100,000.

E. Miscellaneous multisystem diseases:

1. Cystic fibrosis (CF) >1 in 5,000.
2. Congenital hypothyroidism (CH) >1 in 5,000.
3. Biotinidase deficiency (BIOT) >1 in 75,000.
4. Congenital adrenal hyperplasia (CAH) >1 in 25,000.
5. Classical galactosemia (GALT) >1 in 50,000.

F. Newborn screening by other methods than blood testing:

1. Congenital deafness (HEAR) >1 in 5,000.

D. Disorders added in last decades (USA):

1. Severe combined immune deficiency (SCID) — added in 2009.
2. Critical congenital heart defects (Screened using pulse oximetry) — added in 2010.
3. Pompe disease — added in 2013.
4. Mucopolysaccharidosis type I — added in 2015.
5. X-linked adrenoleukodystrophy — added in 2018.
6. Spinal muscular atrophy — added in 2018.

More than 1 in 300 newborns have a condition detectable by newborn screening!

Selective screening and targeted screening. The key distinguishing feature of mass screening is that eligibility is based on a characteristic associated with the increased risk of the condition being detected, e.g., such as occupation or ethnicity.

A. Tandem mass spectrometry.

The introduction of tandem mass spectrometry (MS/MS) in the 1990s for population-based newborn screening has enabled to detect an increased number of metabolic disorders in a single process by using dried blood-spot specimens routinely collected from newborns. MS/MS technology enables improvements and consolidation of metabolic screening methods to detect amino acid disorders (e.g., PKU, maple syrup urine disease, and homocystinuria) in newborns, and does so with a low false-positive rate. MS/MS technology expands the metabolic disorder screening panel by incorporating an acylcarnitine profile, which enables detection of fatty acid oxidation disorders (e.g., medium-chain acyl-CoA dehydrogenase [MCAD] deficiency) and other organic acid disorders. MS/MS can reliably analyze approximately 20 metabolites in one short-duration run (i.e., ~2 minutes) and provide a comprehensive assessment from a single blood-spot specimen.

B. Cytogenetic methods:

• «Classic» karyotyping;

• Molecular cytogenetics.

Karyotyping is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome-wide snapshot of the individual's chromosomes. Karyotypes can reveal changes in the chromosome number. Careful analysis of karyotypes can also reveal more subtle structural changes, such as chromosomal deletions, duplications, translocations, or inversions.

The test can be performed on almost any tissue, including:

• Amniotic fluid (amniocentesis is needed).

• Blood.

• Bone marrow (a bone marrow biopsy is needed).

• Placenta.

If it is necessary to:

1. Check an unborn baby for genetic disorders.

2. Diagnose a genetic disease in a baby or a young child.

3. Find out if a chromosomal defect is preventing a woman from getting pregnant or is causing miscarriages.

4. Check a stillborn baby (a baby that died late in pregnancy or during birth) to see if a chromosomal defect was the cause of death.

5. Diagnose or make a treatment plan for certain types of cancer and blood disorders.

6. Examine a female with primary ovarian dysfunction.

7. Do before receiving assisted reproductive technology.

Molecular cytogenetics:

1. Fluorescence in situ hybridization (FISH) is a way to visualize and map the genetic material in the individual's cells, including specific genes or portions of genes. This may be used for understanding a variety of chromosomal abnormalities and other genetic mutations. The first step is to prepare short sequences of he single-stranded DNA that matches a portion of the gene the researcher is looking for. These are called probes. The next step is to label these probes by attaching one of the number of colors of fluorescent dye. DNA is composed of two strands of complementary molecules that bind to each other like chemical magnets. Since the researchers' probes are single-stranded, they are able to bind to the complementary strand of DNA, wherever it may reside on the person's chromosomes. When a probe binds to a chromosome, its fluorescent tag provides a way for researchers to see its location. Locus pecific probes bind to a particular region of a chromosome. This type of probe is useful when scientists have isolated a small portion of a gene and want to determine on hich chromosome the gene is located, or how many copies of a gene exist in a particular genome. Alphoid or centromeric repeat probes are enerated from repetitive sequences found in the middle of each chromosome. Researchers use these probes to determine whether an individual has the correct number of chromosomes. These probes can also be used in combination with «locus specific probes» to determine whether an individual is missing genetic material from a particular chromosome. Whole chromosome probes are ollections of smaller probes, each of which binds to a different sequence along the length of a given chromosome. Using multiple probes

labeled with a mixture of different fluorescent dyes, scientists can label each chromosome in its own unique color. The resulting full-color map of the chromosome is known as a spectral karyotype. Whole chromosome probes are particularly useful for examining chromosomal abnormalities, for example, when a piece of one chromosome is attached to the end of another chromosome.

2. Array comparative genomic hybridization (aCGH). Chromosomal microarray may detect more clinically significant chromosomal imbalances than karyotyping in a shorter time but does not detect inversions, triploidies or low mosaicisms. CMA and karyotype are complementary in this case, together permitting a more accurate diagnosis and genetic counseling than if only one method is used. Whole genome arrays are designed to cover the entire human genome. Whole genome arrays have been constructed mostly for research applications and have proven their outstanding worth in gene discovery. They are also very valuable in screening the genome for DNA gains and losses at an unprecedented resolution. Targeted arrays are designed for a specific region(s) of the genome for the purpose of evaluating that targeted segment. It may be designed to study a specific chromosome or chromosomal segment or to identify and evaluate specific DNA dosage abnormalities in individuals with suspected microdeletion syndromes or subtelomeric rearrangements.

3. Sequencing. In genetics and biochemistry, sequencing means to determine the primary structure (sometimes incorrectly called the primary sequence) of an unbranched biopolymer. Sequencing results in a symbolic linear depiction known as a sequence which succinctly summarizes much of the atomic-level structure of the sequenced molecule. Full genome sequencing provides information on a genome that is much larger than by DNA arrays, the previous leader in genotyping technology. For humans, DNA arrays currently provide genotypic information on up to one million genetic variants, while full genome sequencing will provide information on all six billion bases in the human genome, or 3,000 times more data.

