ESSENTIALS OF RARE DISEASES

BASIC GUIDELINE FOR PHYSICIANS

Editors:
Serdar Ceylaner, Gülay Ceylaner, and Béla Melegh

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Türkiye Klinikleri

If you hear hoofbeats outside, think horses and zebras.
If you can't find a zebra, remember okapis.

Persons with undiagnosed and undefined diseases are also waiting for diagnosis and service.

Why shouldn't the okapi, which looks like a mixture of zebra, deer and giraffe and is unknown to many people, be the symbol of undiagnosed and undefined diseases?

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CONTENTS

ESSENTIALS OF RARE DISEASES
BASIC GUIDELINE FOR PHYSICIANS
Including - Basic genetics for all medical doctors

“Rare, but still human”

Dear rare disease volunteer,

This booklet has been prepared to guide you through the key aspects of rare diseases and to serve as your roadmap. It does not cover every rare disease and everything about this issue but focuses on essential information. Studying rare diseases is challenging, yet it is a skill that can be acquired, and solutions can be found.

The main principles outlined here include:

- Believing that medicine needs to evolve,
- Being willing to abandon some old habits,
- Keeping in mind the possibility that “your patient may have a rare disease,” and consulting more experienced colleagues within European Reference Networks
- Understanding that research on rare diseases involves working until the etiology, whether rare or common, is identified. This approach contributes to providing the highest quality and most cost-effective healthcare services, as in any other diseases,
- Recognizing that a definitive diagnosis ensures the correct treatment, protects against the risk of recurrence in the next generations of the patient and their family, and leads to a longer and higher-quality life,
- Realizing that only through multidisciplinary collaboration, patients with rare diseases can be served
- Striving to increase accessibility to new diagnostic technologies, such as genomic technologies. If you do not have such a team, please use your e-mail,
- Supporting patients with rare diseases in organizing themselves,
- Feeling like a member of rare disease community,
- Engaging in preventive medicine using new-generation technologies.

These studies will make you feel more capable as a physician, enable you to participate in scientific studies of rare diseases, and make you feel like a member of a multi-disciplinary team.

The European exam is one of the important steps in the formation of a rare disease community. This exam aims to measure core competencies. This is the first step. Once a community with competencies is formed, it is possible to draw new road maps. Physicians who specialize in certain areas and on rare diseases see the rare disease world within the boundaries of their field of expertise. We must expand our world and become a general physician, but one who specializes in a particular field.

The rare disease physician must constantly ask “why?”. Why are my patient’s symptoms different? Why did the treatment fail? Why is the patient still not happy. Why did he/she experience complications? etc.

In this booklet;

A little summary on some of the titles to help you to learn easier, and further readings are suggested.

The main aim to help you to understand these basic principles of rare diseases and prepare you for the European exam. In future versions of this booklet, we plan to add chapters for all medical specialties to add their own rare diseases and their red flags.

If you are new, welcome to the rare disease community.

We recommend you to;

Please read UEMS- Multidisciplinary Joint Committee of Rare and Undiagnosed Diseases (MJC-RUD) documents

- Description of Competency
- ETR
- Syllabus

• https://mjcrud-uems.pte.hu/

• https://mjcrud-uems.pte.hu/content/etrs

- This booklet gives you a summary of some important topics and some further reading suggestions. Rather than providing information about rare diseases, the summaries are intended to provide you with experiences regarding rare diseases and to help you gain the necessary perspective for rare disease diagnosis.
- Considering that a significant portion of rare diseases are genetic diseases, try to master the basic information about genetics.
- The aim of the European exam is not to question the details about rare diseases, but to determine that the physician knowledge on the rare disease red flags and uses the necessary algorithms when evaluating a patient who may have a rare disease.

Some important notes;

A significant portion of rare diseases are diseases that have a genetic basis. Therefore, this booklet about rare diseases contains a lot of genetic information and links to resources containing some genetic information are included. UEMS Medical Genetics exam is an exam to certify the equivalence of medical genetics specialty diplomas across Europe and is organized by the Section of Medical Genetics (SMG). Two of three editors also a member of SMG and part of the SMG examination committee. U.E.M.S. - Multidisciplinary Joint Committee - Rare And Undiagnosed Diseases (MJC-RUD) exam is a competency evaluation exam. It is not intended to lead to a specialist degree and is open to other specialists as well as Medical Geneticists. You can see the details on the MJC-RUD website.

UEMS does not have any plans or efforts to prepare books for exams. This study was not conducted on behalf of UEMS or due to an assignment given to it. This booklet aims to provide both a scientific contribution and a perspective for those who will take the exam. It is important for all specialists dealing with rare diseases to have a good grasp of the general perspective and to know rare disease flags.

Another important issue is that since the author's native language is not English and he is trying to prepare a book with a low budget, the content was prepared by the author and grammar checks were made by artificial intelligence. I think that Medical Geneticists like us, who have a field of expertise that loves new technologies, and rare disease enthusiast physicians who look to the future, should internalize these technologies more and more every day and devote our financial resources and time to our patients and science.

Finally, as a writer, I would like to express my gratitude to Prof Jean Calleja Agius, who encouraged and motivated the creation of this booklet; to Prof Reinold (Rijk) Gans, Prof Ambrogio Fassina and Dr Hasan Baş for their contributions and suggestions by reading the content in addition to their motivation. I would like to express my gratitude to my dear wife, my love and my dear companion, Assoc. Prof. Gülay Ceylaner, who not only contributed to the content of the book but also drew the graphics, was always by my side with her moral support, and took all my other work away from me while I was preparing the book. I would like to give a special thanks to my dear friend and president of MJC-RUD, Prof Béla Melegh, who has supported me in the field of rare diseases, to which I have devoted my professional life, and who has been with me and guided me at all stages of the book process.

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RARE DISEASES GENERAL INFORMATION

The catchphrase "1 in every 10 persons" serves as a powerful reminder to alert medical professionals to the significance of rare diseases. While each rare disease may seem uncommon on its own, when taken together, they are actually quite prevalent. These conditions are characterized by a frequency of less than 1 in 2000 individuals and often have genetic origins. Many lack specific diagnostic tests and can pose life-threatening risks, leading to long-term consequences. Astonishingly, 75% of rare disease cases affect children, and tragically, around 30% of them do not survive beyond the age of 5. Moreover, there exists a considerable population of undiagnosed adults, creating a hidden problem akin to an iceberg. The range of rare diseases encompasses 6000-8000 different types, affecting 25 million people in the USA and 55 million in Europe.

Misdiagnosis is a common issue, with many individuals receiving generic labels such as "epilepsy" or "cerebral palsy" that merely describe symptoms rather than pinpointing the underlying condition. Rare diseases often mimic other illnesses, making accurate diagnosis a challenging task. Approximately 20% of patients in outpatient clinics suffer from rare diseases due to their chronic nature, necessitating repeated medical visits. Worryingly, 20% of infant deaths can be attributed to rare disorders. The journey to diagnosis is arduous, taking an average of 5.7 years, highlighting the prevalence of genetic factors in many chronic conditions. These statistics underscore the ubiquity of rare and genetic diseases.

Patients afflicted with rare diseases tirelessly seek solutions, even though specific treatment options are available in only 5% of cases. However, accurate and early diagnosis can significantly enhance both life expectancy and the quality of life for most individuals.

In our daily medical practice, we are often reminded to consider common conditions when faced with medical issues ("when you hear hoofbeats, think horses, not zebras"). Nonetheless, rare diseases are the zebras of the medical world, and they are symbolized as such in the realm of rare diseases. Doctors are trained to favor simpler explanations, but it is evident that we may be overlooking critical diagnoses far too frequently.

Please visit the links below.

- https://rareportal.org.au/healthcare-professionals/
- https://www.eurordis.org/information-support/what-is-a-rare-disease/

1.1. PERSONALIZED MEDICINE

In the field of medicine, there is no one-size-fits-all approach for patients. It is essential to consider various factors, including the individual's biological, medical, and social circumstances, before offering any medical advice. These considerations encompass:

1. **Coexisting Medical Conditions:** Assessing whether the patient has any other existing diseases or conditions that might influence their treatment plan.
2. **Drug Metabolism Variability:** Recognizing differences in drug metabolism rates among individuals, which can affect the choice and dosage of medications.
3. **Allergies:** Identifying any allergies or adverse reactions to specific medications or substances.
4. **Socioeconomic Factors:** Taking into account the patient's economic situation, including their ability to afford prescribed medications, access to proper nutrition, and living conditions.
5. **Dietary Habits:** Considering the patient's nutritional habits, such as interactions between certain foods and medications (e.g., grapefruit affecting the metabolism of some drugs).
6. **Underlying Causes of Disease:** Investigating the underlying causes of the disease, as conditions like migraines can result from various factors, including channelopathies or autoimmune diseases. Additionally, treatment success may vary depending on whether genetic variants in the same gene result in a loss-of-function or gain-of-function effect in certain types of epilepsy.
7. **Potential Associated Conditions:** Recognizing

the possibility of associated conditions or findings related to the disease in question. For example, pneumothorax can occur in isolation or may be linked to underlying syndromes like Birt-Hogg-Dube syndrome or Marfan syndrome.

In cases of chronic diseases, it is particularly crucial to discuss options such as genomic screening for the patient to detect etiologic factors, the detection of any additional underlying conditions, and the evaluation of pharmacogenetics to tailor treatment plans effectively. This comprehensive approach ensures that medical advice and treatment strategies align with the unique circumstances and needs of each individual patient.

Further Reading

- https://www.genome.gov/genetics-glossary/Personalized-Medicine
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6366451/

1.2. PRECISION MEDICINE-PREDICTIVE MEDICINE

One of the fundamental principles in medical practice is to minimize surprises and take proactive precautions. This approach can be identified as predictive medicine, where possibilities are investigated, and preventive measures are taken before problems arise. Precision medicine, on the other hand, involves evaluating whether a patient will benefit from a specific treatment.

For instance, in the context of chronic diseases, before prescribing a long-term medication, it is wise to consider the patient's family history of kidney disease, especially when the chosen drug is eliminated from the kidneys. In such cases, even if the immediate evaluation of kidney function appears normal, conducting genomic tests to identify potential kidney disease risks within the family is a proactive step. Integrating genomic medicine practices into medical care discussions is essential for preventing distressing, life-threatening, and costly complications down the road.

Rare cancers constitute a significant portion, approximately 20%, of all cancer cases. This prevalence can sometimes lead to misdiagnoses or inadequate diagnoses in cancer patients. Furthermore, the same type of cancer can originate from different genetic mechanisms, making treatment planning safer and more precise when guided by molecular genetic findings. In addition to identifying the organ of origin and histopathological characteristics of the cancer, understanding the underlying genetic mechanisms/mutations or mutational load is crucial for targeted drug selection, prognosis determination, and recognizing potential drug resistance. Conducting these genetic studies before initiating treatment plans exemplifies the practice of precision medicine.

Further Reading

- https://www.ncbi.nlm.nih.gov/books/NBK441941/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8106271/
- https://www.england.nhs.uk/long-read/genomics/

1.3. "RED FLAGS" OF RARE DISORDERS

This list encompasses various general and specific symptoms that may indicate the presence of underlying medical conditions, especially those that are rare or uncommon. These symptoms and factors include:

General Indicators:

- **Early or Late Onset Symptoms:** Symptoms appearing earlier or later than expected.
- **Progressive Disease:** Unexplained conditions/symptoms that worsen over time.
- **Chronic Disorders:** Long-lasting medical symptoms that are unexplained but do not resolve with time.
- **Atypical Course:** Unusual clinical features or progression.
- **Positive Family History:** A family history of similar medical problems.
- **Complications:** Additional and unusual health problems in the course of an underlying disease.
- **Treatment Failure:** Lack of response to prescribed treatments that usually work.
- **Anomalies:** Unusual physical or biological characteristics.
- **Multiple Symptoms in Different Systems:** Coexistence of symptoms affecting various organ systems (e.g., diabetes and deafness).
- **Different Chronic Disorders in the Same Family:** Multiple family members experiencing diverse

chronic conditions, potentially indicative of a shared genetic mechanism.

- **Recurrent Symptoms:** Frequent episodes of specific symptoms (e.g., vomiting, ataxia, paralysis).
- **Unexpected Diseases:** Occurrence of conditions that are rare or unusual (e.g., male breast cancer).
- **Unexpected Drug Side Effects:** Unexpected adverse reactions to medications.
- **Prolonged ICU Necessity:** Extended stays in the Intensive Care Unit.
- **Frequent Hospitalization:** Repeated hospital admissions with similar or different clinical presentations.
- **Extreme or Exceptional Disease Presentations:** Uncommonly severe of a common disease.
- **Surprising Lab Values:** Unusual or unexpected laboratory test results.

Specific Symptoms:

- **Repeated Pregnancy Losses or Adverse Pregnancy Outcomes:** Frequent miscarriages or complications during pregnancy.
- **Delayed Milestones in Childhood:** Delays in achieving developmental milestones.
- **Intellectual Disability:** Impaired cognitive functioning.
- **Unusual Growth Patterns:** Atypical growth, including short stature or tall stature.
- **Distinctive Facial Features:** Unique facial characteristics associated with specific conditions.
- **Epilepsy:** Recurrent seizures.
- **Vision or Hearing Problems:** Impaired vision or hearing.
- **Frequent Infections:** Frequent and recurrent infections.
- **Persistent Digestive Problems:** Chronic gastrointestinal symptoms.
- **Developmental Regression:** Loss of previously acquired skills or abilities.
- **Memory Loss:** Impaired memory function.
- **Recurrent Thrombosis or Bleeding:** Blood clotting or bleeding issues.
- **Early Onset Cancers:** Cancer diagnosis at a young age.
- **Multiple Primary Cancers:** Developing multiple distinct cancers.
- **Skin Pigment Anomalies:** Unusual skin pigmentation patterns.
- **Unusual tumors, Benign Tumors, or Multiple Polyps:** Abnormal growths or tumors.
- **Combination of Specific Disorders and Symptoms Associated with Rare Diseases:** e.g., renal clear cell carcinoma, retinitis pigmentosa, autism, and infertility.
- **School Concerns:** Academic or behavioral issues in school.
- **Early or Delayed Puberty:** Premature or delayed onset of puberty.
- **Sudden Death:** Unexpected and unexplained fatalities.

These symptoms and factors often require thorough medical evaluation to diagnose underlying conditions, especially when they persist or recur. Early detection and diagnosis are crucial for effective treatment and management.

References

- Baxter MF, Hansen M, Gration D, Groza T, Baynam G. Surfacing undiagnosed disease: consideration, counting and coding. Front Pediatr. 2023 Oct 25;11:1283880. doi:10.3389/fped.2023.1283880. PMID: 38027298; PMCID: PMC10646190.

CLASSIFICATION OF GENETIC DISEASES

2.1. VARIANT TYPES

Variants in genes can take various forms, ranging from changes in single nucleotides to alterations (SNP's) involving multiple adjacent or distant nucleotides. Additionally, variants can affect the copy number of an entire gene or multiple genes due to large deletions or duplications (CNV's). While there are no strict rules, partial changes in copy number typically result in a loss of gene function. Conversely, when the copy number of an entire gene increases, it's often referred to as a gain-of-function mutation. If the entire gene is lost, it usually leads to a loss-of-function mutation. In some cases, copy number changes can impact multiple adjacent genes, resulting in what's known as contiguous gene syndrome. When changes occur in more than one gene simultaneously, a person may exhibit multiple diseases simultaneously, resulting in a complex clinical presentation that combines the features of these disorders. In some instances, alterations in DNA regions located within copy number variation regions, whose functions are currently unknown, can give rise to clinical manifestations different from what would be expected based on the affected genes.

Furthermore, some genes contain repeat regions with a small number of nucleotides that can influence the gene's function speed. Expanding or reducing the number of these repeats can lead to a gain or loss of function. Changes in repeat numbers can occur for various reasons, particularly during meiosis due to mismatching. These variants can be detected through sequence analysis or fragment analysis. However, in cases where the repeat number is extremely large, as seen in conditions like fragile X syndrome, specialized techniques such as Southern blotting may be necessary. These variations are responsible for diseases referred to as "repeat number diseases."

Further Reading

- https://www.nature.com/scitable/topicpage/genetic-mutation-441/

CHOOSING GENETIC AND LABORATORY METHODS

When selecting a laboratory method for investigating a genetic disease, several considerations come into play. One of the primary decisions is whether to opt for targeted tests or general screening tests. The choice between the two depends on various factors.