Applications of array comparative genomic hybridization (aCGH):

1. Genomic abnormalities in cancer. Genetic alterations and rearrangements occur frequently in cancer and contribute to its pathogenesis. Array CGH may also be applied to the monitoring of the progression of tumors.

2. Submicroscopic aberrations. Prader-Willi syndrome (PWS) is a paternal structural abnormality involving 15q11-13, while a maternal aberration in the same region causes Angelman syndrome. In both syndromes, the majority of cases (75%) are the result of a 3–5 Mb deletion of the PWS/AS critical region.

3. Prenatal genetic diagnosis. The use of array CGH as a tool for pre-implantation of genetic screening is becoming an increasingly popular concept. It has the potential to detect CNVs and neuploidy in eggs, sperm or embryos which may contribute to a failure of he embryo to successfully implant, to miscarriage or the conditions such as Down syndrome (trisomy 21).

Whole Genome Sequencing sequences the complete DNA of an organism — all coding (exons) and noncoding (intron) nuclear DNA as well as mitochondrial DNA.

This allows researchers to generate large quantities of data, which can then be analyzed. The exome makes up only 1.5% of the whole human genome, however, includes ALL protein coding genes found in the exome. Since most genetic disorders are correlated with mutations in protein coding genes, most physicians and scientists who use sequencing technologies for diagnostic purposes start with an analysis of the exome. Exome sequencing and analysis typically takes less time than whole genome sequencing at less than half of he cost. However, researchers have found that DNA variations outside the exons can affect gene activity and protein production and lead to genetic disorders--variations that whole exome sequencing would miss. While many more genetic changes can be identified with whole exome and whole genome sequencing than with select gene sequencing, the significance of much of the information is unknown.

Exome sequencing is used for molecular diagnosis of rare monogenic disorders, identification of de novo variants implicated in omplex diseases such as autism, tumor/normal analysis to identify driver genes in cancer.

Targeted Gene Sequencing.

Targeted gene sequencing panels are useful tools for analyzing specific mutations in a sample. Focused panels contain a select set of genes or gene regions that have known or suspected associations with the disease or phenotype under study. Rapid targeted gene panels might provide a simpler alternative to rES and rGS, yet with comparable diagnostic rates.

Now there is available:

1. Cardiac Arrhythmias and Cardiomyopathy Research Panel. 92 genes.
2. Deafness Research Panel v2. 126 genes.
3. Autism Research Panel. 236 genes.
4. Dermatology Research Panel v2.
5. Dysmorphia-Dysplasia Research Panel v2. 519 genes.
5. Inborn Errors of Metabolism Research Panel v2.
7. Noonan Research Panel.
8. Ophthalmic Research Panel. 316 genes.
9. Renal Research Panel v2. 154 genes.
10. Endocrine Research Panel v2.
11. Epilepsy Research Panel. 386 genes.
12. And more…

How to take a decision?

1. Suspected chromosomal disorder — chromosome microchip.
2. Suspicion of a specific disease — targeted gene sequencing.
3. Uncertain clinical presentation — exome sequencing.
4. Nothing found, but you are sure of the genetic nature of the disease — genome sequencing.
5. RNA-seq methods are available for studying many different aspects of RNA biology, including single-cell gene expression, translation (the translatome) and RNA structure (the structurome).
6. So, allele-specific expression (ASE) refers to the characteristic of preferentially expressing a parental allele in the hybrid due to variations in regulatory sequences from the parental genomes. The expression difference caused by ASE may lead to phenotypic variation depending on the function of the genes.
7. DNA methylation is one of several epigenetic mechanisms that cells use to control gene expression.
8. DNA methylation analysis can help researchers gain valuable insight into gene regulation and identify potential biomarkers. Aberrant DNA methylation has been implicated in many disease processes, including cancer, obesity, and addiction.
9. The methyl marks are heritable, certain methylation patterns have transgenerational effects.

Personalized medicine is a broad and rapidly advancing field of health care that is informed by each person's unique clinical, genetic, genomic, and environmental information [Chan I.S., Ginsburg G.S. Personalized medicine: progress and promise. Annu Rev Genomics Hum Genet 2011; 12:217-44].

Personalized medicine is an emerging practice of medicine that uses an individual's genetic profile to guide decisions made regarding the prevention, diagnosis, and treatment of disease. Knowledge of a patient's genetic profile can help doctors select the proper medication or therapy and administer it using the proper dose or regimen. It is sometimes called precision medicine or personalized care. The term «personalized medicine» is older than precision medicine, but nowadays precision medicine is preferred.

Personalized medicine is being advanced through data from the Human Genome Project. The Human Genome Project was an international project that mapped and sequenced the entire human genome. It was announced in 1990 and completed in April 2003. Data from the project are freely available to researchers and others interested in genetics and human health.

The HGP has now been succeeded by the Human Variome Project, which seeks to compile and share the huge variation in human DNA sequence worldwide.

This is potentially possible since whole exome sequencing (WES) and whole genome sequencing (WGS) have been increasingly performed in several population studies.

For example, the 100,000 Genomes Project in the United Kingdom have achieved its goal of 100,000 genomes sequenced.

The «father» of Personalized Medicine term is Kewal K. Jain. Francis S. Collins, M.D., Ph.D. says: «Personalized medicine is a fantastic opportunity to take a «one size fits all» approach to diagnostics and drug therapy and prevention and turn it into an individualized

approach. We all are similar, of course, but we are also different. And the idea that medicine would be applied in a fashion that ignores those differences can't be any more correct than going to the shoe store and buying any old pair of shoes without checking the size. Lots of work to do here, but maybe the biggest revolution in medicine in a very long time».

Personalized medicine based on «4 P» model (Source: adapted from Future of Cancer Care, BCRF):

1. Predictive- determining the risk/susceptibility and treatment response.

2. Preventive-allowing early intervention to prevent the disease altogether.

3. Personalized-according to the genetic make-up of the person and their disease.

4. Participatory- involving the patient in decisions on prevention or treatment.

Personalized medicine enables to:

1) determine if a person has a higher risk of developing a disease and consequently apply suitable prevention strategies;

2) diagnose a disease earlier after onset, thereby allowing more effective treatment options;

3) enhance therapeutic efficacy by ensuring that the most appropriate medicine is used, and that the dosage regimen takes into account any genetic variations, which may influence how the medicine is metabolized;

4) avoid «trial and error» and reduce the probability of medicine-related side effects and complications by matching the treatment to a patient's genomic profile.