If there is a strong suspicion or a high probability of a specific disease based on clinical indicators, and there exists a laboratory method that effectively confirms that particular disease, it is generally more cost-effective and efficient to conduct a targeted test. In such cases, the aim is to confirm the diagnosis quickly and accurately.

Conversely, when the clinical presentation can be caused by variants in more than one gene, or when it is challenging to definitively diagnose the disease based on symptoms and family history alone, it is advisable to consider screening methods as a first step. Screening methods are designed to explore multiple possibilities simultaneously, allowing for a broader evaluation of potential genetic factors contributing to the condition. This approach can be particularly useful when there is uncertainty about which specific genetic variant might be responsible for the observed clinical picture.

In summary, the choice between targeted tests and screening methods in genetic disease investigation depends on the clinical context and the level of certainty in diagnosing a particular disease. Targeted tests are suitable for cases where a clear diagnosis is likely, while screening methods are more appropriate when multiple genes or genetic factors need to be explored due to diagnostic complexity or uncertainty.

Further Reading

- https://bio.libretexts.org/Bookshelves/Genetics/Online_Open_Genetics_(Nickle_and_Barrette-Ng)/08%3A_Techniques_of_Molecular_Genetics
- https://accessmedicine.mhmedical.com/content.aspx?bookid=3128&sectionid=262427637
- https://www.youtube.com/watch?v=BH8Y5qKgNOE

The analysis of copy number variants (CNVs) and sequence variants in genetic diseases involves various methods, each tailored to specific diagnostic needs:

Copy Number Variant (CNV) Analysis:

1. **Array-Based Methods (Array-CGH and SNP-Array):** These methods involve scanning the entire genome to investigate copy number changes. Both techniques are effective at detecting CNVs, with SNP-Array being more capable of identifying smaller copy number changes that affect part of a gene and loss of heterozygosities (LOH) which may be related to uniparental disomies (UPD).
2. **Next-Generation Sequence Analysis (NGS):** NGS-based methods are increasingly favored as they can detect smaller CNVs affecting gene portions. NGS is becoming more widely used due to its cost-effectiveness and ability to study numerous genes simultaneously.
3. **Chromosome Analysis:** This method is effective for identifying very large copy number changes, chromosomal numerical disorders, such as Down syndrome, or structural chromosomal anomalies. It is relatively cost-effective, making it suitable for family screening studies.

Sequence Analysis:

1. **Sanger Sequence Analysis:** This traditional method is used for sequencing small genes or specific gene regions. It is still employed for routine analysis.
2. **Next-Generation Sequence Analysis (NGS):** NGS is preferred for its cost-efficiency and the ability to study a larger number of genes simultaneously. Both methods require the enrichment of gene regions of interest.

Enrichment Methods:

- **PCR-Based Enrichment:** In this method, small DNA sequences called primers mark the regions to be amplified, and enzymes are used to amplify the desired regions billions of times.

- **Capture-Based Enrichment:** DNA is fragmented into small pieces, and probes capture the regions of interest while separating them from other DNA regions. This method is commonly used for studying multiple genes or DNA regions simultaneously, and it allows for copy number analysis.

Exome Sequencing:

This method targets the coding regions and exon-intron boundaries of almost all genes simultaneously using the capture-based approach. It enables the examination of more than 20,000 genes at once.

Genome Sequencing:

DNA is fragmented into small pieces and directly sequenced using next-generation sequencing devices. Software compares the obtained sequences with normal ones to identify differing regions, which are further analyzed to assess their impact on gene function.

Examples of Targeted Analysis:

- **Repeat Number Diseases:** For diseases involving repeat number variations, methods like fragment analysis, Southern blotting, or PCR-based techniques are employed to determine repeat numbers.
- **PCR and Real-Time PCR:** These methods are used to directly detect specific mutations, particularly deletions.
- **Multiplex Ligation-dependent Probe Amplification (MLPA):** MLPA is widely used for targeted copy number analysis, especially when assessing multiple regions of genes. It is commonly applied in diseases like Spinal Muscular Atrophy (SMA) where deletion or duplication mutations are prevalent.
- **Specific Variant Types:** In diseases results from various types of variants, screening often begins by checking for the most common variant type. For example, Duchenne muscular dystrophy primarily involves copy number variants, with MLPA being the first step test. The remaining cases, which may involve SNP variants, can be detected through sequence analysis.

These methods offer a range of diagnostic capabilities, allowing healthcare professionals to effectively analyze genetic diseases and tailor their approach to the specific clinical context.

SOURCES FOR DETECTION OF EXPECTED VARIANT TYPES IN A DISEASE

When selecting laboratory techniques in genetics, it's crucial to consider disease-related mechanisms as a priority, although there are no rigid guidelines or boundaries in this regard. To establish priorities, it is advisable to effectively utilize specific data sources. Physicians should incorporate resources like GeneReviews, GTR, and OMIM into their daily practice for reference. However, there may arise situations where alternative methods are indispensable for diagnosing patients. Occasionally, it becomes necessary to identify single-gene disorders through chromosome analysis. To make such determinations, collaborating with specialists in the field is essential when reaching a precise diagnosis becomes challenging.

- https://www.ncbi.nlm.nih.gov/gtr/
- https://www.omim.org/
- https://www.ncbi.nlm.nih.gov/clinvar/

SINGLE GENE DISEASES AND THEIR RED FLAGS

Single gene diseases are characterized by the involvement of only one gene, distinct from chromosome diseases. Common diagnostic methods like chromosome analysis, Array-CGH, and SNP array are often ineffective for these conditions. Instead, disease-specific gene analyses tailored to both the disease and the individual should be considered. It is advisable to collaborate with a geneticist when planning such analyses.

In autosomes, there exist two copies of each gene. However, in gonosomes (X and Y chromosomes), males typically have a single copy of most genes, except those found in the pseudoautosomal region where some genes are present in duplicate. In contrast, females possess two copies of all genes, although in many cases, the second copy remains silent.

Key genetic terms to understand include:

- **Heterozygous:** When a variant is observed in one of the two gene copies.
- **Homozygous:** When both copies of the gene possess the same variant.
- **Compound heterozygous:** When different variants are found in the same gene.
- **Compound heterozygous at the cis position:** When two variants are present in one of the two gene copies.
- **Compound heterozygous at the trans position:** When two variants are present in separate copies of the gene.
- **Hemizygous:** This term applies when a person has a single gene copy, which has a variant. It is often used in the context of X chromosomal variants in males (X-linked diseases). Additionally, it is used when a mutation occurs in genes with only one remaining copy due to deletion or loss of one of the two chromosomes.

5.1. INHERITANCE PATTERNS OF SINGLE GENE DISORDERS

Autosomal Dominant: In these cases, disease manifestation is possible with heterozygous variants, and it can be more severe in homozygotes. Heterozygous individuals have a 50% chance of passing the variant to their offspring. Clinical variations, even within families (difference in expression), and incomplete penetrance, where not everyone with the variant displays symptoms, can be observed in autosomal dominant diseases.

Autosomal Recessive: Diseases in this category are triggered by homozygous or compound heterozygous variants, while heterozygotes are carriers who have either no or mild symptoms, as seen in beta thalassemias. When both parents are carriers, there's a 25% chance of having an affected child and a 50% chance of having carrier children. Most autosomal recessive diseases show little clinical variation within families, and those with homozygous or compound heterozygous variants are typically affected. Some heterozygous cases may exhibit symptoms, often due to factors like an undetectable second variant, mosaic variants, or dominant negative effects.

X-linked: In these disorders, men with hemizygous variants and homozygous or compound heterozygous women are affected. Women with X chromosome deletion or 45,X chromosome structure (Turner syndrome) may also exhibit symptoms due to hemizygous variants. Heterozygous women may or may not show symptoms, depending on X-inactivation controlled by the XIST gene. Clinical manifestations in women are generally less severe than in males. Heterozygous women have a 50% chance of passing the variant to their offspring, with daughters expected to be heterozygotes and sons expected to be affected.

5.2. CLINICAL VARIABILITY IN GENETIC DISORDERS

Single Gene Diseases: These disorders arise from variants in a single gene, pushing a person beyond the threshold needed to trigger the disease. The impact of environmental factors varies across different conditions, affecting clinical symptoms in some instances. Typically, the severity of the disease is determined by the degree of loss or gain of function in the protein or RNA produced by the affected gene. However, when various genes or proteins within the same biochemical pathway influence the tolerance to damage, it can result in milder clinical manifestations. If a patient exhibits a more severe or milder clinical presentation than expected, it's important to consider the influence of other genes or environmental factors on their condition (modifying factors).

Family Variability: In families with affected individuals, the clinical presentations can vary significantly, sometimes leading to different disease diagnoses. This variation is especially pronounced in dominant diseases, where nearly all family members may exhibit distinct clinical presentations. For example, out of ten known disease symptoms, one family member may display only two, while another may show five – this variability is referred to as "variable expression." Some individuals within the family may even carry the variant without displaying any symptoms, due to a phenomenon known as "penetrance." Low penetrance means that some variant carriers in the family remain symptom-free. If, for instance, 20 out of 50 individuals with the variant exhibit symptoms of any severity, the penetrance can be calculated as 40%. In contrast, recessive diseases tend to show more similarity in clinical presentation among family members and across different families. Clinicians must consider this clinical variability and spectrum to ensure accurate diagnoses, as it is a common occurrence in genetic disorders.

5.3. IMPORTANT NOTES:

Dominant Diseases: Dominant diseases can be inherited from either the mother or father, or they can originate from a new "de novo" variant. These diseases typically result from point mutations that occur in sperm, often when the father is older, although other factors can contribute.

Autosomal Recessive Diseases: In autosomal recessive diseases, one variant typically comes from the mother, and the other from the father. Alternatively, one variant may be inherited from a parent, while the other could be a new "de novo" variant. In some cases, the second variant may exist as mosaic, affecting only some cells. If the variant is not detectable or is present in very few blood cells, additional tissue samples may be necessary to identify the second variant. An example of this is Familial Mediterranean Fever. Finding a heterozygous variant is not definitive for diagnosis; it provides supportive evidence but not a conclusive diagnosis.

Genetic Testing Limitations: Most genetic tests focus on coding exons and exon-intron boundaries, potentially missing variants outside of these regions. A negative genetic test result does not rule out a disease entirely. Further discussion and additional genetic tests may be necessary if the clinical picture aligns with the disorder, but other similar disorders should also be considered.

Diverse Genetic Causes: Sometimes, the same clinical presentation of a disease can result from different genetic causes. For instance, neural tube defects, often influenced by multifactorial factors, can also stem from certain single gene diseases like Meckel syndrome. While folic acid administration can reduce the risk of neural tube defects in some pregnancies, it may not be effective in others. Therefore, further investigations are required to pinpoint the exact cause in such cases.

5.4. RED FLAGS OF SINGLE GENE DISORDERS

1. **Positive Family History in Autosomal Recessive Disorders:** A family history showing similar clinical symptoms in multiple family members can suggest the possibility of autosomal recessive disorders.
2. **Positive Family History in Autosomal Dominant Disorders:** In contrast, a positive family history displaying variable clinical symptoms among family members may indicate autosomal dominant disorders.
3. **Clinical Differences Between Genders in X-Linked Disorders:** X-linked disorders often present with more severe clinical features in males and milder symptoms in females.
4. **Gender Ratio in X-Linked Disorders:** In pedigrees for X-linked disorders, there may be a decreased male-to-female ratio due to the X-linked inheritance pattern.
5. **Presence of symptom(s) or disease in OMIM Database:** Utilizing the OMIM database, symptom-based searches can provide diagnostic possibilities for genetic disorders based on the observed symptoms. Checking the symptoms of all patients in OMIM helps to diagnose rare and frequent disorders.
6. **Symptoms Indicative of Genetic Disorders:** Certain symptoms, such as epilepsy, cholestasis, congenital anomalies, cardiomyopathies, and arrhythmia, can serve as red flags signaling potential genetic disorders.

CHROMOSOMAL DISEASES AND RED FLAGS - BALANCED/UNBALANCED CONCEPT

Chromosomal Diseases Overview: Chromosomes serve as the organized carriers of genes and other DNA regions. Chromosomal diseases often affect numerous genes, sometimes even hundreds, collectively determining the clinical presentation in affected individuals. While some chromosomal diseases are easily recognizable, like Down syndrome or trisomies 13 and 18, others are less apparent. The size and content of the affected chromosomal segment play a critical role in the severity of symptoms. Chromosome analysis is the primary diagnostic method, and in some cases, additional techniques are needed, especially for detecting copy number variations.

Autosomal and Sex Chromosomes: Humans have 22 pairs of autosomal chromosomes, numbered from 1 to 22, and a pair of sex chromosomes, X and Y.

Balanced and Unbalanced Chromosomal Changes: Chromosomal changes are categorized as balanced or unbalanced. Unbalanced changes typically result in clinical symptoms, while a balanced change in a parent can lead to unbalanced chromosomal structures in their offsprings. Since chromosomal changes often impact multiple genes, they tend to produce severe clinical outcomes.

Mosaicism: In certain cases, different types of cells with varying chromosomal structures can exist within an individual's body, a phenomenon known as mosaicism.

Types of Numerical Chromosomal Changes: Numerical changes in chromosomes can be of two main types: Ploidies involve multiples of 23 chromosomes, such as triploidy (69 chromosomes) or tetraploidy (92 chromosomes), while aneuploidies involve changes that are not multiples of 23, such as trisomy (three copies of a chromosome) or monosomy (a single copy of a chromosome).

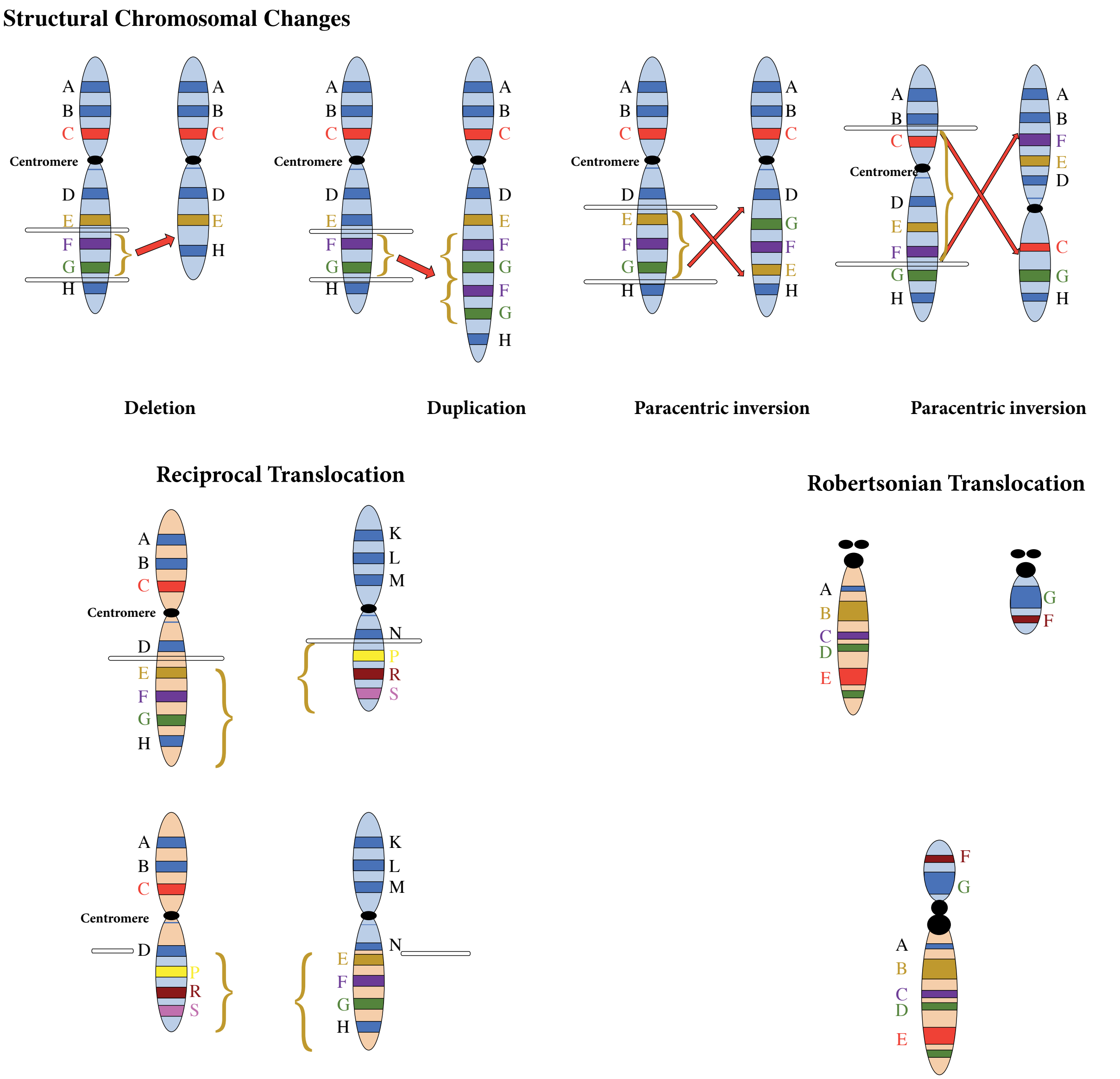
Structural Chromosomal Changes: Structural changes in chromosomes result from breaks in the chromosome structure and can include translocation (exchange of segments between chromosomes), inversion (a chromosome breaks in two places, and the middle part is reversed and reattaches), deletion (loss of a segment of a chromosome), duplication (gain of a chromosomal segment), and insertion (addition of a chromosomal segment from another chromosome). If translocation involves acrocentric chromosomes, it's termed Robertsonian translocation, and if it alters segments within chromosomes, it's referred to as reciprocal translocation.

Microdeletions and Duplications: These genetic anomalies result from the loss or addition of tiny, submicroscopic segments within chromosomes. The clinical manifestations vary based on the number and content of the genes affected by these changes. Similar to chromosomal diseases, they can be categorized into two groups: recognizable diseases, such as DiGeorge syndrome, and those that are not easily identifiable through examination. Variants occurring in genetic hotspots often lead to similar clinical presentations among patients and are recognizable. Conversely, variants located in non-hotspot regions can result in variable clinical pictures due to differences in the size and content of the affected chromosomal segment.

Diagnostic Methods: Diagnosis of these conditions can involve various techniques, including chromosome analysis, Array-CGH (comparative genomic hybridization), SNP-Array (single nucleotide polymorphism array), whole exome sequencing, and whole genome sequencing. When clinical findings suggest recognizable diseases like DiGeorge syndrome, targeted tests like FISH (fluorescence in situ hybridization) or MLPA (multiplex ligation-dependent probe amplification) can be particularly helpful. However, for clinical presentations that cannot be readily recognized, more comprehensive approaches like Array-CGH, SNP-Array, whole exome sequencing, and whole genome sequencing are often employed for diagnosis.

6.1. MECHANISMS OF CHROMOSOMAL DISEASES

Copy Number Changes: Variations in copy numbers can result in either the loss or gain of gene function. Unbalanced variants often lead to clinical symptoms, whereas most balanced variants merely increase



the risk of having offsprings with unbalanced chromosomal structures.

Locus Effect: The arrangement of genes side by side is crucial for their cooperative functioning. Alterations in this order can disrupt not only a single gene but also the coordination between multiple genes, particularly those sharing control regions.

6.2. RED FLAGS OF CHROMOSOMAL DISORDERS AND COPY NUMBER VARIATIONS

Clinical Indicators:

- **Resembling Recognizable Syndromes:** Clinical presentations that closely resemble known chromosomal or microdeletion-duplication syndromes.
- **Diverse Clinical Presentations in Children of Unaffected Parents:** Cases where two different severe clinical pictures are observed in children born to unaffected parents.
- **Pregnancy-Related Red Flags:**
  - History of Pregnancy Loss: A history of previous pregnancy losses.
  - Infertility: Difficulty conceiving.
  - Prenatal Features
  - Short Stature: Stunted growth.
  - Developmental Delay and Failure to Thrive: Delayed development and failure to thrive in infancy.

- Congenital Anomalies and Dysmorphic Features: Birth defects and unusual physical features.
- Intellectual disability: Subnormal intellectual functioning.
- Ambiguous Genitalia: Genital abnormalities causing uncertainty in gender.
- Amenorrhea: Absence of menstruation in females.
- Gynecomastia: Abnormal breast development in males.
- Developmental Defects of Secondary Sexual Features: Abnormalities in the development of secondary sexual characteristics.

6.3. PREGNANCY RELATED RED FLAGS

- **Advanced Maternal Age:** Pregnancy occurring in older mothers.
- **Congenital Anomalies:** Fetal structural abnormalities.
- **Soft Markers in Ultrasounds:** Soft markers observed in prenatal ultrasounds, although the risk should be assessed considering relevant literature, as not all soft markers lead to high risk.
- **Positive Maternal Serum Screening Test:** Positive results in 1st or 2nd trimester maternal serum screening tests, often combined with increased nuchal translucency (NT).
- **Positive Family History:** A family history indicating a risk of chromosome abnormality.
- **Parent Carrier of Balanced Chromosomal Aberration:** One or both parents carrying balanced chromosomal rearrangements.
- **Ultrasound Findings:** Ultrasound findings such as intrauterine growth retardation, polyhydramnios (excessive amniotic fluid), or oligohydramnios (insufficient amniotic fluid).

Further Reading

- https://www.ncbi.nlm.nih.gov/books/NBK563293/
- https://www.ncbi.nlm.nih.gov/books/NBK557691/

REPEAT DISORDERS AND RED FLAGS

Trinucleotide repeat disorders are a group of neuropsychiatric conditions caused by abnormal expansions of trinucleotide repeat sequences in specific genes. Notably, around 30% of the human genome consists of microsatellite repeat sequences. When the number of these repeat sequences within certain genes surpasses a certain threshold, it can lead to either a gain or loss of function, resulting in pathological consequences. What sets these disorders apart is their dynamic nature, and they are often referred to as dynamic mutations. This dynamic aspect becomes evident as the number of repeat sequences can increase from one generation to the next, often worsening the clinical presentation in subsequent generations. This phenomenon is termed "anticipation."

Trinucleotide repeat disorders are generally classified into two types:

1. **1. Type 1:** These disorders involve the expansion of polyglutamine repeats, with an abnormal repetition of the CAG trinucleotide sequence.
2. **2. Type 2:** These are diseases characterized by non-polyglutamine (non-polyQ) repeat expansions.

In some of these disorders, the expanded repeat sequence is located within the non-coding region of the gene. Notable examples within this group include Fragile X syndrome, myotonic dystrophy, and certain cerebellar ataxias such as SCA8, SCA10, and SCA12.

Among these disorders:

1. Friedreich's Ataxia accounts for approximately 50% of all hereditary ataxia cases. It is inherited in an autosomal recessive manner, with a carrier rate estimated to be around 1 in 29,000 individuals.
2. Fragile X syndrome has been reported to affect one in every four thousand Caucasian men.
3. Spinocerebellar Ataxia has an estimated prevalence of 3 in 100,000 individuals.
4. Myotonic Dystrophy is observed with a frequency of approximately 1 in 7,400 in the European population.

These disorders vary in their clinical presentations and genetic mechanisms, but they share the common feature of trinucleotide repeat expansions contributing to their pathogenesis.

7.1 FRAGILE X

Fragile X syndrome is a genetic disorder that affects a significant portion of the population, with varying frequencies among different racial groups. In the Caucasian population, the frequency is approximately 1 in 8,000 individuals. However, it has been reported to be more prevalent in African Americans, particularly in certain regions like Atlanta and Georgia, where the rate can be as high as 1 in 2,500 individuals.

Clinical features of Fragile X syndrome include:

- Moderate to severe intellectual disability
- Macroorchidism
- Long face
- Macrotia
- Mandibular prognathia

Many of these physical and cognitive characteristics become noticeable during adolescence and early adulthood.

The underlying genetic cause of Fragile X syndrome is associated with the number of CGG repeats in the non-coding region of the FMR1 gene:

- 5-44 CGG repeats are considered within the normal range.
- 45-54 repeats fall into the intermediate range.
- 55-200 repeats are categorized as premutation.
- >200 repeats are classified as full mutation.

In individuals carrying a full mutation, the FMR1 gene is hypermethylated, leading to reduced production of the Fragile X Mental Retardation Protein (FMRP). This deficiency in FMRP is responsible for the clinical manifestations of Fragile X syndrome.

Female premutation carriers have the potential to pass the mutation on to the next generation, either as a premutation or it can expand into a full mutation. In contrast, male carriers typically transmit the mutation with relative stability and are less likely to pass on a full mutation. In some cases, carriers of the Fragile X premutation may exhibit two distinct clinical conditions:

1. **Fragile X-associated Ataxia and Tremor Syndrome (FXTAS):** This is a condition characterized by movement problems, such as tremors and abnormality of coordination, typically observed in older individuals who carry the premutation.
2. **Fragile X-related Primary Ovarian Insufficiency (FXPOI):** In some female premutation carriers, there may be an association with early onset of premature ovarian insufficiency, leading to infertility and hormonal imbalances.

Fragile X syndrome is a complex genetic disorder that can manifest in various ways, and its inheritance and clinical presentation can be influenced by the number of CGG repeats and other genetic factors.

7.2. MYOTONIC DYSTROPHY Type 1

Myotonic dystrophy type 1 (DM1) is a complex multisystem disorder that affects various body systems, including skeletal and smooth muscles, the eye, heart, endocrine system, and central nervous system. DM1 can manifest in three different clinical forms: mild, classic, and congenital.

1. Mild Form: In its mild form, DM1 typically presents with the following characteristics:

- Cataracts
- Mild myotonia, which involves continuous, mild muscle contractions
- Muscle symptoms are more pronounced after periods of rest and improve with muscle activity, known as the "warming-up phenomenon"
- Selective involvement of specific muscle groups, such as those in the forearm, hand, tongue, and jaw
- Importantly, individuals with the mild form of DM1 tend to have a normal lifespan, and the condition does not significantly impact life expectancy.

2. Classic Form: The classic form of DM1 is characterized by more severe symptoms, including:

- Muscle weakness and wasting
- Myotonia
- Cataracts
- Cardiac conduction abnormalities
- This form of DM1 can lead to significant disabilities in adulthood and may result in a shortened lifespan.

3. Congenital Form: The congenital form of DM1 is the most severe and is evident from birth. It presents with the following features:

- Severe hypotonia (muscle weakness)
- Respiratory failure shortly after birth
- Early mortality, often within the first few weeks or months of life
- Reduced fetal movements during pregnancy
- Additional prenatal findings on ultrasound, such as polyhydramnios (excess amniotic fluid), talipes equinovarus (clubfoot), or borderline ventriculomegaly (enlarged brain ventricles)

The diagnosis of DM1 is typically based on the number of CTG repeats in the DMPK gene. Normally, there are 5-34 CTG repeats. The classification based on the number of repeats is as follows:

- **35-49 repeats:** Premutation
- **50 and above repeats:** Full mutation

The number of CTG repeats is correlated with the severity of the disease, and it has been observed that individuals born to older mothers tend to exhibit more severe symptoms. These symptoms can impact various aspects of life, including motor skills (sitting, walking), feeding, breathing, and the development of conditions like scoliosis (abnormal curvature of the spine). DM1 is an inherited genetic disorder with autosomal dominant inheritance, meaning that it can be passed down from affected individuals to their offspring.

7.3. SPINOCEREBELLER ATAXIA

It includes a group of hereditary ataxia syndromes characterized by degenerative changes in the cerebellum and sometimes the spinal cord. Although different figures are reported in different regions, the average rate is 3 per hundred thousand. It is known that there are more than 40 types. SCA3 is the most common type.

It is an autosomal dominant transmission. The three main findings of SCA include ataxic gait and incoordination, nystagmus/visual problems, and dysarthria.

There is no current FDA-approved treatment for SCA.

It can develop due to mutations in many different genes. Some of these occur due to an increase in the number of CAG repeats in the related gene.

7.4. FRIEDREICH ATAXIA

Friedreich ataxia (FRDA) is a rare autosomal recessive neurodegenerative disease characterized by a range of symptoms, including progressive gait disturbance and ataxia-associated limb muscle weakness. Here are some key features of FRDA:

1. Clinical Presentation: FRDA typically presents with the following clinical features:

- Progressive gait disturbance and ataxia, which affects coordination and balance.
- Limb muscle weakness, particularly in the lower extremities.
- Absence of lower extremity reflexes, such as the knee and ankle reflexes.
- Extensor plantar responses, which can be detected during neurological examination.
- Dysarthria, leading to difficulty in speech articulation.
- Decreased vibration sense and proprioception, affecting sensory perception.

2. Age of Onset: Symptoms of FRDA often begin before the age of 25, with the average age of onset falling between 10 and 15 years old.

3. Prevalence: FRDA is a rare condition, occurring in approximately 1 in 50,000 individuals.

4. Variable Features: In addition to the core symptoms mentioned above, FRDA can also manifest with various additional features:

- Visual deficits may occur, affecting vision.
- Scoliosis, an abnormal curvature of the spine, can develop.
- Pes cavus, a condition where the arch of the foot is unusually high, may be present.
- Cardiomyopathy (heart muscle disease) is observed in approximately two-thirds of individuals with FRDA.
- Diabetes mellitus is observed in around 30% of cases.

5. Atypical Cases: In approximately 25% of cases, individuals with FRDA may exhibit atypical clinical features. These atypical cases often have a later onset of symptoms, and tendon reflexes may appear to be preserved in contrast to the typical absence of lower extremity reflexes.

6. Genetic Testing: Diagnosis of FRDA is typically confirmed through genetic testing. More than 95% of individuals with FRDA have an abnormally expanded GAA repeat variant located in intron 1 of the FXN gene. Importantly, this variant is present in both maternal and paternal copies of the gene in affected individuals.

7. Compound Heterozygous Variants: In a small percentage (approximately 5%) of individuals with FRDA, compound heterozygous variants may be detected. This means that there is an expanded GAA repeat on one allele that can cause the disease, along with the presence of another intragenic pathogenic variant on the other allele.

Friedreich ataxia is an autosomal recessive disorder, which means that affected individuals inherit two mutated copies of the FXN gene, one from each parent. The expansion of the GAA repeat sequence in the FXN gene leads to impaired production of frataxin, a protein involved in mitochondrial function, and results in the characteristic features of the disease. While there is currently no cure for FRDA, management focuses on addressing symptoms and providing supportive care to improve the quality of life for affected individuals.

7.5. HUNTINGTON'S DISEASE

Huntington's disease (HD) is a devastating neurodegenerative disorder characterized by a range of motor, cognitive, and psychiatric symptoms. Here are key points about Huntington's disease:

1. Age of Onset: The average age of onset for Huntington's disease is typically around 45 years of age. However, there can be considerable variability, and some individuals may develop symptoms earlier or later in life.

2. Progression: HD is a progressive disease, and after symptoms begin, the average survival is generally considered to be around 15 to 18 years. The disease leads to a gradual decline in motor function, cognition, and overall quality of life.

3. Genetic Basis: Huntington's disease is caused by pathogenic variants of the HTT gene located on chromosome 4. This common variant involves an abnormal expansion of CAG repeats within the gene. CAG codes for the amino acid glutamine, and the expansion results in an abnormally long polyglutamine repeat in the huntingtin protein (HTT). This mutant protein forms aggregates in the brain, disrupting various cellular processes.

4. Clinical Presentation: The symptoms of Huntington's disease can vary between individuals, but common features include:

- **Chorea:** Involuntary, rapid, jerky movements that are characteristic of the disease.
- **Cognitive Decline:** Changes in memory, reasoning, and other cognitive functions.
- **Personality Changes:** Alterations in behavior and personality, which can include irritability, mood swings, and social withdrawal.
- **Psychiatric Symptoms:** Symptoms such as depression, anxiety, and psychosis can occur.

5. Stages: The progression of HD can be divided into stages:

- **Early Stage:** Initially, symptoms may be subtle, with mood changes and mild motor symptoms.
- **Middle Stage:** Chorea becomes more pronounced, and individuals may have difficulty with coordination and daily activities.
- **Late Stage:** Severe motor disability and cognitive decline are characteristic of this stage, and individuals become largely dependent on others for care.

6. Genetic Testing: Diagnosis of Huntington's disease is confirmed through genetic testing. Normal individuals typically have 26 or fewer CAG repeats in the HTT gene. A repeat count of 27-35 is considered intermediate range, and they may expand in subsequent generations (anticipation), leading to the onset of clinical symptoms. Individuals with 36 or more CAG repeats are considered to have pathogenic alleles and are at high risk of developing the disease. While repeat counts of 36-39 are associated with reduced penetrance, 40 or more repeats are considered as "full penetrance" and they are most certainly expected to result in Huntington's disease.