The genetic information required for targeting a treatment in personalized medicine is obtained through genetic testing. The testing can be differentiated into somatic (for mutations acquired in the DNA of somatic cells after conception; these are not heritable) and germ — line (for mutations in the DNA of the germ cells, which are heritable).

Examples of genetic and genomic testing in personalized medicine are:

A. Pre-symptomatic risk assessment:

1. BRCA1/2 testing for breast cancer.

2. Lynch syndrome testing for hereditary colon cancer.

3. Long QT interval.

4. Spinal Muscular Atrophy.

B. Diagnosis:

1. Beta thalassemia.

2 Fusion genes and rearrangements including BCR-ABL, E2A-PBX1, TEL-AML1, and MLL in pediatric leukemia.

3. Gene expression profiles define subtypes of breast cancer.

4. Human Papilloma Virus detection.

5. Hepatitis C detection.

6. PCR detection of micro-organisms (bacteria, fungi).

C. Prognosis:

1. Fragile X syndrome (the number of trinucleotide repeats predicts severity).

2. Gene expression signatures and prognosis in breast cancer.

3. Gene expression analysis and lymphoma prognosis.

D. Treatment and pharmacogenomics:

• Pharmacogenomic application:

1. CYP 2C19*2 variant (rs4244285) associated with diminished clopidogrel response.

2. Rs2395029 testing for HLA-B*5701 allele, correlated with hypersensitivity to abacavir treatment for HIV + patients.

• Therapies for targeted gene mutations in cancer:

1. EGFR point mutations in lung cancer and glioblastoma treated by cetuximab, gefitinib, erlotinib, panitumumab, and lapatinib.

2. KIT, PDGFR mutations in sarcoma, glioma, liver and renal cancer, melanoma treated by imatinib, nilotinib, sunitinib, sorafenib.

3. BRAF mutations in melanoma treated by RAF inhibitors.

4. BCR-ABL translocation in chronic myelogenous leukemia treated by imatinib.

5. KRAS wild-type status correlated with resistance to EGFR inhibition.

6. PARP inhibitors in BRCA mutant breast, ovarian, prostate, and pancreatic cancer.

7. Herceptin (Trastuzumab) in HER2 + breast cancer.

New tools for detection, diagnosis, and treatment of disease. Omics technologies seek to define and explain the molecular mechanisms of the human body:

1) genomics (the study of genes and their function);

2) glycomics (the study of cellular carbohydrates);

3) lipidomics (the study of cellular lipids);

4) metabolomics (the study of molecules involved in cellular metabolism);

5) pharmacogenomics (the study of how genetic variations influence an individual's response to medicines);

6) proteomics (the study of proteins).

Biomarkers — measurable indicators of healthy and pathological processes in the body, can belong to different types of biochemical molecules such as proteins, DNA, RNA, lipids; novel genetic biomarkers are continually being discovered (Single-nucleotide polymorphisms (SNPs) for example). They may be used to:

1) dentify and diagnose a disease as early as possible (diagnostic biomarkers), detect a person's risk of developing a disease (risk biomarkers);

2) make out the evolution or progression of a disease, i.e., if it is indolent or aggressive (prognostic biomarkers);

3) assess the response to and toxicity of a treatment (predictive biomarkers).

The identification of biomarker consists of a series of phases: biomarker discovery, verification, and clinical validation, which require availability of high quality and well characterized patient samples. Therefore, it is needed to have a large collection of patient samples with patient's clinical, pathologic and outcome data. So, an important tool for biomarker discovery and validation is biobanking. A biobank collects and stores biological material and associated medical and epidemiological information on a great number of people (donors). It organizes samples and data in a systematic way so they can be used for the purpose of medical-scientific research and diagnosis. Biobanks typically apply some sort of anonymization to assure the privacy of donors, and include governance structures (e.g., ethics committees) and procedures (e.g., consent) to protect participants' rights and interests.

Challenges in implementation of personalized medicine are:

1. Translational gap: observers point out that progress in translating molecular-biology breakthroughs into clinical practice («from bench to bedside») is slow.

2. Data protection, confidentiality and right to information.

3. Regulatory clarity: stakeholders have stressed the need for a clear regulatory framework for personalized medicine — to ensure it respects the principle of universal and equal access to healthcare, to avoid discrimination of patients based on genetic data (e.g., by insurance companies or employers), and to regulate genetic/genomic information for commercial purposes.

3. Cost: molecularly targeted treatments tend to be expensive since, by definition, they are suitable for only a limited number of patients.

Clinical translation of genetic markers can take place even in the absence of a complete understanding of their functional biologic significance. Human disease susceptibility is the result of rare genetic variants of high penetrance as well as common genomic variants of low penetrance. Genetic and genomic variants may manifest phenotypically in non-Mendelian patterns. The genomic model of human disease, like the multifactorial genetic model, will incorporate environmental as well as genetic modifiers. Analytic validity of genotyping cannot be taken for granted.

Behavioral science is needed to inform the translation of genetics (and genomics) to preventive practice, in order to maximize benefit and to avoid the consequences of incomplete risk communication.

The individual response to probabilistic genetic information varies and depends on context. Genetic or genomic markers with proven analytic and clinical validity and a strong biological rationale may not meet evidence-based standards of clinical utility.

The promise of personalized, genomic medicine should be informed by the past decade of experience developing genetically targeted therapies.

As it was in the case for genetic counseling for complex disorders, new models for «genomic counseling» are most responsibly offered first in a research and then in a clinical context. The same range of ethical issues is applied to «personalized genomics» as to personalized genetics. The final goal is the «genetic card of a child's health». This genetic card may help in the personalized prevention and treatment of patients.

1. At what age can hereditary diseases appear?
1) in the embryonic period;
2) in childhood;
3) in adulthood;
4) in old age;
5) at any age.

2. Mitochondrial genome:
1) is represented by one circular DNA molecule;
2) encodes 13 thousand proteins;
3) is represented by RNA molecules;
4) encodes enzymes responsible for repair.