7. Inheritance: HD follows an autosomal dominant inheritance pattern. This means that an affected individual has a 50% chance of passing the mutated gene to their offspring. The probability of clinical symptoms in offspring depends on the size of the CAG repeat and the concept of penetrance. Those with larger repeat expansions have a higher likelihood of developing symptoms.

8. Ethical Considerations: Genetic testing for Huntington's disease in individuals without clinical symptoms can pose ethical dilemmas due to the lack of a cure or effective treatments. Preimplantation genetic diagnosis (PGD) is an option for individuals with pathogenic alleles who wish to have children without passing on the disease.

9. Counseling: Individuals at risk of Huntington's disease, especially those considering genetic testing, should receive comprehensive genetic counseling. This includes discussing the implications of testing, potential emotional and psychological impacts, and the importance of informed decision-making.

Huntington's disease is a complex and challenging condition, both for affected individuals and their families. Supportive care and multidisciplinary management can help improve the quality of life for those living with HD. Additionally, ongoing research into potential therapies offers hope for the future management of this condition.

7.6. RED FLAGS OF REPEAT DISORDERS

- Movement disorders
- Progressive neurodegeneration
- Myotonia
- Intellectual disability especially in males
- Increased disease severity and/or early onset in new generations (anticipation)
- Unexplained muscle disorders by whole exome or whole genome studies (e.g. Oculopharyngodistal myopathy)

Further Reading

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6485936/

EPIGENETIC DISEASES AND RED FLAGS

Genomic imprinting and uniparental disomy are fascinating aspects of genetics and epigenetics that play crucial roles in the regulation of gene expression and can lead to various genetic and epigenetic disorders. Here are some key points about genomic imprinting and uniparental disomy:

Genomic Imprinting:

1. **Definition:** Genomic imprinting is an epigenetic phenomenon where certain genes are marked for expression or repression based on their parental origin. This means that specific genes are "turned on" or "turned off" depending on whether they are inherited from the mother or the father.
2. **Methylation:** The most common mechanism of genomic imprinting involves DNA methylation, where methyl groups are added to specific regions of DNA. Methylation can silence a gene and prevent it from being expressed.
3. **Parent-Specific Expression:** Imprinted genes typically have one functional allele (gene copy) and one silenced allele. The active allele is derived from one parent, while the silenced allele is derived from the other parent.
4. **Non-Mendelian Inheritance:** Genomic imprinting follows a non-Mendelian inheritance pattern because the expression of genes depends on their parental origin and the presence of DNA methylation marks.

Prader-Willi and Angelman Syndromes: One of the most well-known examples of genomic imprinting is found on chromosome 15. If a deletion occurs on chromosome 15 and is inherited from the mother, it results in Angelman syndrome. However, if the same deletion is inherited from the father, it leads to Prader-Willi syndrome. These two syndromes have distinct clinical presentations.

8.1. UNİPARENTAL DİSOMY (UPD):

1. **Definition:** Uniparental disomy (UPD) refers to a situation where an individual inherits both copies of a particular chromosome (or part of a chromosome) from a single parent, rather than one copy from each parent.
2. **Causes:** UPD can occur due to several mechanisms, including trisomy rescue (where one of three copies of a chromosome is eliminated), monosomy rescue (where a missing chromosome is duplicated), and gamete complementation (where two gametes from one parent fuse).
3. **Implications:** UPD can have significant consequences, especially if the affected chromosome contains imprinted genes. When both copies of an imprinted gene are inherited from the same parent (either both from the mother or both from the father), it can disrupt the normal regulation of gene expression and lead to various genetic and epigenetic disorders.
4. **Chromosomes with Imprinted Genes:** Chromosomes 6, 7, 11, 14, 15, 16 and 20 contain imprinted genes, and UPD involving these chromosomes can result in an increased risk of related epigenetic disorders.
5. **Epigenetic Diseases:** Epigenetic diseases, including those related to genomic imprinting and UPD, can sometimes manifest later in life due to environmental factors, such as smoking, poor living conditions, and exposure to certain substances. However, these conditions can still be passed on to offspring if the affected genes are imprinted.

Understanding genomic imprinting and uniparental disomy is essential for comprehending the complexity of gene regulation and the potential genetic and epigenetic disorders that can arise when these mechanisms are disrupted. These phenomena highlight the intricate interplay between genetics and epigenetics in shaping an individual's phenotype and health outcomes.

8.2. RED FLAGS OF EPIGENETIC DISORDERS

- Recognizable genetic syndromes like Prader-Willi syndrome, Angelman syndrome, etc.

- Unexplained clinical findings like hypotonia, intellectual disability or structural anomalies by extensive genetic tests like whole exome or whole genome sequencing
- Trisomy of 6, 7, 11, 14, 15, 16 and 20 in chorionic villus samples but normal amniotic chromosomes
- Positive non-invasive prenatal test (NIPT) for aneuploidy of chromosomes 6, 7, 11, 14, 15, 16 and 20.
- Intrauterine growth retardation

Further Reading

- https://www.cdc.gov/genomics-and-health/about/epigenetic-impacts-on-health.html?CDC_AAref_Val=https://www.cdc.gov/genomics/disease/epigenetics.htm
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1392256/

MITOCHONDRIAL DISEASES AND RED FLAGS

Mitochondria indeed play a unique role in cellular function and have their own genetic material, separate from the nuclear DNA. Here's a summary of the key points you mentioned:

9.1. MITOCHONDRIAL GENETICS:

1. **Mitochondrial DNA (mtDNA):** Mitochondria have their own DNA, consisting of genes that code for various proteins essential for energy production and cellular functions.
2. **Mitochondrial Ribosome and RNA:** Mitochondria possess their ribosomes and RNA, allowing them to produce some of their own proteins using their mtDNA.
3. **Nuclear-Encoded Mitochondrial Genes:** While mitochondria can produce some proteins from their mtDNA, many mitochondrial proteins are encoded by genes located in the cell's nuclear DNA. These proteins are synthesized in the cytoplasm and then imported into the mitochondria.

9.2. INHERITANCE PATTERNS:

1. **Maternal Inheritance:** Mitochondrial DNA is inherited exclusively from the mother to her offsprings. This means that variants in mtDNA can be passed from mother to both of her sons and daughters.
2. **Clinical Variation:** Variants in both mitochondrial DNA and nuclear mitochondrial genes can lead to similar clinical presentations. The severity of these conditions can vary widely, with the mother sometimes being mildly affected or unaffected, particularly in heteroplasmic disorders.
3. **Homoplasmy vs. Heteroplasmy:** In cells with homoplasmy, all mitochondria have the same variant. In contrast, heteroplasmy refers to the presence of a mix of mutated and non-mutated mitochondria in the same cell.
4. **Random Distribution (Bottleneck Theory):** The phenomenon of random distribution of mitochondria during cell division leads to variations in the ratio of mutated mitochondria passed on to daughter cells. This phenomenon is often referred to as the "bottleneck" effect.
5. **Tissue-Specific Variation:** Due to random distribution, some tissues or organs may have a higher proportion of mutant mitochondria, leading to more pronounced clinical symptoms in those specific tissues.

9.3. DIAGNOSTIC APPROACHES:

1. **Mitochondrial DNA Sequencing:** Mitochondrial DNA sequence analysis can be helpful for diagnosis, especially in homoplasmic conditions where the variant is present in all mitochondria.
2. **Mitochondrial MLPA:** Multiplex Ligation-dependent Probe Amplification (MLPA) is another technique used to analyze mitochondrial DNA copy number and identify deletions or duplications.
3. **Sampling in Heteroplasmic Cases:** In heteroplasmic cases, obtaining samples from the most symptomatic organs or tissues may be necessary to accurately assess the proportion of mutated mitochondria and aid in diagnosis.

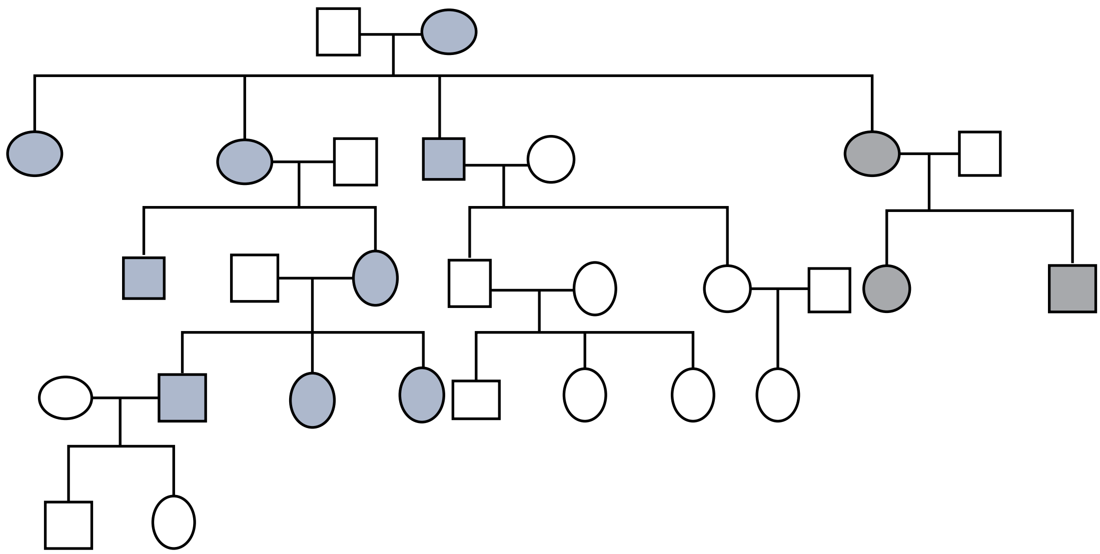
Understanding mitochondrial genetics and inheritance patterns is crucial for diagnosing and managing mitochondrial disorders, which can have a wide range of clinical presentations and often involve complex genetic and cellular mechanisms.

9.4. RED FLAGS OF MITOCHONDRIAL DISORDERS

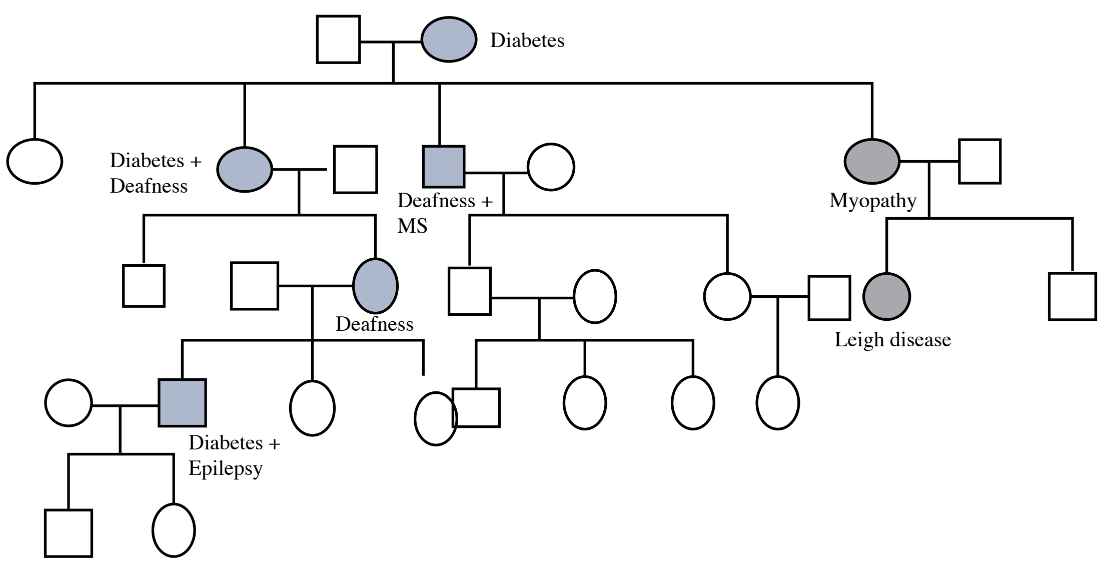
The list of red flags associated with mitochondrial disorders highlights the diverse and often complex clinical presentations of these conditions. It's important to note that mitochondrial disorders can affect various systems and organs in the body, leading to a wide range of symptoms. Here's a summary of the red flags to consider:

1. **Hypotonia:** Reduced muscle tone or weakness can

mitochondrial disease (homoplasmic)



mitochondrial disease (heteroplasmic)



be an early sign of mitochondrial disorders, particularly in infants and young children.

2. **Second Wind Phenomenon:** This refers to a sudden and significant improvement in aerobic exercise tolerance after a brief rest or sleep. It can be a notable feature in some mitochondrial disorders.
3. **Ptosis after Exercise or Fatigue:** Drooping eyelids (ptosis) that worsen with exercise or fatigue can be indicative of mitochondrial involvement, especially in the eye muscles.
4. **Diabetes:** Mitochondrial disorders may affect the endocrine system and lead to conditions like diabetes.
5. **Epilepsy:** Seizures and epilepsy can be associated with mitochondrial disorders, and they may present in various forms.
6. **Sudden Infant Death:** Mitochondrial disorders can lead to sudden and unexplained infant deaths, which is a particularly distressing manifestation.
7. **Chronic Diarrhea:** Gastrointestinal symptoms, including chronic diarrhea, can occur in some mitochondrial disorders.
8. **Retinal Issues (Blindness/Retinitis Pigmentosa):** Vision problems, including blindness and retinitis pigmentosa (a degenerative eye condition), may be present in mitochondrial disorders.
9. **Cardiac Failure:** Mitochondrial disorders can impact the heart, potentially leading to cardiac failure or other cardiovascular issues.
10. **Neurodevelopmental Issues:** Mitochondrial disorders may be associated with neurodevelopmental disorders such as autism, language delay, learning disabilities, and intellectual disability.
11. **Leukodystrophy:** Some mitochondrial disorders can affect the white matter of the brain, leading to leukodystrophy, a group of disorders involving abnormal white matter development.
12. **Cerebral Palsy:** In some cases, mitochondrial disorders can mimic cerebral palsy or contribute to its development.
13. **Deafness:** Hearing impairment or deafness may occur as a result of mitochondrial disorders.
14. **Myoglobinuria:** Elevated levels of myoglobin in the urine (myoglobinuria) can be a symptom of muscle involvement in mitochondrial disorders.
15. **Encephalopathy:** Brain dysfunction or encephalopathy can manifest as cognitive or neurological symptoms.
16. **Tubulopathy:** Impaired renal tubular function (tubulopathy) may be seen in mitochondrial disorders.
17. **Hepatopathy:** Liver dysfunction or hepatopathy can occur in some mitochondrial disorders, affecting liver function.
18. **Ketoacidosis:** Metabolic disturbances, including ketoacidosis (high levels of acidic ketones in the blood), may be observed.
19. **Movement Disorders, Especially Ataxia:** Movement abnormalities, including ataxia (loss of coordination), can be part of the clinical picture.

It's crucial to recognize that mitochondrial disorders can affect individuals differently, and the presence of one or more of these red flags should prompt further evaluation and consideration of mitochondrial disease, especially when other causes have been ruled out. Early diagnosis and intervention can be crucial in managing these complex conditions.

Further Reading

https://www.ncbi.nlm.nih.gov/books/NBK1224/

Databases and Tools

- https://www.mitomap.org/MITOMAP

Expert annotated mitochondrial DNA variants, including disease information

- https://mseqdr.org/mitobox.php

MULTIFACTORIAL DISEASES AND RED FLAGS

These disorders result from a combination of multiple genes and environmental factors. These diseases often result from the interplay of multiple genes, each contributing a small effect, along with environmental triggers or risk factors. Examples include neural tube defects and isolated pes equinovarus. These diseases affect about 60% of the population and include conditions like atherosclerosis, diabetes, cleft lip and palate, and obesity. Genetic predisposition interacts with environmental factors to determine disease risk. Managing environmental factors can sometimes prevent or reduce clinical symptoms. Rheumatological diseases are notable examples, influenced by both genetic background and environmental factors, often leading to similar or different types of rheumatological problems in families.

Polygenic Risk Scores (PRS): Recent research has focused on calculating PRS to assess multifactorial diseases using genomic methods. Artificial intelligence-supported software is being explored to assist in these studies. One challenge is that certain genetic changes may increase risk in some populations but not in others, necessitating data collection efforts across various societies to better understand these patterns.

Further Reading

- https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-021-02229-5
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6673602/
- https://www.nature.com/articles/s41584-022-00904-2
- https://www.genome.gov/Health/Genomics-and-Medicine/Polygenic-risk-scores
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7612115/

Here are some key points related to multifactorial diseases:

1. **Complex Etiology:** Multifactorial diseases are characterized by their complex etiology, where both genetic and environmental factors play a role. The genetic component may involve several genes, each with a minor influence on disease susceptibility.
2. **Population Distribution:** The incidence of multifactorial diseases in the population follows a bell curve, with most individuals having an average risk, while a smaller proportion is at higher or lower risk. This distribution reflects the influence of multiple factors.
3. **Familial Clustering:** Multifactorial diseases tend to run in families. Close relatives of affected individuals have a higher risk of developing the disease compared to the general population. However, the risk decreases as the degree of relatedness decreases.
4. **Threshold Value:** The concept of a threshold value is essential in understanding multifactorial diseases. Below a certain threshold of risk factors, an individual may not develop the disease or may have only mild symptoms. Exceeding this threshold increases the likelihood and severity of the disease.
5. **Gender-Specific Risks:** In some multifactorial diseases, there are gender-specific differences in risk. For example, certain conditions may be more common in one gender than the other. Having the disease in the less commonly affected gender increases the risk of recurrence in the family.
6. **Recurrence Risk:** The risk of recurrence in families with affected individuals depends on various factors, including the number of affected relatives, the severity of the disease, the gender of the affected person, and consanguinity (relatedness of parents).
7. **Preventive Measures:** Some multifactorial diseases can be influenced by lifestyle and environmental factors. Implementing preventive measures, such as a healthy diet, regular exercise, and avoiding known risk factors, can help reduce the risk of developing these diseases.
8. **Genetic Counseling:** Families with a history of multifactorial diseases may benefit from genetic counseling to assess the risk of recurrence and un-

derstand potential preventive strategies.

Examples of multifactorial diseases include:

- Congenital heart diseases
- Cleft lip and palate
- Coronary heart disease
- Diabetes
- Neural tube defects
- Rheumatological diseases

In summary, multifactorial diseases are influenced by a combination of genetic and environmental factors, making them challenging to study and understand. Genetic counseling and preventive measures are essential components of managing these complex conditions, especially in families with a history of such diseases.

Further Reading

- https://www.nature.com/scitable/topicpage/complex-diseases-research-and-applications-748/

Level of relationship	Risk of recurrence
Population risk	0,1%
First cousin of affected person	0,3%
Niece or nephew of affected person	0,8%
Child of affected person	3,5%
Sibling of affected person	4%
Monozygotic twin of affected person	40%

10.1. RED FLAGS OF MULTIFACTORIAL DISORDERS

These conditions encompass a wide range of health issues and demonstrate the diverse nature of diseases influenced by both genetic and environmental factors. Here's a brief overview of some of the multifactorial diseases you mentioned:

1. **Neural Tube Defects:** These are congenital malformations of the brain, spine, or spinal cord, such as spina bifida or anencephaly. Folic acid supplementation before and during pregnancy can reduce the risk.
2. **Clubfoot:** A congenital condition characterized by abnormal positioning of the foot, which can be influenced by genetic and environmental factors.
3. **Cleft Lip/Palate**: Birth defects that affect the upper lip and/or roof of the mouth. Both genetic and environmental factors play a role.
4. **Cardiac Anomalies:** A wide range of heart defects that may involve structural abnormalities or functional issues. Genetic predisposition and environmental factors can contribute to these anomalies.
5. **Diabetes:** Both type 1 and type 2 diabetes have genetic components, but lifestyle factors, such as diet and physical activity, also play a significant role.
6. **Cancers:** Many cancers have multifactorial causes, including genetic mutations and environmental exposures (e.g., smoking, UV radiation).
7. **Hypertension and Hypercholesterolemia:** High blood pressure and elevated cholesterol levels can result from a combination of genetic factors and lifestyle choices.
8. **Alzheimer's Disease:** A neurodegenerative disorder influenced by complex genetic and environmental interactions. Age is a significant risk factor.
9. **Rheumatologic Disorders:** Conditions like rheumatoid arthritis and lupus may have a genetic predisposition, but environmental triggers can contribute to disease onset.
10. **Osteoporosis:** Reduced bone density leading to fractures. Genetics, hormonal factors, and lifestyle choices influence the risk.
11. **Autoimmune Disorders:** Conditions where the immune system mistakenly targets the body's own tissues. Genetic susceptibility and environmental actors are involved.
12. **Asthma and Allergies:** Conditions characterized

by airway inflammation and allergic reactions. Genetic predisposition and environmental allergens are contributing factors.

13. **Multiple Sclerosis:** An autoimmune disease affecting the central nervous system. Both genetic and environmental factors are believed to be involved.

These examples highlight the complexity of multifactorial diseases and the need for a comprehensive approach to their prevention, diagnosis, and treatment, considering both genetic and environmental influences.

Further Reading

- https://www.nature.com/scitable/topicpage/multifactorial-inheritance-and-genetic-disease-919/

DIAGNOSIS WITH FAMILY TREE (PEDIGREE) AND DIFFERENTIATION OF ENVIRONMENTAL FACTORS/ GENETIC DISEASES WITH FAMILY TREE

Pedigree analysis is indeed a crucial tool in clinical genetics and provides valuable insights into the inheritance patterns and patterns of disease within families. Here are some additional points highlighting the importance of pedigree analysis:

1. **Risk Assessment:** Pedigrees help assess the risk of a genetic disorder in an individual based on their family history. This information can guide genetic counseling and testing decisions.
2. **Carrier Detection:** Pedigrees can reveal carriers of autosomal recessive disorders, even if they do not exhibit symptoms themselves. Identifying carriers is essential for family planning and counseling.
3. **Genotype-Phenotype Correlations:** By studying multiple generations within a family, pedigree analysis can help establish correlations between specific genetic mutations and the resulting clinical manifestations.
4. **Prenatal Diagnosis:** In cases of known genetic conditions in a family, pedigree analysis assists in determining the risk of passing on the condition to future generations and informs decisions about prenatal testing.
5. **Consanguinity:** Pedigrees can highlight consanguineous (related by blood) marriages within families, which can increase the risk of autosomal recessive disorders due to shared genetic ancestry.
6. **Disease Mapping:** In research settings, pedigree analysis can be used for disease gene mapping, helping to identify the location of disease-causing genes in the genome.
7. **Inheritance Patterns:** Pedigree analysis is crucial for classifying the inheritance patterns of genetic disorders, including autosomal dominant, autosomal recessive, X-linked, or mitochondrial inheritance.
8. **Genetic Counseling:** Genetic counselors use pedigree information to provide families with accurate information about the genetic risks they may face, helping them make informed decisions about their health and family planning.
9. **Population Studies:** In population genetics, pedigree analysis can provide insights into the prevalence and distribution of specific genetic traits within a population.

Overall, pedigree analysis plays a fundamental role in clinical genetics, genetic counseling, and the understanding of genetic diseases, helping both healthcare professionals and families make informed decisions about genetic testing, family planning, and disease management.

Further Reading

- https://journals.lww.com/djo/fulltext/2023/33010/the_role_of_pedigree_charting_and_analysis_in.4.aspx

CONCEPT OF CONSANGUINEOUS MARRIAGE

Consanguineous marriages, which involve individuals who are closely related by blood, can indeed have a significant impact on the occurrence and severity of genetic diseases within populations. Here are some key points regarding the effects of consanguinity on genetic diseases:

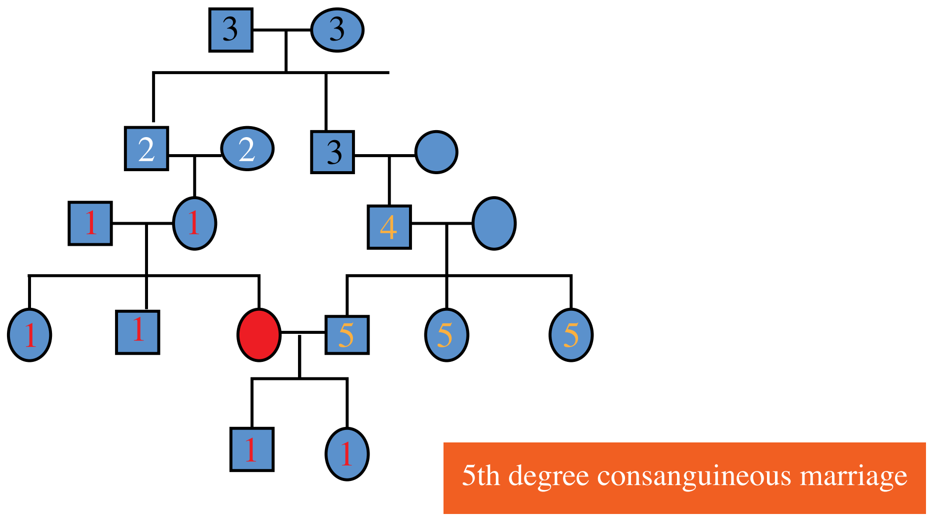
1. **Increased Risk of Autosomal Recessive Disorders:** Consanguineous marriages increase the likelihood of both parents carrying the same autosomal recessive disease gene. When both parents are carriers, there is a 25% chance that their offspring will inherit two copies of the disease-causing gene, leading to the development of the disorder. This results in an increased incidence of autosomal recessive disorders in populations with a tradition of consanguinity.
2. **Homozygosity for Recessive Mutations:** In consanguineous offspring, there is a higher probability of inheriting two identical disease-causing alleles from a common ancestor. This increases the risk of homozygosity for recessive mutations and, consequently, the likelihood of manifesting the disease in a severe form.
3. **Genetic Diversity:** Consanguinity can lead to a reduction in genetic diversity within a population. Reduced genetic diversity can limit the range of genetic variation and increase the prevalence of specific genetic diseases within the population.
4. **Complex Inheritance Patterns:** While consanguinity predominantly impacts autosomal recessive disorders, it can also influence the expression of other genetic diseases, including autosomal dominant and X-linked disorders. In some cases, it can lead to the emergence of more severe phenotypes of dominant disorders through the inheritance of two identical disease-causing alleles.
5. **Genetic Counseling:** In regions where consanguineous marriages are common, genetic counseling and carrier screening programs become essential. These programs can help individuals understand their genetic risks and make informed decisions regarding family planning.
6. **Population-Specific Genetic Diseases:** Consanguinity can contribute to the prevalence of population-specific genetic diseases, which may not be as common in populations with greater genetic diversity.
7. **Public Health Considerations:** Public health initiatives and educational campaigns in regions with high rates of consanguinity can help raise awareness about the potential genetic risks and encourage genetic counseling and testing.

Overall, consanguinity can have a significant impact on the genetic health of populations, and understanding its implications is crucial for both healthcare providers and individuals in affected regions. Genetic counseling, carrier screening, and family planning can help mitigate some of the risks associated with consanguineous marriages.

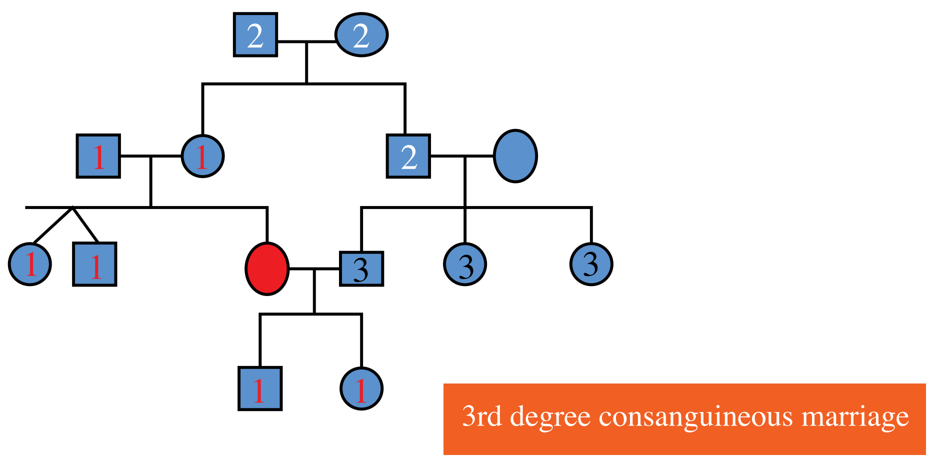
Further Reading

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3419292/

Pedigree analysis



Family tree (pedigree) analysis



READING REPORTS AND VARIANT CLASSIFICATION. WHAT DOES RISK FACTOR MEAN?

Understanding the impact of genetic variations and the classification of variants is essential in the field of genetics and genomics, as it plays a critical role in diagnosing and managing genetic disorders. Here are some additional points to consider:

1. **Functional Significance:** It's crucial to determine the functional significance of genetic variants. Not all genetic variations result in disease or have clinical significance. Some variants may be neutral or benign, and their presence alone may not warrant medical intervention.
2. **Genotype-Phenotype Correlation:** Establishing a clear genotype-phenotype correlation is essential for understanding how specific genetic variants contribute to the development and progression of diseases. This correlation helps in making accurate diagnoses and predicting disease outcomes.
3. **Variant Interpretation:** Variant interpretation involves a comprehensive analysis of genetic data, combining clinical information, family history, and laboratory findings. It often requires a multidisciplinary approach, including genetic counselors, clinicians, and molecular geneticists.
4. **Reporting and Communication:** Effective communication between genetic testing laboratories and healthcare providers is vital. Genetic reports should convey complex genetic information in a clear and understandable manner, helping clinicians make informed decisions about patient care.
5. **Variant Databases:** Access to variant databases and resources like ClinVar and HGMD (Human Gene Mutation Database) is valuable for variant interpretation. These databases contain information about known genetic variants and their clinical significance.
6. **Clinical Relevance:** Genetic testing should always consider the clinical relevance of the identified variants. For example, identifying a variant associated with a specific disease may not be clinically relevant if the patient does not exhibit symptoms or family history of that disease.
7. **Consanguinity and Founder Effect:** In populations with a history of consanguinity, certain genetic disorders may have a higher prevalence due to the presence of founder effect. A founder effect occurs when a small group of migrants establish a new population and certain variants becomes abundant in the next generations. Awareness of these founder variants is essential for accurate diagnosis.
8. **Clinical Utility:** Before ordering genetic tests, clinicians should consider the clinical utility of the information obtained. Will the test results impact patient management, treatment decisions, or genetic counseling?
9. **Ethical and Counseling Considerations:** Genetic testing raises ethical and counseling considerations. Patients and their families should be informed about the potential implications of genetic testing results, including the psychological and social aspects.
10. **Interprofessional Collaboration:** Genetic testing often requires collaboration among various healthcare professionals, including geneticists, genetic counselors, pediatricians, internists, and specialists, to provide comprehensive patient care.

Understanding genetic variations and their implications is an evolving field, and ongoing research continues to expand our knowledge of the human genome. Effective integration of genetic information into clinical practice is crucial for improving patient care and the management of genetic disorders.

Further Reading

- https://genomemedicine.biomedcentral.com/articles/10.1186/s13073-019-0688-9
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10055485/#:~:text=From%20an%20initial%20estimate%20of,suggests%20as%20few%20as%20~19%2C000%20
- https://varnomen.hgvs.org/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544753/
- https://www.nature.com/articles/s41431-021-00903-z

ACMG Classification of Variants:

Pathogenic Variant: Variants that are almost certainly disease-causing.

Likely Pathogenic Variant: Variants with strong evidence of being pathogenic. Family screening, clinical matching, and sometimes functional analyses are needed.

Variants of Uncertain Significance: Variants with limited or conflicting evidence about pathogenicity. If the patient's clinical findings are compatible with the disease and there is no suspicion of a similar disease, then this variant can be considered "likely pathogenic."

Benign and Likely Benign Variants: These are variants not recommended to be included in reports. They are not expected to cause disease.

Risk Factor: Although they are not the cause of the disease themselves, these variants increase the likelihood of the disease if present. They can usually cause clinical findings together with environmental factors and other genetic factors. Thrombophilia variants can be given as examples.

There are various guidelines for writing genetic reports. These guides explain in detail what information should be included in the reports and for what purposes. Although people who write genetic reports are generally familiar with these guidelines, clinicians are generally not knowledgeable about them. It will be easier for clinicians to master these guidelines to ensure that reports are read correctly and to make specific requests from the genetic center they work with.