3. Dolichocephaly is:
1) the long, narrow skull with prominent forehead and nape;
2) an increase in the longitudinal size of the skull relative to the transverse;
3) an increase in the transverse size of the skull with a relative decrease in the longitudinal size;
4) expansion of the skull in the occipital part and narrowing in the frontal part.

4. Epicant is:
1) fused eyebrows;
2) widely spaced eyes;
3) the vertical skin fold at the inner corner of the eye;
4) narrowing of the palpebral fissure.

5. Oligodactyly is:
1) a lack of fingers;
2) the finger splice;

3) one or more fingers missing;
4) an increase in the number of fingers.

6. Cryptorchism is:
1) non-clogging of the urethra;
2) undescended testicles into the scrotum;
3) genital underdevelopment.

7. Arachnodactyly is:
1) the shortening of fingers;
2) changing in the shape of fingers;
3) an increase in the length of fingers.

8. Cytogenetic study is carried out to confirm a diagnose in:
1) diseases caused by changes in the number and structure of chromosomes;
2) multifactorial diseases;
3) hereditary metabolism diseases;
4) hereditary connective tissue diseases.

9. How are called chromosomes with the end positioning centromere?
1) metacentrics;
2) submetacentrics;
3) dicentrics;
4) acrocentrics.

10. The area of using the cytogenetic methods is:
1) diagnostics of chromosomal syndromes;
2) identification of the primary biochemical defect of a mutant gene;
3) identification of heterozygous gene carriers;
4) diagnostics of monogenic syndromes.

Key

1) 5. **2)** 1. **3)** 1. **4)** 3. **5)** 3. **6)** 2. **7)** 3. **8)** 1. **9)** 4. **10)** 1.

Case Study 1. Duchenne Muscular Dystrophy Carrier and Prenatal Testing

Case Study. The Mr. C came to the Ob-Gyn to talk with you about pre-conception genetic counseling. He is 32 years old without significant family history of genetic disorders in his family. His wife, Dr. C. is 27 years old and she has a 17-year-old brother with Duchenne Muscular Myotrophy. What you can say about risks for the next pregnancy?

Family History: Dr. C is the eldest child in a family, four girls and one boy (who is affected with DMD). Her mother had one brother, who also had DMD and died at the age of 20. No other members of her large family have DMD.

1. What is the type of inheritance of DMD?
2. Describe the pathogenesis of DMD?
3. What is the significance of Creatine Kinase in affected males? In carrier females?
4. What type of genetics testing do you choose for this family?
5. What is the presentation of the maternal uncle's disease? How would genetic testing change if there was no other incidence of DMD in the family?

Write a pedigree of this family.

The family come back to your office for the results of testing and tells you that the wife is 11 weeks' pregnant. During the visit, you tell her that she is a carrier of the same dystrophin mutation as her affected brother. She tells you that she wants to know the mutation status of her fetus.

1. What is the prenatal testing in this situation?
2. What about the prenatal diagnosis?
2. What does «nondirective» mean in the context of genetic counseling?

Case Study 2. Hereditary Pancreatitis and Genetic Analysis

A mother and father with their 8-year-old daughter came to the emergency. Their child had been having a severe abdominal pain for the previous few days. At first, they thought that this is a iral disease but however, the pain had become worse and the child refused to eat. The patient had had frequent mild episodes of abdominal pain before but without any problems. The arents worried because the pain appeared to be much worse that time than in revious episodes. The patient stated, «My belly hurts and I feel as if I am going to throw up». The child had no remarkable medical or surgical history. She is vaccinated according to schedule in due time and visited a pediatrician regularly. She attended school and studied well. Her mother and father were both young. In her family history there were frequent abdominal pains in her mother and maternal grandmother who had recently died from cancer. The mother was not sure about the type of cancer but supposed it had been a pancreatic one. The mother stated that she had been admitted to hospital for several abdominal pain episodes. She was told that she had had pancreatitis but had never been told about the cause of the disease. She and her husband drank from one to two alcoholic beverages per week. The father and the patient's younger sibling were both in good health. On physical exam a child suffered from moderate to severe pain. Vital signs were significant for a mild fever but were stable. On palpation the child had severe pain over the mid epigastrium with guarding. Bowel sounds were absent. You administered a CT scan and standard lab tests. The CT scan showed pancreatic stranding consistent with chronic pancreatitis. The patient was referred to a gastroenterologist for consultation and an ERCP was performed. A pancreatic duct stricture and stone were present. The duct was dilated and stented and the stone was removed. The patient's pain disappeared in a few days.

1. What is the epidemiology and prevalence of Hereditary Pancreatitis.
2. What about the presentation and disease progression of patients with hereditary pancreatitis.
3. Describe the type of inheritance and what kind of the genes involved into hereditary pancreatitis.
4. Write about the pathophysiology and diagnosis of hereditary pancreatitis.
5. Write a plan for genetic counseling of family members for patients with hereditary pancreatitis.

What are the available treatments for patients with hereditary pancreatitis?

Case study 3. Alpha-1 Antitrypsin Deficiency and Genetic Analysis

A 45 year old male come to your for a complaints of increased dyspnea on exertion for the last several months. He reports he has always believed he has had some form of asthma or chronic lung infection, but lately he has had a great deal of difficulty performing any activity without shortness of breath. He denies cough, hemoptysis, chest pain, dysphasia, weight loss, night sweats, or fevers. He states he has no significant past medical or surgical history. He knows no family members who have died prematurely or who have had asthma. The patient states he use to smoke a few cigarettes a day while he was young but quit a few years ago. He does not drink alcohol and takes no medication. On physical exam you note mild expiratory wheezes and clubbing but note no other abnormal findings. Chest X-ray is significant for flattened diaphragm and large lung fields with basal hyperlucency. Pulmonary function tests are significant for a lower than expected FEV1/FEV ratio. All other blood work is within normal limits except for mild to moderate elevation of liver transaminases.

1. What about the epidemiology and prevalence of α1-antitrypsin deficiency?
2. How the pathogenesis revealed in situation with α1-antitrypsin deficiency?
3. What is the usual presentation and progression of a patient with α1-Antitrypsin deficiency?
4. What would you do to diagnose α1-Antitrypsin deficiency?
5. What is the type of inheritance and the common phenotypes in α1-Antitrypsin deficiency?
6. Which patients and family members of patients with α1-Antitrypsin deficiency should be screened for AAT?
7. Do we have any treatments for patients with α1-Antitrypsin deficiency?