The data obtained during the interpretation of genetic tests can sometimes cause confusion. For example, in autosomal recessive diseases, if the clinical picture matches, finding a heterozygous variant should be considered supportive of the diagnosis but cannot confirm it. However, in cases where the patient's parents are relatives, finding a heterozygous variant for a disease that is very rare in the population should be evaluated with suspicion and requires investigation of other similar diseases and the possibility of digenic disease. Although finding a homozygous variant in a dominant disease can be observed in some mild mutations, it should still be viewed with suspicion and should prompt an investigation of similar diseases.

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

- https://www.nature.com/articles/gim201530

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5707196/

Recommendations for reporting results of diagnostic genomic testing

- https://www.nature.com/articles/s41431-022-01091-0

APPROACHES TO VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) AND VARIANTS THAT ARE NOT COMPATIBLE WITH CLINICAL FINDINGS FOR THE CLINICIANS

VUS can indeed pose challenges, as they may not provide clear guidance for patient management. Your proposed algorithm takes into consideration various factors that can help clinicians better understand the clinical relevance of these variants. Here are some additional thoughts on your approach:

1. **Clinical Matching:** Ensuring a good clinical match between the identified genetic variant and the patient's symptoms is a critical first step. It's essential to rule out the possibility of other disorders or phenocopies that may present with similar clinical features.
2. **Segregation Analysis:** Investigating how the genetic variant segregates within the family can provide valuable insights into its pathogenicity. Patterns of inheritance and penetrance rates can help distinguish between pathogenic and non-pathogenic variants.
3. **Consulting Databases:** Utilizing resources like ClinVar, gnomAD, and other variant databases is essential. These databases provide information on the clinical significance of variants based on previously reported cases. Collaborating with experts in the field can also provide valuable guidance.
4. **Functional Studies:** Assessing the potential for functional studies of the gene in question is an excellent approach. Functional studies can help establish the functional impact of a variant and its role in disease pathogenesis.
5. **Literature Review:** Regularly reviewing the literature for updates on the gene and its associated symptoms is crucial. New research findings may provide additional context or insights into the clinical relevance of a variant.
6. **Pathway Analysis:** Examining the relationships between the focused gene and other genes within related pathways is a valuable strategy. Some disorders may involve multiple genes within a common biological pathway.
7. **Natural History:** Understanding the natural history of the disorder is essential, as some genetic conditions may have variable age of onset or clinical presentations. Longitudinal data on patient outcomes can help in making more informed assessments.
8. **Penetrance:** Carefully considering the penetrance rates, especially in dominant disorders, is crucial. Low penetrance may indicate that a variant is less likely to be pathogenic.
9. **Mild Mutations:** Being aware of mild or hypomorphic variants is essential, as some variants may not cause severe disease but can still be clinically relevant.

Incorporating all of these factors into the variant interpretation process can help clinicians make more informed decisions regarding patient care, including diagnosis, prognosis, and treatment recommendations. Additionally, the ongoing advancement of genetic and genomic research may lead to reclassification of variants, further highlighting the importance of regular updates and collaboration with experts in the field.

Further Reading

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9158111/
- https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2810999

GERMLINE AND SOMATIC VARIANTS

These two types of variants differ in their origin, presence in the body, and potential inheritance to the next generation:

Germline Variants (Germinal Variants):

- **Origin:** These variants are inherited from one's parents and are present in every cell of the individual's body.
- **Timing:** Germline variants occur in the germ cells (sperm or egg) or in the very early stages of embryonic development.
- **Presence:** They are present in all tissues and cells, including germ cells (sperm or egg), which means they can be detected from any human tissue.
- **Inheritance:** Germline variants have the potential to be passed on to the individual's offspring. If a mutation occurs in a germ cell, it can be transmitted to the next generation.

Somatic Mutations:

- **Origin:** Somatic variants arise after the embryonic stage, typically as a result of various factors such as environmental exposures, DNA replication errors, or other cellular processes.
- **Timing:** Somatic variants can occur at any point during an individual's life, from early embryonal development to adulthood.
- **Presence:** These variants are limited to specific tissues or cells where they originated. They are not present in all cells throughout the body.
- **Inheritance:** Somatic variants are not typically inherited by the individual's offspring in case of they are not present in the germ cells (sperm or egg). To be inherited, a somatic mutation must occur in the germ line (germinal mutation).

Understanding the distinction between these two types of variants is crucial in genetics and clinical practice. Germline variants are particularly significant because they can be passed on to future generations, potentially leading to hereditary diseases, while somatic variants are often responsible for the development of sporadic conditions, including many cancers.

Further Reading

- https://www.ncbi.nlm.nih.gov/books/NBK557896/
- https://www.genomicseducation.hee.nhs.uk/genotes/knowledge-hub/constitutional-germline-vs-somatic-tumour-variants/

CHROMOSOME VARIANTS

These are important concepts in genetics, and they involve changes in the number of chromosomes in a cell or organism. Here's a brief recap:

16.1 ANEUPLOIDY (NUMERICAL CHANGES NOT A MULTIPLE OF 23):

- Aneuploidy refers to a condition where there is an abnormal number of chromosomes in a cell or organism.
- Types of aneuploidies include trisomy (three chromosomes of a particular type), tetrasomy (four chromosomes), pentasomy (five chromosomes), monosomy (single chromosome), and nullisomy (loss of both copies of a chromosome).
- A common example of trisomy is Down syndrome, which results from an extra chromosome 21 (three copies instead of the usual two).
- Aneuploidy can lead to genetic disorders and developmental abnormalities.

16.2. PLOIDY (NUMERICAL CHANGES THAT ARE A MULTIPLE OF 23):

- Ploidy refers to changes in the number of whole sets of chromosomes. These changes are usually a multiple of the haploid number, which is 23 in humans.
- Triploidy (69 chromosomes), tetraploidy (92 chromosomes), and haploidy (23 chromosomes) are examples of ploidy changes.
- These changes often result in non-viable embryos or severe developmental abnormalities.

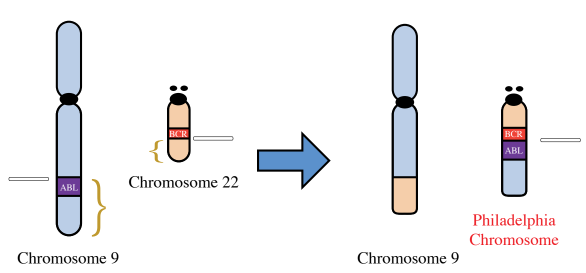
16.3. BALANCED CHROMOSOMAL VARIANT:

- A balanced chromosomal variant means there is no change in the total amount of chromosomal material or genes.
- While a balanced chromosomal variant may not cause problems for the individual, it can lead to the transmission of unbalanced chromosomes to their offsprings during meiosis, potentially causing genetic disorders.

16.4. UNBALANCED CHROMOSOMAL VARIANTS AND COPY NUMBER VARIANTS (CNVS):

- Unbalanced chromosomal variants involve gains or losses of chromosomal material, which may include genes.
- Some unbalanced variants may not have clinical consequences if they do not affect critical genes.
- In autosomal dominant disorders, deletions can lead to the loss of a functional allele, potentially causing loss-of-function disorders. In gain-of-function disorders, duplications can result in an abnormal increase in gene dosage.
- Copy Number Variants (CNVs) refer to variations in the number of copies of a particular DNA segment in an individual's genome. CNVs can include deletions or duplications of chromosomal regions.

You also mentioned other mechanisms of chromosomal disorders, such as position effects, gene breakage, and fusion variants, which can contribute to genetic diseases, including certain cancers. These mechanisms can disrupt normal gene function or lead to abnormal protein production.



Overall, this provides a comprehensive overview of numerical variants and their potential implications in genetics and clinical medicine.

UPD refers to the situation where an individual inherits both copies of a chromosome (or a chromosomal segment) from one parent instead of one copy from each parent. This can have significant implications for gene expression and can result in various genetic disorders depending on the specific chromosomes involved.

Here's a summary of your explanation:

1. Monosomy Correction and Uniparental Isodisomy:

- In cases where monosomy correction occurs during embryonic development, two copies of the same chromosome from one parent may be inherited.
- This leads to "uniparental isodisomy," where both copies of the chromosome are identical.
- Most of the genetic variants on these chromosomes are detected as homozygous.
- If a pathogenic mutation is present in an autosomal recessive gene on these chromosomes, autosomal recessive diseases may develop due to the lack of a functioning copy from the other parent.

2. Trisomy Correction and Uniparental Heterodisomy:

- In cases of trisomy correction, different variants on two chromosomes may result, and they are often heterozygous.
- This situation is referred to as "uniparental heterodisomy."
- Uniparental heterodisomy is associated with several specific genetic syndromes, including Prader-Willi syndrome, Angelman syndrome, Beckwith-Wiedemann syndrome, and Russell-Silver syndrome.
- These syndromes result from the specific inheritance patterns and epigenetic alterations associated with uniparental heterodisomy.

Understanding these mechanisms of uniparental disomy and its potential consequences is crucial for diagnosing and managing various genetic disorders, as it can significantly impact gene expression and disease risk.

16.5. STRUCTURAL CHROMOSOMAL VARIANTS

1. **Translocation:** This involves the exchange of chromosomal segments between two or more chromosomes. If it occurs within more than two chromosomes, it's termed a complex chromosomal translocation.
2. **Inversion:** An inversion refers to the reversal of a chromosomal segment within the same chromosome. Depending on whether the inverted segment includes the centromere or not, it can be classified as pericentric (with centromere) or paracentric (without centromere) inversion.
3. **Deletion:** Deletion involves the loss of a segment of a chromosome, leading to the absence of specific genetic material.
4. **Duplication:** Duplication refers to the gain or replication of a chromosomal segment, resulting in extra genetic material.
5. **Insertion:** Insertion occurs when a segment of one chromosome is inserted into another chromosome.
6. **Isochromosome:** In an isochromosome, one arm of the chromosome is deleted, while the other arm is duplicated, leading to a symmetric chromosome.
7. **Ring Chromosome:** A ring chromosome forms when both ends (telomeres) of a chromosome are deleted and the remaining sticky ends of the chromosome fuse to create a ring-like structure. This often results in the deletion of critical genetic material and severe clinical consequences.
8. **Contiguous Gene Syndrome:** This occurs when deletion or duplication affects a group of neighboring genes, leading to a combination of clinical features. Microdeletion and duplication syndromes can cause such contiguous gene syndromes, where the deletion or duplication of multiple genes results in a complex clinical presentation.

Your explanation provides a valuable overview of these chromosomal structural abnormalities, which can have significant implications for an individual's health and development, depending on the specific genes and chromosomal regions involved.

Further Reading

- https://www.biologyonline.com/dictionary/chromosomal-mutation

SNP VARIANTS

1. **Genes:** Genes are segments of DNA that contain the instructions for building proteins. They are composed of both coding regions (exons) and non-coding regions (introns).
2. **Exons:** Exons are the coding regions of genes where the actual genetic code for producing proteins is found. These regions are transcribed into RNA and then translated into amino acids, which form the basis of proteins.
3. **Nucleotides:** DNA is made up of four nucleotide bases: adenine (A), cytosine (C), guanine (G), and thymine (T). In RNA, thymine is replaced by uracil (U). The specific sequence of these nucleotides in a gene determines the genetic code.
4. **Codons:** In the genetic code, three nucleotides form a codon. Each codon corresponds to a specific amino acid, which is the building block of proteins. There are 64 possible codons, including start and stop codons.
5. **Mutation:** Any change in the sequence of nucleotides in a gene is called a mutation. Mutations can be caused by various factors, including environmental factors, replication errors, or exposure to radiation. Mutations can have different effects, ranging from no impact to altering the function of a protein.
6. **Variant:** A differentiation of DNA sequence compared to the reference DNA. Genetic variants may or may not have an effect on proteins.
7. **Amino Acid Chain:** The sequence of amino acids, as determined by the genetic code, forms a polypeptide chain. These chains fold into specific three-dimensional structures to create functional proteins.

Understanding the genetic code and how it relates to the synthesis of proteins is crucial in genetics and molecular biology. Variants in the genetic code can lead to genetic disorders or contribute to genetic diversity within a population.

Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine	UGU	Cysteine
UUC	Phenylalanine	UCC	Serine	UAC	Tyrosine	UGC	Cysteine
UUA	Leucine	UCA	Serine	UAA	Stop	UGA	Stop
UUG	Leucine	UCG	Serine	UAG	Stop	UGG	Tryptophan
CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine
CUC	Leucine	CCC	Proline	CAC	Histidine	CGC	Arginine
CUA	Leucine	CCA	Proline	CAA	Glutamine	CGA	Arginine
CUG	Leucine	CCG	Proline	CAG	Glutamine	CGG	Arginine
AUU	Isoleucine	ACU	Threonine	AAU	Asparagine	AGU	Serine
AUC	Isoleucine	ACC	Threonine	AAC	Asparagine	AGC	Serine
AUA	Isoleucine	ACA	Threonine	AAA	Lysine	AGA	Arginine
AUG (Start)	Methionine	ACG	Threonine	AAG	Lysine	AGG	Arginine
GUU	Valine	GCU	Alanine	GAU	Aspartate	GGU	Glycine
GUC	Valine	GCC	Alanine	GAC	Aspartate	GGC	Glycine
GUA	Valine	GCA	Alanine	GAA	Glutamate	GGA	Glycine
GUG	Valine	GCG	Alanine	GAG	Glutamate	GGG	Glycine

1. **Nucleotide Substitution:** In this type of mutation, one nucleotide is replaced by another. Depending on the specific substitution, it can lead to different outcomes:
    - **Missense Variants:** These substitutions result in an amino acid change in the protein, potentially affecting its function.
    - **Nonsense Variants:** These substitutions create a premature stop codon in the genetic code, leading to a truncated and often non-functional protein.
    - **Silent Variants:** These substitutions do not alter the amino acid sequence and, as a result, may not affect protein function.
2. **Variants Disrupting mRNA Processing:** Mutations can occur in regions that are crucial for mRNA transcription, processing, or translation. These variants can interfere with the proper functioning of the mRNA and its subsequent translation into a protein.
3. **Deletion and Insertion of Nucleotides:** These variants involve the addition or removal of nucleotides. If the number of nucleotides added or deleted is a multiple of three, it may not cause a frameshift effect and may result in the addition or removal of an amino acid. If the number is not a multiple of three, it can lead to a frameshift variant, altering the entire reading frame of the genetic code.
4. **Splice Variants:** Variants in exon-intron junction regions can affect the splicing process during mRNA maturation. This can result in the inclusion or exclusion of specific exons, leading to variations in the protein's length or function.
5. **Rearrangements:** Structural changes in genes, such as translocations or inversions, can disrupt the normal production or function of gene products. In some cases, gene rearrangements are part of natural processes, such as antibody production in the immune system.
6. **Dynamic Variants (Trinucleotide Repeat Disorders):** These variants involve the expansion of repetitive sequences of three nucleotides (trinucleotide repeats) within a gene. The length of these repeats can change from one generation to the next, leading to diseases with variable severity due to the expansion of these repeats.

Understanding these types of variants is essential in genetics and molecular biology, as they underlie various genetic disorders and contribute to genetic diversity within populations.

Stop codons, frame shift variants and splice variants mostly cause loss-of-function effect, but it is not an exact rule. Missense variants may cause both LOF and GOF.

Further Reading

- https://www.nature.com/scitable/topicpage/genetic-mutation-441/

Type of Variant	Original Sequence	Variant Sequence	Amino Acid Changes
Silent	ATG C**GA** TTA CCG TGC	ATG C**AA** TTA CCG TGC	No amino acid change
Nonsense	ATG CG**A** TTA CCG TGC	ATG CG**T** TTA CCG TGC	CGA (Arg) → CGT (Stop)
Missense	ATG C**GA** TTA CCG TGC	ATG C**CA** TTA CCG TGC	CGA (Arg) → CGG (Pro)
Frameshift	ATG **C**GA TTA CCG TGC	ATG **GAT TAC CGT GC**	Various amino acid changes

THE MAN SAW THE DOG HIT THE CAN	Normal
THE MAN SAW THE DOT HIT THE CAN	Missense
THE MAN SAW THE DOG *	Nonsense
THE MAN SAW THE DOG * THE CAN	Deletion of 3 nucleotides
THE MAN SAW THE FAT DOG HIT THE CAN	Insertion 3 nucleotides
THE MAN SAW THE *OGH ITT HEC AN	Frameshift

WHY DO VARIANTS CAUSE DISEASES? THE LOGIC OF INHERITANCE

The impact of variant, domain, mild variant, and similar types of variants that affect the clinical picture and modifying factors

We will delve into the concept of variants and their role in formation of diseases, focusing on the example of hemoglobinopathies, which are prevalent medical conditions.