Case study 1

1. What is the type of inheritance of DMD?

DMD is an X linked recessive disorder. As with all XLR disorders, it is usually diagnosed in males, and rarely manifests clinically in females. Females are usually carriers of the mutation, which they can pass along to their offspring. 50% of the males born to a female carrier will have DMD, and 50% of males will be unaffected. Similarly, any female born to a carrier has a 50% chance of being a carrier herself.

Unlike some genetic diseases like Sickle Cell Anemia, DMD is caused by many different mutations. Thus, there is no single site on the Dystrophin gene that molecular diagnostic labs can look at to determine if an individual carries a mutation. This makes testing for DMD mutations more extensive than that for Sickle Cell Anemia.

2. Describe the pathogenesis of DMD?

DMD is caused by a mutation in the dystrophin gene — usually a large, out-of-frame deletion. Dystrophin is a structural protein associated with the support scaffolding of plasma membrane in muscle cells. Absent dystrophin causes a weakening of muscles, particularly skeletal and cardiac muscles. With contractions, the muscle cells burst, leaking their contents to the interstitium. Accordingly, the muscles respond with the so-called «pseudo-hypertrophy» to maintain muscle mass.

Clinically, DMD typically manifests between 3–4 years of age with a positive Gower's sign and pseudohypertrophy of the calves. DMD is a progressive neuropathy. Males affected with DMD typically utilize a wheelchair full time by middle school, and continue to deteriorate physically until their demise, which usually occurs in their early twenties due to cardiac or respiratory failure.

Beckers Muscular Dystrophy (BMD) is a less severe variant of DMD. In these cases, the mutation usually involves a frame deletion or insertion that codes for a dystrophin protein with some

functional capacity. These men typically present later in childhood and have a slower, less severe progression of disease.

Currently, there is no cure for DMD or BMD. Current studies indicate that corticosteroid use can increase functional ability in males affected with DMD. However, the side effects associated with steroid use frequently dissuade physicians and patients from using this medication. In addition to steroids, current therapies are aimed at maintaining function ability for as long as possible in males with DMD by using wheelchairs, respirators, and pharmacotherapies to keep heart and lung function as high as possible.

3. What is the significance of Creatine Kinase in affected males? In carrier females?

CK levels are usually extremely high in young men affected with DMD — usually 50–100 times normal. This is due to leakage of muscle enzymes into the interstitium when weakened sarcolemmas burst. Definitive diagnosis is made via muscle biopsy or genetic testing (the last is preferably now). Young women with elevated CKs have a presumptive diagnosis as a DMD carrier. While these young women are functionally normal and have no abnormal biopsy results, approximately 50% of their muscle cells will have abnormal dystrophin in childhood. By the time they are young adults, these muscle cells will have died, and the remaining functional 50% will remain. Thus, it is only during childhood that CK levels in carrier females will be elevated. Of note, some carrier females will not have grossly elevated CK levels. Therefore, a low CK test result reduces, but does not eliminate, the risk for carrier status in females.

4. What type of genetics testing do you choose for this family?

Testing of the carrier status in Dr. C is the appropriate procedure at present. DMD is definitively diagnosed with a molecular-genetics testing. Thus, the specific mutation affecting an individual is typically known. If her brother's mutation is known, PCR evaluation or DNA sequencing testing is an option. If the specific mutation has not been worked out but a biopsy is still available, the first step would be to determine the mutation in her brother, then screen Dr. C's DNA for the same mutation.

Sometimes a patient in Dr. C's case wants to determine carrier status after her affected family member has died without a muscle biopsy available. In this instance, linkage analysis can be used to determine her risk of carrier status.

5. What is the presentation of the maternal uncle's disease? How would genetic testing change if there was no other incidence of DMD in the family?

Alive maternal uncle makes it overwhelmingly likely that Dr. C's mother is a carrier of the disease. If her brother were the first and only incidence of DMD in this pedigree, things could get tricky. Haldane's rule states that 1/3 of XLR mutations arise spontaneously. Thus, it is imperative to establish where the mutation began. If the first incidence of mutation were in Dr. C's brother's DNA only, her risk for carrier status could be that of the normal population. However, if the mutation first emerged in the P1 generation, with Dr. C's mother as a carrier, Dr. C could have a 50% chance of being a carrier. Finally, even a negative test result for carrier status in the P1 female does not reduce Dr. C's risk to mutation of the normal population. Gonadal mosiacism is a phenomenon where a population of ova in Dr. C's mother's ovaries all carry the same dystrophin mutation. This occurs in 15% cases. In these instances, Dr. C has greater risk for being a carrier of this mutation.

6. What is the prenatal testing in this situation?

Firstly we have to understand the sex of the fetus. If it is female, she has a very small chance of DMD. If the fetus is male, things get more complicated.

7. What about the prenatal diagnosis?

The best option is the testing of the fetal DNA. Retrieval of fetal DNA may come from an amniocentesis, PUBS, or CVS. Each of these three tests has a different time frame for when during gestation they may be performed. Chorionic Villus Sampling (CVS) can ccur between 10–12 weeks' gestational age. Amniocentesis can occur after 15–16 weeks' gestational age, and Percutaneous Umbilical Cord Sampling can occur after 18 weeks' gestational age. Once the fetal cells have been cultured, they may be tested via sequencing or PCR or blotting techniques to determine if the fetus has a dystrophin mutation.

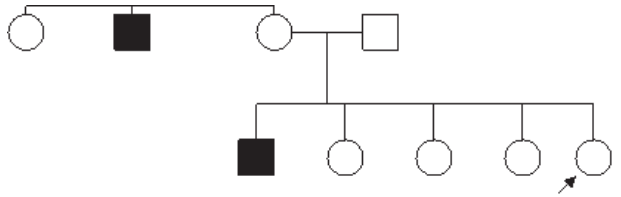
Would it be appropriate to determine carrier status if the fetus were a female? What about after the baby girl is born? When would you recommend carrier testing for Dr. C's daughter?

No and no. Current genetic testing guidelines for carrier status testing indicate that these tests should be performed when the female is considering her own reproductive choices.

8. What does «nondirective» mean in the context of genetic counseling?

Genetic counseling involves presenting patients with facts, educating them about risks of procedures and mutation/disease status, and allowing the patient to make an informed choice. Nondirective methods mean that genetic counselors do not make recommendations.

The pedigree:



Case Study 2

1. What is the epidemiology and prevalence of Hereditary Pancreatitis.

In pediatrics we have a lot of diseases which can go to chronic pancreatitis such as cystic fibrosis and α1-antitrypsin deficiency. However, there are subsets of patients who have chronic pancreatitis with unknown etiology. These patients are diagnosed with idiopathic pancreatitis. A subset of these patients will have a family history of chronic pancreatitis, and several studies have found a genetic link in chronic pancreatitis families. It is difficult to estimate the incidence and prevalence of the disease due to its similarity in presentation to other causes of chronic pancreatitis. It is estimated that of childhood cases of chronic pancreatitis (CP), idiopathic CP accounts for approximately 40 to 60%, and hereditary pancreatitis (HP) accounts for approximately 20 to 25%. Using the childhood population is useful since it avoids the confounding variable of alcohol which predisposes patients to chronic pancreatitis in older populations. Gender distribution appears to be equal. To date, approximately 100 families and 600 individuals have been reported.

2. What about the presentation and disease progression of patients with hereditary pancreatitis.

The symptoms sometimes developing before age 5 and most will have symptoms before age 20. Patients with HP tend to pre-

sent earlier than patients with idiopathic chronic pancreatitis. One review found 63% percent of chronic pancreatitis patients have had 5 or more emergency department visits or hospitalizations, and 56% had 5 or more episodes of documented pancreatitis before the diagnosis of HP was made. Children who are symptomatic will present with abdominal pain, nausea, vomiting, and anorexia. Pain in children is usually not chronic as found in adults. The pain will usually reoccur, but due to the vague symptomology in this age population, the diagnosis is difficult to make. In children, the pain is often sudden in onset and accompanied by decreased bowel sounds. Adults will tend to have a similar presentation to chronic pancreatitis including chronic pain. Many will be depended on opioid pain medication. One study found 52% were taking narcotic analgesic agents on a chronic basis. Pancreatic insufficiency is common with bulky stools. Insulin dependent diabetes is common. Calcification, pseudocysts formation, necrosis, and infection will all tend to develop with older age. Eventually, many of these patients will be diagnosed with pancreatic cancer. Pancreatic cancer prevalence is 50–60 times greater in HP patients than in the general population.

3. Describe the type of inheritance and what kind of the genes involved into hereditary pancreatitis.

Hereditary pancreatitis is believed to be inherited in an autosomal dominant manner. However, some data would suggest other patterns. It has an 80% penetrance in the next generation. One study has shown approximately 3.4% of the analyzed 5,600 genes from pancreatic RNA had deregulated expression in CP patients suggesting a genetic link. Although several mutations have already been found, there are other possible genetic deformities that could play a role in the pathogenesis of HP.

One of the genes that received the most attention is PRSS1, also referred to as serine protease 1, a trypsinogen gene that is responsible for one of the most secreted proteins of the pancreas. The gene is located on the long arm of chromosome 7 in an area of the chromosome known to contain numerous trypsinogen genes. Although there are a number of different mutations associated with this gene, the R122H and N29I mutations have been found in a number, but not all, of CP patients.

The SPINK1, or PSTI, gene may also play a role in normal pancreatic function by inactivating trypsin activity. It is located

on chromosome 5. A mutation on this gene called N43S has also been found in CP patients, many of whom lack the PRSS1 mutants. 25–40% of patients with idiopathic CP carry N34S on one or both alleles. There are other possible genetic deformities that could also play a role in the pathogenesis of HP.

The CFTR gene and the mutations associated with cystic fibrosis produce a documented effect on the pancreas. The pancreas ability to secrete enzymes and bicarbonate due to mutations in ion and water transport across membranes can lead to retention of pancreatic enzymes and digestion of the pancreas.

4. Write about the pathophysiology and diagnosis of hereditary pancreatitis.

Mutations of the PRSS1 gene have been associated with chronic pancreatitis. Many of the mutant proteins are susceptible to autoactivation and resists autolysis compared to other trypsinogens proteins. The R122H mutation is believed to make activated trypsin resistant to hydrolytic inactivation and allowing continued digestion of the pancreas and associated enzymes. It is also possible that increased autoactivation may play a role in R122H pathophysiology. The N29I mutation is also associated with increased autoactivation as well as resistant degradation, although there are several conflicting studies on the exact mechanism of action. One study has found CP patients have a significant difference in genotypes corresponding to high TGF-(beta)1 producer phenotypes. Since TGF-(beta)1 is associated with production and regulation of the extracellular matrix, excess TGF-(beta)1 may be associated with fibrogenesis seen in CP.

Genetic disposition for pancreatitis patients who consume large amounts of alcohol is in an area on continuing study. One model, the SAPE model, attempts to explain why some patients who consume large amounts of alcohol have CP while others do not. Patients who consume large amounts of alcohol may put the acinar cells under stress. Under stressful conditions cytokines, adhesion molecules, activated neutrophils, lymphocytes, and monocytes, and pancreatic stellate cells are released into the pancreas. After the acute inflammatory phase, a late phase follows where anti-inflammatory proteins, including TGF-(beta)1 are released. Any genetic predisposition to an over inflammatory or insufficient anti-inflammatory response in conjunction with repeated stress of the pancreas could lead to fibrosis and CP.

The diagnosis of HP is based on clinical suspicion. A physician must be able to rule out other common causes of pancreatitis in patients who present repeat episodes such as with alcohol abuse, gallstones, and hypertriglyceridemia. Radiology, blood test for pancreatic inflammation, and stool studies for fat should all be used in the diagnostic process. Physicians should have a strong suspension for children who present with recurring pancreatitis or malabsorption not responding to treatment. A few laboratories can test for the most common mutations known to cause hereditary pancreatitis.