Hemoglobin disorders can be categorized as follows:

1. **Structural changes (e.g., sickle cell anemia, hemoglobin D):** Some hemoglobin variants exhibit no clinical symptoms, while others result in mild or severe clinical manifestations.
2. **Thalassemias (reduced production of globin molecules):**
   - Alpha Thalassemia
   - Beta Thalassemia
3. **Hereditary Persistent Fetal Hemoglobin (Heterochronic expression)**
4. **Extreme destruction**
5. **Porphyria:** In this condition, enzymes responsible for heme synthesis malfunction, leading to the accumulation of precursor molecules.
6. **Methemoglobinemia:** This disorder causes hemoglobin to bind to oxygen but fail to release it, resulting in oxygen deficit in tissues, often manifesting as blue or purple discoloration in the skin, nails, or lips. In severe cases, methemoglobinemia can be life-threatening, especially when compounded by anemia (modifier effect).

The most common genetic disease mechanisms include:

1. **Loss of function:** Occurs due to mutations in coding regions, regulatory regions, or critical gene regions, or through gene deletion. The severity of the disease is directly related to the degree of loss of function. An example in hemoglobinopathies is gene deletions in alpha thalassemia.
2. **Gain of function:** Characterized by increased production of a normal protein. For instance, the APP gene associated with Alzheimer's is found on chromosome 21, which is triplicated in Down syndrome, potentially leading to early development of Alzheimer's.
3. **Gaining a new feature or function (Novel property):** In sickle cell anemia, the Glu6Val variant does not affect oxygen transport but causes abnormal protein aggregation in low-oxygen environments, leading to cell deformation.
4. **Expression at the wrong time (Heterochronic expression):** Abnormal expression of specific hemoglobin molecules during embryonic stages can lead to developmental abnormalities, such as Hereditary Persistence of Fetal Hemoglobin (HPFH).
5. **Expression in the wrong place (Ectopic expression):** In some hemoglobinopathies, hemoglobin is produced in organs where it is not typically synthesized during a specific life stage, like in the liver.

Genetic Heterogeneity: Genetic heterogeneity refers to the presence of various genetic defects that result in similar or identical phenotypes. It can be classified into allelic heterogeneity (multiple disease-causing variants at the same locus influencing the disease) and locus heterogeneity (variants at different loci causing the same phenotype, such as in ciliopathies).

Clinical or Phenotypic Heterogeneity: This phenomenon occurs when the same variant in a gene leads to different clinical outcomes in different individuals. For instance, in ciliopathies, the same variant may cause Meckel syndrome in one family member and Joubert syndrome in another.

Modifier genes: Modifier genes influence the severity of a disease caused by variants in other genes, typically within the same pathway. For example, in beta thalassemia patients with a deletion in the alpha globulin gene, the clinical picture is improved because the increased alpha globulin content enhances oxygen-carrying capacity. Alpha thalassemia deletion, if present simultaneously, further alleviates the clinical symptoms.

Penetrance: Penetrance refers to the degree to which variants manifest as clinical phenotypes. Variants with low penetrance may not lead to clinical symptoms in some individuals, while fully penetrant variants cause a phenotype in all individuals. For example, a variant with 30% penetrance results in a clinical picture in 30% of affected cases. Low penetrance is common in dominant disorders.

Variable expressivity: Variants in genes with variable expressivity may not produce identical clinical phenotypes in affected individuals. Different family members may exhibit varying subsets of symptoms in disorders that typically cause multiple symptoms. This is common in dominant disorders but less common in some recessive disorders.

Digenic inheritance: In certain autosomal recessive disorders, two variants in distinct genes can produce the same phenotype, often involving genes that function together.

Mild variant: Some variant may have a less severe impact on gene function, leading to milder or different phenotypes.

Location of the variant: Proteins are composed of domains, and variants in different domains may result in more, less, or entirely different clinical manifestations. The LMNA gene provides examples of phenotype variations associated with variants in distinct domains.

https://www.omim.org/entry/150330?search=LMNA&highlight=lmna

Further Reading:

Genetic Modifiers and Rare Mendelian Disease

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7140819/

How can gene variants affect health and development?

- https://medlineplus.gov/genetics/understanding/mutationsanddisorders/mutationscausedisease/

Individual Genetic Heterogeneity

- https://www.mdpi.com/2073-4425/13/9/1626

RARE DISEASES AND DIAGNOSTIC APPROACH

The initial and most crucial step in diagnosing rare diseases involves a comprehensive patient history, examination of previous medical records, symptom evaluation, systematic examination, and the use of imaging techniques to visualize internal organs. In cases with multisystem involvement, various clinical assessments and laboratory tests are conducted across multiple specialties. It is essential to document all these evaluations, identify affected organs, and pinpoint any issues within these organs. If a preliminary clinical diagnosis is suspected, it should be supported by a few simple laboratory tests that can corroborate this initial diagnosis. However, when there is no preliminary diagnosis, it becomes necessary to explore databases like OMIM and review medical literature by documenting the patient's symptoms. It's important to note that diagnostic guidelines are generally less useful in this patient group, as they aim to diagnose most, but not all, patients and do so cost-effectively. In the case of rare diseases that are not frequently diagnosed, these approaches may not yield satisfactory results. One should be prepared for unexpected findings. When diagnosis remains elusive despite these approaches, it is advisable to consider using whole-genome sequencing or similar genomic screening tests, given that a significant proportion of rare diseases have a genetic basis.

In situations where the patient history suggests infection or exposure to toxic substances, the optimal approach involves conducting relevant infectious agent screenings and planning meta-genomic analyses for infections that cannot be detected through conventional methods. Toxicological screening protocols should be especially designed for patients where environmental factors are suspected to play a significant role. When multiple toxic factors are at play simultaneously, classic symptoms may not be evident, and in such cases, general screenings are often more appropriate.

If no conclusive results are obtained from these screening protocols, two possibilities should not be discounted: the likelihood of it being a different variant of a known disease or an entirely new, undefined disease. If an undefined disease is suspected, functional examinations should be carried out on the systems believed to be affected, guided by the data acquired and existing literature. In recent years, the use of RNA-Seq or transcriptome analysis has gained prominence in such diagnostic protocols. Additionally, metabolomics studies can assist in identifying the affected metabolic systems. Throughout all these stages, experience plays a vital role. Therefore, in patients with undiagnosed diseases, seeking input from experienced teams familiar with rare, undiagnosed, and undefined diseases is an essential approach to expedite the diagnostic process for the patient.

It is crucial to bear in mind that tests alone do not make a diagnosis; rather, a diagnosis is formed through the collective assessment of laboratory findings and clinical evaluations. Even apparently clear mutations, laboratory data, or similar findings may lead to incorrect conclusions. Confirming each result with clinical findings and other pertinent laboratory methods serves as the foundation of diagnosing rare diseases.

Further Reading

- https://genomemedicine.biomedcentral.com/articles/10.1186/s13073-022-01026-w

THE PATH FROM SYMPTOM TO RARE DISEASE DIAGNOSIS

Where are these rare diseases? Where are they hiding?

20.1. MIGRAINE EXAMPLE

Insufficient diagnosis is indeed a prevalent issue in the field of medical practice. Often, certain "symptoms" are used as clinical diagnoses, such as in the case of migraine. However, relying solely on symptom-based diagnoses can lead to various problems, including complications, ineffective treatments, and more. To address these issues, it's essential to continually ask "why" and delve deeper into the underlying causes of the condition:

1. Why did migraine develop in this particular individual?
2. Why does this person experience additional symptoms like eczema alongside migraines?
3. Why are migraine attacks more frequent than the typical pattern? Is there an underlying secondary triggering periodic disorder?
4. Why do some other family members have epilepsy, and could there be a genetic link?

The challenge lies in the absence of therapeutic guidelines for migraines based on their underlying etiology, despite existing literature on the subject. Etiological factors have been explored in migraine cases, and some of the most common etiologic factors include:

- **Single gene diseases:** Certain genetic variants or disorders have been associated with migraines, indicating a genetic basis for some cases.
- **Susceptibility genes:** Genetic variants that make individuals more prone to developing migraines have been identified.
- **Autoimmune diseases:** In some instances, migraines may be linked to autoimmune conditions, highlighting the role of the immune system.
- **Epilepsy-related genes:** There are overlaps between genes associated with epilepsy and those linked to migraines, suggesting potential shared genetic factors.
- **Mitochondrial migraine:** Dysfunction in the mitochondria, the energy-producing structures within cells, has been implicated in some migraine cases.

Understanding the underlying causes of migraines and their association with these etiologic factors is crucial for tailoring more effective treatments and management strategies for individuals affected by this condition. It underscores the need for personalized medicine and a deeper exploration of the factors contributing to a patient's specific migraine experiences.

Migraine often coexists with autoimmune diseases, highlighting the intricate interplay between the immune system and neurological conditions. Here are some noteworthy observations and considerations:

1. **Migraine and Autoimmune Diseases:** Migraine has been found to accompany various autoimmune diseases. For instance:
   - It has been reported with a frequency of 14-46% in patients with Multiple Sclerosis (MS).
   - In cases of Systemic Lupus Erythematosus (SLE), migraine has been reported with a frequency ranging from 17-52%.
2. **Gluten Enteropathy and Migraine:** Gluten enteropathy, also known as celiac disease, has a population prevalence of 1 in 100 (according to NHS data). Migraine is among the common neurological findings associated with this condition.
3. **Effect of Autoimmune and Autoinflammatory Therapies on Migraine:** Research on the impact of therapies for autoimmune and autoinflammatory disorders on migraine attacks is limited but critical. Some studies have reported successful results, indicating that treating the underlying autoimmune condition may alleviate migraines. However, there are also instances where the effect of these therapies on migraine is not well-established, necessitating further investigation.
4. **FMF and Epilepsy:** Familial Mediterranean Fever (FMF) has been identified as a triggering mechanism for frequent migraine attacks. However, the precise impact of FMF on the success of migraine

Disorders associated with migraine				
Migraine, familial hemiplegic, 1; FHM1	Migraine, familial hemiplegic, 2; FHM2	Monosodium glutamate sensitivity	Loeys-dietz syndrome 6; LDS6	Monocarboxylate transporter 1 deficiency; MCT1D
Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis; SANDO	Basal ganglia calcification, idiopathic, 4; IBGC4	Retinal dystrophy, optic nerve edema, splenomegaly, anhidrosis, and migraine headache syndrome; ROSAH	Mitochondrial DNA depletion syndrome 20 (MNGIE type); MTDPS20	Neuropathy, hereditary, with liability to pressure palsies; HNPP
Migraine, familial hemiplegic, 3; FHM3	Glut1 deficiency syndrome 2; GLUT1DS2	Amyloidosis, hereditary, transthyretin-related	Hemifacial atrophy, progressive; HFA	Tremor, hereditary essential, 1; ETM1
Headache associated with sexual activity; HAS	Brain small vessel disease 1 with or without ocular anomalies; BSVD1	Paroxysmal nonkinesigenic dyskinesia 1; PNKD1	Angiomatosis, diffuse corticomeningeal, of Divry and Van Bogaert	Hypomagnesemia, hypertension, and hypercholesterolemia, mitochondrial
Vasculopathy, retinal, with cerebral leukoencephalopathy and systemic manifestations; RVCLS	Cluster headache, familial	Phosphoglycerate kinase 1 deficiency	Anisocoria	Ataxia and polyneuropathy, adult-onset
Cerebral arteriopathy, autosomal dominant, with subcortical infarcts and leukoencephalopathy, type 1; CADASIL1	Alternating hemiplegia of childhood 1; AHC1	Episodic ataxia, type 6; EA6	Cerebral cavernous malformations; CCM	Mitochondrial complex I deficiency, mitochondrial type 1; MC1DM1
Cyclic vomiting syndrome; CVS	Mitochondrial DNA depletion syndrome 7 (hepatocerebral type); MTDPS7	Alternating hemiplegia of childhood 2; AHC2	Epilepsy, focal, with speech disorder and with or without impaired intellectual development; FESD	Mitochondrial complex v (ATP synthase) deficiency, mitochondrial type 1; MC5DM1
Advanced sleep phase syndrome, familial, 2; FASPS2	Dravet syndrome; DRVT	Combined oxidative phosphorylation deficiency 16; COXPD16	Xeroderma pigmentosum, complementation group f; XPF	Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; MELAS
Spinocerebellar ataxia 6; SCA6	Alpha-methylacyl-coa racemase deficiency; AMACRD	Basal ganglia calcification, idiopathic, 5; IBGC5	Epilepsy, familial adult myoclonic, 2; FAME2	Myoclonic epilepsy associated with ragged-red fibers; MERRF
Seizures, benign familial infantile, 2; BFIS2	Peroxisome biogenesis disorder 14b; PEX14B	Stormorken syndrome; STRMK	Fetal akinesia, respiratory insufficiency, microcephaly, polymicrogyria, and dysmorphic facies; FARIMPD	Telangiectasia, hereditary hemorrhagic, type 2; HHT2
Major depressive disorder; MDD	Telangiectasia, hereditary hemorrhagic, type 1; HHT1	Dystonia 9; DYT9	Loeys-Dietz syndrome 3; LDS3	Deafness, autosomal dominant 34, with or without inflammation; DFNA34
Episodic ataxia, type 2; EA2	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant 3; PEOA3	Basal ganglia calcification, idiopathic, 7, autosomal recessive; IBGC7	Hermansky-pudlak syndrome 6; HPS6	Developmental and epileptic encephalopathy 99; DEE99
Periodic fever, menstrual cycle-dependent	Chiari malformation type I	Sulfide:quinone oxidoreductase deficiency; SQORD	Episodic kinesigenic dyskinesia 1; EKD1	Spinocerebellar ataxia 50; SCA50
PHACE association	Meniere disease			

Genes associated with increased risk for migraine						
Location	Phenotype	Inheritance	Phenotype mapping key	Phenotype MIM number	Gene/Locus	Gene/Locus MIM number
1q31	{Migraine, familial hemiplegic, 4}	AD	2	607516	*MGR6*	607516
1q31	{Migraine with or without aura, susceptibility to, 6}	AD	2	607516	*MGR6*	607516
4q24	{Migraine with or without aura, susceptibility to, 1}	AD	2	157300	*MGR1*	157300
4q31.22-q31.23	{Migraine, resistance to}	AD	3	157300	*EDNRA*	131243
5q21	{Migraine, susceptibility to, 8}		2	609570	*MGR8*	609570
6p21.33	{Migraine without aura, susceptibility to}	AD	3	157300	*TNF*	191160
6p21.1-p12.2	{Migraine with or without aura, susceptibility to, 3}	AD	2	607498	*MGR3*	607498
6q25.1-q25.2	{Migraine, susceptibility to}	AD	3	157300	*ESR1*	133430
10q22-q23	{Migraine, with or without aura, susceptibility to, 12}	AD	2	611706	*MGR12*	611706
10q25.3	{Migraine, with or without aura, susceptibility to, 13}	AD	3	613656	*KCNK18*	613655
11q24	{Migraine with aura, susceptibility to, 9}		2	609670	*MGR9*	609670
14q21.2-q22.3	{Migraine without aura, susceptibility to, 4}	AD	2	607501	*MGR4*	607501
15q11.2-q12	{Migraine with aura, susceptibility to, 7}		2	609179	*MGR7*	609179
17p13.1	{Migraine with or without aura, susceptibility to, 10}	AD	2	610208	*MGR10*	610208
18q12.1	{Migraine with or without aura, susceptibility to, 11}	AD	2	610209	*MGR11*	610209
19p13	{Migraine with or without aura, susceptibility to, 5}	AD	2	607508	*MGR5*	607508
Xq	{Migraine, familial typical, susceptibility to, 2}	XL	2	300125	*MGR2*	300125

therapy remains unclear and warrants more comprehensive research.

In light of these observations, it is evident that additional studies and research are essential to better understand the relationships between autoimmune diseases, autoinflammatory conditions, and migraine. Investigating the effects of therapies for these common clinical problems on migraine management can provide valuable insights into improving treatment strategies and enhancing the quality of life for individuals affected by these conditions. This holistic approach to patient care underscores the importance of considering the broader medical context when addressing migraine and related disorders.