5. Write a plan for genetic counseling of family members for patients with hereditary pancreatitis.

The following are recommendations for screening for HP from the Consensus Committees of the European Registry of Hereditary Pancreatic Diseases, Midwest Multi-Center Pancreatic Study Group, International Association of Pancreatology. The committees recommend pre and post-test counseling as well as informed consent of all patients undergoing genetic testing.

The indication for cationic trypsinogen (PRSS1) gene mutation analysis in a symptomatic adult patient should be any of the following:

• Recurrent (2 or more separate, documented episodes with hyper-amylasaemia) attacks of acute pancreatitis for which there is no explanation.

• Unexplained (idiopathic) chronic pancreatitis.

• A family history of pancreatitis in a first-degree (parent, sib, child), or second-degree (aunt, uncle, grandparent) relative.

For patients with pancreatitis eligible for a research protocol.

The indications for testing in asymptomatic persons include enhanced counseling, and the person must have a first-degree relative with a defined HP gene mutation. The person should be over 16 years of age and able to make an independent and fully informed decision.

The guidelines for symptomatic children are as follows:

• An episode of documented pancreatitis of unknown etiology and severe enough to require hospitalization.

• Two or more documented episodes of pancreatitis of unknown etiology.

• An episode of documented pancreatitis occurring in a child where a relative is known to carry an HP mutation.

• A child with recurrent abdominal pain of unknown etiology where the diagnosis of HP is a distinct clinical possibility.

• Chronic pancreatitis of unknown etiology, where the diagnosis of HP is a distinct clinical possibility.

The committees gave no specific recommendations on prenatal testing.

6. What are the available treatments for patients with hereditary pancreatitis?

Treatment mimics the care given to a patient with chronic pancreatitis.

Patients should be advised to avoid alcohol, discontinue smoking, and to eat small meals that are low in fat.

Pancreatic enzyme supplements relieve pain in some patients, are generally safe, and are thus a reasonable next step for patients with continued pain. Treatment may be more likely to be successful in those without involvement of large ducts and those with idiopathic pancreatitis.

Treatment for steatorrhea is restriction of fat intake (to less than 20 g per day).

Pain control with short courses of opioids and amitriptyline has been found to be effective. Long- acting opioids are preferred over short-acting ones.

Nerve blocks, ERCP to relieve obstruction, or surgery can be considered for patients who continue to have pain despite appropriate pain medication.

Screening for cancer has been debated and some studies have suggested screening with CT every year for patients over 40. However, the optimal screening strategy has not been defined, and it is unknown whether early detection of cancer at a resectable stage is possible. There are no data on pancreatectomy as a prophylactic measure to reduce the mortality from pancreatic cancer.

Case study 3

1. What about the epidemiology and prevalence of Alpha-1 Antitrypsin deficiency.

Although Alpha-1 Antitrypsin deficiency (AAT) is traditionally believed to be a disease found in northern European populations, the disease has been described throughout the world in all racial subgroups. Worldwide it is believed there are 116 million carriers and 3.4 million cases of deficiency. It is estimated there are

70,000–100,000 affected individuals in the United States with a similar number of affected individuals in Europe. There is a strong belief many cases of AAT have gone undiagnosed or misdiagnosed as emphysema, bronchiectasis, or cirrhosis. AAT is typically diagnosed when neonates present with liver disease of unknown etiology or when adults develop early emphysema.

2. How the pathogenesis revealed in situation with a1-Antitrypsin deficiency.

Alpha-1 Antitrypsin is a serine protease inhibitor synthesized in the liver. The protein reaches the lungs by diffusion from the circulation and by local production in macrophages and bronchial epithelial cells. Its role is to inhibit several enzymes including elastase, collagenase, and trypsin. These enzymes are located in neutrophils and are especially active in the lower respiratory tract.

In the liver the cause of disease only tends to occur in individuals with Z phenotypes. A substitution of a lysine for a glutamic acid in the Z protein widens the protein β-sheet and allows polymerisation, linking of one α1-antitrypsin molecule to the β-sheet of another molecule in an irreversible process. This polymerisation within the hepatocyte prevents its secretion. Only about 15% of Z-mutated antitrypsin is secreted into the plasma leading to a buildup of this protein in the endoplastic reticulum. The buildup of this mutated protein is cytotoxic to hepatocytes and leads to cirrhosis. It is unknown why some patients with this phenotype develop lung or liver disease and other do not. Although there are very few studies available, there are no known risk factors, including the consumption of alcohol, other than male sex that have been identified as risk factors for development of chronic liver disease in adults or children with homozygous AAT. There is some data suggesting cofactors such as HCV and alcohol are necessary to promote chronic liver disease in heterozygous AAT.

Emphysema in Alpha-1 Antitrypsin deficiency is thought to result from an imbalance between neutrophil elastase in the lung, and the elastase inhibitor AAT. There are two proposed mechanisms leading to lung damage. The AAT protein is much less effective than the wild type protein at inactivating elastin, and there is a decreased amount of the protein in the lung due to the buildup of the protein in the hepatocytes. Smoking is thought to accelerate lung injury by increasing the amount of elastase.

3. What is the usual presentation and progression of a patient with α1-Antitrypsin deficiency?

AAT deficiency can present in a number of different organ systems. Classic findings include lung disease, liver disease, and skin lesions, which is typically panniculitis, an inflammation of fat which can present as a necrotizing lesion or as subcutaneous nodules. Other manifestations of AAT include vasculitis, glomerulonephritis, fibromuscular dysplasia, and lung, colorectal, and bladder cancers. Many patients with severe AAT will only have one manifestation of the disease. Most patients who have lung disease do not develop liver disease, and most patients with liver disease do not develop lung disease.

The typical presentation of AAT is an adult patient with early onset panacinar emphysema. As described above, several studies have found smokers tend to have a higher probability of developing emphysema (2). It is estimated 1–2% of all COPD patients have inherited severe AAT (4). The typical finding on plain films is large lung fields with bilateral basal hyperlucency.

In adults, liver disease may not present clinically until the signs and symptoms of chronic liver disease manifest. In contrast, liver disease is the highest cause or morbidity and mortaility in AAT patietns under 20. Liver disease can present in the neonatal period as cholestasis with or without jaundice and hepatomeagly leading to cirrhosis. However, one study found although 83% of AAT patients had abnormal liver enzymes during childhood, only 17% had serious liver disease. AAT may also predispose to hepatocellular carcinoma by accumulation of protein within the cells and favoring a cancer prone state by surviving with intrinsic damage.

The prognosis of AAT depends on the severity of the disease, response to treatments including the availability of lung or liver transplantation, and ability to avoid exposure to cigarette smoke and other lung irritants. Mortality rates for patients with a severe deficiency of AAT vary among different studies, presumably due to differences in study populations. Relatively normal survival appears possible for nonsmoking asymptomatic individuals. A fall in FEV1 appears to correlate with overall mortality.

4. What would you do to diagnose α1-Antitrypsin deficiency?

The key to a diagnosis is a strong clinical suspension in any child who has liver disease or any adult who presents with early onset

emphysema, usually before the age of 45. Due to the vague symptoms, it is common to miss or delay the diagnosis. In a recent survery of AAT patients, one study found an average delay of 5.6 years between the first onset of symptoms and diagnosis.

There are several tests used to confirm the diagnosis of ATA.

Blood test- the first line screening test. Subnormal concentrations in serum of α1-antitrypsin can be found in patients with deficiencies. Normal amounts in blood are about 150–350 mg/dl. Patients whose serum concentrations fall below 80 mg/dl are at increased risk of lung disease. The blood levels of AAT are not a highly sensitive test. Plasma levels are often normal in heterozygotes and may transiently increase to normal even in homozygous patients during periods of systemic inflammation.

Histology- The Periodic acid-Schiff stain (PAS) stains both glycogen and AAT globules. When the glycogen is dissolved with a diastase, liver biopsies with an abnormal amount of AAT protein within the hepatocytes will satin a dark, red/purple. Immunohistochemical and electron microscopy can also be used to detect abnormal amounts of AAT protein trapped in the cell on the golgi apparatus.

Genotype testing- the gold standard. All patients who meet the recommendations listed below should have genetic screening. Isoelectric focusing on gel electrophoresis can be used to isolate the AAT genotype and corresponding phenotype. Commercial kits are now available to detect the Z and S alleles.

5. What is the type of inheritance and the common phenotypes in α1-Antitrypsin deficiency?

AAT is inherited in a co-dominant manner. The gene is located on chromosome 14 and results in a substitution of amino acids resulting is a misfolded protein that tends to polymerized as described above. The gene has been designated by the name Pi. The different mutations result in phenotypes which been assigned a letter based on the allele's ability to migrate in a gel electrophoresis. Normal alleles are assigned a letter M giving the normal phenotype the designation PiMM. Patients who have a deficiency have several possible allele formations. The most common allele deficiency associated with emphysema and liver disease is the Z allele. In this allele, a glutamic acid has been replaced by a lysine. Patients with this mutation are given the designation Pi*Z if heterozygous or PiZZ if homozygous. Other common alleles leading to disease are PiF, PiS, and PiNull.

The Null allele is used when a patient has no detectable AAT in the serum. This finding is very rare and patients with this phenotype have the most severe form of lung disease, but since there is no protein to accumulate in the hepatocytes, they do not have liver disease.

6. Which patients and family members of patients with α1-Antitrypsin deficiency should be screened for AAT?

As always with testing for genetic disease, all of the implications need to be discussed with the patient and family before they consent to testing. There are well known psychological and financial risks to any type of genetic testing.

The following are the recommendation for testing and screening by the American Thoracic Society and European Respiratory Society (ATS-ERS):

Type A recommendations: genetic testing should be performed:

• Symptomatic adults with emphysema, COPD, or asthma that does not respond to treatment with bronchodilators.

• Individuals with unexplained liver disease including children.

• Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors such as smoking or toxin exposure.

• Adults with necrotizing panniculitis.

• Siblings of patients with AAT deficiency.

Type B recommendation: genetic testing should be discussed and could be reasonable accepted or declined:

• Adults with bronchiectasis without evident etiology.

• Adolescents with persistent airflow obstruction.

• Asymptomatic individuals with persistent airflow obstruction and no risk factors.

• Adults with C-ANCA positive vasculitis.

• Individuals with a family history of COPD or liver disease not known to be attributed to AAT deficiency.

• Distant relatives of an individual who is homozygous for AAT deficiency.

• Offspring or parents of an individual with homozygous AAT deficiency.

• Siblings, offspring, parents, or distant relatives of an individual who is heterozygous for AAT deficiency.

• Individuals at high risk of having AAT deficiency-related diseases.

• Individuals who are not at risk themselves of having AAT deficiency but who are partners of individuals who are homozygous or heterozygous for AAT deficiency.

Type C recommendation: genetic testing is not recommended:

• Adults with asthma in whom airflow obstruction is completely reversible.

• Population screening of smokers with normal spirometry.

Type D recommendation: genetic testing should not be performed:

• Predispositional fetal testing.

• Population screening of either neonates, adolescents, or adults.

7. Do we have any treatments for patients with α1-Antitrypsin deficiency?

At this time, there appear to be two aspects of management, treatment of symptoms and treatment of disease by attempting to raise AAT to a protective level.

Treatment of pulmonary symptoms in individuals with AAT deficiency should include many of the interventions and medications recommended for patients with emphysema:

• Inhaled bronchodilators.

• Preventive vaccinations against influenza and pneumococcus.

• Supplemental oxygen when indicated by conventional criteria, including during commercial air travel.

• Pulmonary rehabilitation for individuals with functional impairment.

• Consideration of lung transplantation for selected individuals with severe functional impairment and airflow obstruction.

• During acute exacerbations of COPD, management should again include usual therapies for COPD patients including systemic corticosteroids and ventilatory support when indicated.

The only treatment at this time for liver disease is liver transplantation.

At this time, there is only one approved treatment aimed at increasing AAT serum concentration, intravenous or aerosolized augmentation therapy which is the infusion of purified pooled human plasma α1-antitrypsin. Based on current evidence, it would appear augmentation therapy is beneficial only in patients with moderate pulmonary disease and offers little benefit to those with severe disease. Augmentation therapy is not recommended for asymptomatic patients. Future areas of study include gene therapy and promotion of hepatic secretion of AAT

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