Some examples of related studies as follows

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8573865/
- https://www.frontiersin.org/articles/10.3389/fneur.2022.944791/full
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6545713/
- https://www.jofph.com/articles/10.11607/ofph.2079

Migraine is often associated with mitochondrial disorders, and it is important to recognize that when migraine is listed as a "diagnosis" in medical records, it can be misleading and inadequate, as it may not address the underlying mitochondrial condition. Various mitochondrial disorders have been linked to migraines, including:

1. Chronic Progressive External Ophthalmoplegia (CPEO)
2. Myoclonic Epilepsy with Ragged Red Fibers (MERRF)
3. Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes (MELAS)
4. Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE)
5. Kearns-Sayre Syndrome
6. Leber Hereditary Optic Neuropathy

These mitochondrial disorders can present with various symptoms, and migraine may be one of the manifestations. Therefore, it is crucial for healthcare professionals to conduct thorough assessments and consider the possibility of an underlying mitochondrial disorder when a patient presents with migraines or other symptoms. Proper diagnosis and management of the specific mitochondrial condition are essential for providing appropriate care and addressing the root cause of the patient's health issues.

Further Reading

- https://www.ncbi.nlm.nih.gov/books/NBK560787/
- Terrin, A., Bello, L., Valentino, M.L. et al. The relevance of migraine in the clinical spectrum of mitochondrial disorders. Sci Rep 12, 4222 (2022).
- https://www.nature.com/articles/s41598-022-08206-z

MULTISYSTEMIC AND MULTISYMPTOMATIC DISORDERS

In diseases that affect multiple organ systems or present with various findings within the same organ system, research methodologies often share similarities but differ in certain aspects. When multiple findings are observed within the same organ system, particularly if some involve anomalies, it raises the possibility of genetic disorders that influence embryological development. While an initial diagnosis can often be made by reviewing the OMIM database or relevant literature based on the observed findings, it's crucial to remain open to unexpected findings, focusing more on the affected organs and organ systems rather than individual symptoms.

For example, a condition that results in kidney anomalies might also lead to kidney agenesis, even if there haven't been reported cases of renal agenesis associated with that particular disease. Therefore, the possibility of such occurrences should not be overlooked.

In patients with findings in multiple organ systems, two scenarios should be considered: either all the findings are caused by the same disease, or some of the findings may be due to different diseases. Failure to consider both possibilities is one of the main reasons for missed diagnoses in this patient group. An important step is searching the OMIM Database and PubMed for all the findings together and in various combinations. When a diagnosis remains elusive through these means, searching for disorders that affect the named organs, rather than focusing solely on symptoms, may lead to a diagnosis. Genetic or environmental factors that impact the development of various organs or organ systems can result in such clinical presentations.

It's essential to recognize the possibility that some disease findings detected during these examinations may be secondary findings. For instance, decreased amniotic fluid during pregnancy is often a symptom of kidney disease, and in the same babies, decreased joint movements (arthrogryposis) can occur as a secondary finding due to restricted movement in the womb. Such secondary findings are referred to as "deformations" in dysmorphology. Additionally, certain anomalies and clinical findings may frequently co-occur, even if their genetic mechanisms or etiologies are not fully understood. This phenomenon is termed "association" in dysmorphology (e.g., VACTERL association). When the same etiological factor results in symptoms affecting more than one organ or organ system, it is referred to as a "syndrome."

In some diseases, unique facial findings or anomalies may be present. While these may be described in a similar manner, the combination of certain findings can result in a distinctive appearance. Therefore, especially in syndromic cases, it is essential to review photographs of previously reported patients with the same condition and compare them to the patient under investigation for a more accurate diagnosis. This approach can provide valuable insights into identifying and understanding specific syndromes and their associated features.

USE OF GENOMIC TECHNOLOGY

The role of genomic studies in medicine has become increasingly evident, showcasing their cost-effectiveness, particularly in cases of chronic diseases and critically ill patients. It is imperative for clinicians to adapt to this evolving landscape and integrate genomic studies, including comprehensive screening methods such as whole genome sequencing and whole exome sequencing, into their diagnostic and differential diagnosis processes. This approach is especially valuable in scenarios like undiagnosed diseases, challenges in differential diagnosis (e.g., isolated elevated liver enzymes), and conditions with multiple genetic etiological factors or unexpected clinical presentations.

Genomic tests should be considered earlier in diagnostic investigations, especially when there is a need to explore the underlying causes of a disease. Studies have demonstrated that genomic testing contributes significantly, ranging from 30% to 65%, to the diagnosis of critically ill patients, both in neonatal and adult intensive care settings. As a result, understanding the nuances of genomic techniques, selecting the appropriate tests, and collaborating with geneticists have become integral aspects of routine clinical practice.

Furthermore, in cases where clinical confirmation is not achieved through molecular methods, there remains the possibility of overlooking a similar disease, emphasizing the importance of a comprehensive genomic approach.

Additionally, genomic screening methods can be employed to investigate the potential presence of a secondary disease that could impact the clinical course of an individual with a chronic condition. Recent advancements, such as pharmacogenetic analysis derived from whole genome sequencing data, offer opportunities for more effective protection against drug-related side effects.

Pre-pregnancy screenings also play a critical role in healthcare, as they are effective tools for reducing the frequency of long-term diseases and minimizing the associated high treatment costs. Embracing genomics as an integral part of medical practice can enhance diagnostic accuracy, facilitate personalized treatment, and ultimately improve patient outcomes across various clinical scenarios.

Further Reading

- https://www.nature.com/articles/s41436-020-0929-8
- https://pubmed.ncbi.nlm.nih.gov/37106068/
- https://www.nature.com/articles/s41525-022-00326-9
- https://www.mcri.edu.au/research/research-areas/genomic-medicine
- https://www.mdpi.com/2227-9067/10/5/824

GENETIC LABORATORY TECHNIQUES

Genetic tests can be broadly classified into two categories: targeted tests and screening tests. These tests serve different purposes and are chosen based on the specific clinical context and objectives:

1. Targeted Tests:

- Targeted tests are utilized for preliminary clinical diagnoses or for conducting detailed examinations of specific genetic targets that have been identified through previous assessments.
- They are focused on assessing a particular gene or genes known to be associated with a specific condition or disorder.
- Targeted tests are typically employed when there is a clear clinical suspicion of a particular genetic disorder based on the patient's medical history, family history, or specific symptoms.
- Examples include single gene sequencing or testing for specific mutations associated with a particular disease.

2. Screening Tests:

- Screening tests are often preferred when the clinical diagnosis is not clear, or there are multiple potential genetic causes for the observed symptoms.
- They are used for broader assessments to identify potential genetic abnormalities or variations that may contribute to a patient's condition.
- These tests are valuable in cases where a wide range of genetic factors may be at play, and there is a need to narrow down the possibilities for further targeted testing.
- Examples include chromosomal analysis and whole exome sequencing.

Regarding chromosome analysis, specifically karyotype analysis:

- Karyotype analysis is a type of chromosomal screening test.
- It requires the examination of metaphase cells, which are cells that have progressed to a specific stage of mitosis.
- Living cells are necessary for this analysis, and it typically involves obtaining cells in active mitosis, where chromosomes are more condensed and easier to visualize.
- Karyotype analysis is useful for detecting chromosomal abnormalities, such as aneuploidy (e.g., Down syndrome) or structural chromosomal rearrangements.

In summary, the choice between targeted tests and screening tests depends on the clinical context and the specific goals of genetic testing. Targeted tests focus on known genetic factors, while screening tests provide a broader assessment to identify potential genetic abnormalities or variations, helping guide further diagnostic steps. Chromosome analysis, like karyotype analysis, is an important screening tool for detecting chromosomal abnormalities and structural variations in living cells.

Molecular Cytogenetics

Cytogenetic techniques play a crucial role in the field of genetics and genomics, helping to identify chromosomal abnormalities and variations. Here are some notable cytogenetic methods:

FISH (Fluorescent In Situ Hybridization):

- FISH involves the fluorescent staining of specific target regions on chromosomes.
- These targeted regions are visualized under a microscope, either on metaphase chromosomes (during cell division) or interphase nuclei (when the cell is not actively dividing).
- FISH is valuable for detecting chromosomal abnormalities and specific genetic markers.

Molecular Karyotyping:

- Molecular karyotyping is a broader term that encompasses various techniques used to analyze the structure and number of chromosomes at the molecular level.
- It can include methods like FISH, array-CGH, and SNP arrays to assess chromosomal abnormalities and variations.

Karyotypes with different resolutions

Array-CGH (Microarray Comparative Genomic Hybridization):

- Array-CGH is a high-resolution technique used to detect copy number variants in chromosome regions.
- It involves placing DNA regions of interest on a solid surface and comparing the DNA of a healthy individual with that of the patient being investigated.
- Deletions or duplications in specific chromosome regions can be identified based on the relative hybridization intensity.
- Different colors are often used to label DNA fragments for quantitative evaluation and comparison of each chromosome region.

SNP-Array (Single Nucleotide Polymorphism Array):

- SNP arrays are used to examine single nucleotide variations across the genome.
- Unlike array-CGH, SNP arrays do not involve a competition between two individuals' DNA; instead, they assess the hybridization of DNA molecules from the patient to DNA molecules bound to a solid surface.
- SNP arrays are particularly useful for detecting single nucleotide polymorphisms and copy number variations in specific genomic regions.

The primary goal of array-based methods, such as array-CGH and SNP arrays, is to detect and characterize copy number changes in chromosome regions. These methods provide high-resolution data that allow for precise delineation of the boundaries of these changes. Pathogenicity assessments of the gene regions affected by copy number alterations are typically conducted using scientific literature and genetic databases.

Note that the resolution of these techniques can vary based on factors like probe and kit selection, and they are instrumental in diagnosing various genetic disorders and providing valuable insights into the genomic landscape of patients.

Southern Blot Analysis

Southern blot analysis is a molecular biology technique primarily used for the detection and quantification of specific DNA sequences within a DNA sample. It was first developed by Edwin Southern in the 1970s and has been a valuable tool in genetic research. While its applications have evolved over time, its most prevalent contemporary use is in the context of triple nucleotide repeat disorders.

Here's how Southern blot analysis works:

1. **DNA Digestion:** The DNA sample of interest is first digested with restriction enzymes, which cleave the DNA at specific recognition sites, resulting in DNA fragments of varying sizes.
2. **Gel Electrophoresis:** The digested DNA fragments are then separated by size through gel electrophoresis. Smaller fragments move more quickly through the gel, while larger fragments migrate more slowly.
3. **Denaturation:** The DNA fragments within the gel are denatured, causing the double-stranded DNA to become single-stranded DNA.
4. **Transfer to Membrane:** After electrophoresis, the DNA fragments are transferred from the gel onto

Method	Resolution	Limitations
Karyotyping-chromosome analysis	5-10 megabase (Mb)	• Chromosomal screening method. • Can't detect nucleotide variants and the variants below the resolution limits.
FISH	About 100 kilobase (Kb)	• Targeted analysis. • Limited to the binding site of the probs. • Can't detect nucleotide variants and the variants below the resolution limits.
Array based methods	20-700 kilobase (Kb)	• CNV screening method. • Can't detect balanced chromosomal variants, low-level mosaicism, and the variants below the resolution limits. • SNP arrays can be used for detection of nucleotide variants but not safe enough for a single variant.
CNV detection by whole exome sequencing	100 bp in exonic regions but can be detect till one nucleotide up to resolution in the region.	• CNV screening method. • Can't detect balanced chromosomal variants and low-level mosaicism.

https://drpress.org/ojs/index.php/HSET/article/view/1789/1709

a solid membrane, typically made of nitrocellulose or nylon. This transfer step is known as "blotting."

5. **Hybridization:** The membrane is then exposed to a specific DNA probe. This probe is a single-stranded DNA or RNA molecule that is complementary to the target DNA sequence of interest. The probe binds specifically to its complementary sequence on the membrane.
6. **Detection:** The presence of the probe on the membrane is detected using various methods, including radioactive or fluorescent labeling of the probe. This allows researchers to visualize the location and quantity of the target DNA sequence.

As mentioned, Southern blot analysis has found contemporary utility in the field of triple nucleotide repeat disorders, such as Huntington's disease and Fragile X syndrome. These disorders are characterized by the expansion of repetitive DNA sequences within specific genes. Southern blots can be used to determine the size of the repeat expansions, which is crucial for diagnosing these disorders and understanding their severity.

While Southern blot analysis was once a widely used method for various applications in molecular biology, it has become less commonly employed for routine genetic testing due to the availability of more practical and high-throughput techniques such as PCR (polymerase chain reaction), DNA sequencing, and next-generation sequencing. These newer methods offer advantages in terms of speed, sensitivity, and automation, making them more suitable for many research and diagnostic purposes.

- https://www.genome.gov/genetics-glossary/Southern-Blot

PCR (Polymerase Chain Reaction):

- Used to amplify specific DNA regions for further analysis.
- A preliminary preparation technique for obtaining sufficient DNA.

Real-Time PCR:

- Measures the rate of DNA amplification in real-time, often using fluorescent markers.
- Widely used for quantitative studies, mutation detection, gene expression analysis, and rapid microbiological diagnosis.

STR (Short Tandem Repeat) Analysis (Microsatellite-Based Analysis):

- Analyzes microsatellite regions in the human genome.
- Used to detect instability in some cancers, investigate chromosomal numerical disorders during pregnancy, and commonly applied in forensic DNA identification.

MLPA (Multiplex Ligation-dependent Probe Amplification):

- Used for targeted copy number analysis and investigation of methylation disorders.
- Frequently employed with commercial probes.
- Applied in the diagnosis of diseases related to methylation mechanisms and those characterized by copy number changes.

DNA Sequencing Analysis:

- Techniques to determine the nucleotide sequence in gene regions.
- Sanger sequencing, followed by next-generation sequencing (NGS), is widely used.
- Used in panel gene analysis, clinical exome sequencing, exome sequencing, and whole genome sequencing.
- Can also be used for copy number variation (CNV) analysis.

Whole Exome Sequencing:

- Focuses on sequencing the coding regions of genes (exomes).
- Valuable for diagnosing patients with uncertain diagnoses or similar clinical presentations caused by different diseases.
- Increasingly used for prenatal diagnosis due to CNV analysis capabilities.

Whole Genome Sequencing:

- Sequences almost the entire human genome.
- Rapidly decreasing in cost, becoming widely used.
- Provides opportunities for mitochondrial DNA analysis and CNV analysis.

Transcriptome Analysis (RNA-Seq):

- Sequences and quantifies mRNA in a sample.
- Identifies splice variants, fusion mutations, and gene expression changes.
- Used in undiagnosed cases, identification of fusion mutations, and studying malignant diseases like leukemia and sarcoma.
- Complements whole genome sequencing data and aids in understanding disease mechanisms.

Further Reading

- https://www.ncbi.nlm.nih.gov/books/NBK132150/
- https://www.youtube.com/watch?v=BH8Y5qKgNOE
- https://www.ncbi.nlm.nih.gov/books/NBK560712/

USE OF DATABASES - DIAGNOSIS AND TEST DETERMINATION

Utilizing databases to efficiently manage the ever-expanding wealth of genetic data is a crucial aspect of routine diagnostic research. Consequently, it is imperative for medical practitioners to explore and make use of these databases. Comprehensive instructional videos on how to navigate these databases are readily available on platforms like YouTube. Numerous databases are at our disposal, and below is a list of the most frequently utilized ones.

Online Mendelian Inheritance in Man (OMIM)

A database of human genes and genetic disorders. NCBI maintains current content and continues to support its searching and integration with other NCBI databases. However, OMIM now has a new home at omim.org, and users are directed to this site for full record displays.

- https://www.omim.org/help/faq
- https://www.youtube.com/watch?v=_uCdmVYcQ_Q
- https://www.youtube.com/watch?v=dFisrzPCl8M

GeneReviews

A compilation of disease descriptions authored by experts and reviewed by peers is available on the NCBI Bookshelf. These descriptions focus on applying genetic testing for diagnosing, managing, and providing genetic counseling to patients and families dealing with particular inherited conditions.

GeneReviews, an international resource designed for busy clinicians, offers clinically relevant and actionable information about inherited conditions. This information is presented in a standardized journal-style format, encompassing aspects of diagnosis, management, and genetic counseling for patients and their families. Each chapter within GeneReviews is authored by one or more experts specializing in the specific condition or disease. These chapters undergo a thorough editing and peer review process before being published online.

ClinVar

This resource serves as a publicly accessible database that records and tracks reported connections between human genetic variations and their associated health outcomes, substantiated by supporting evidence. It includes links to relevant information from various sources such as the NIH Genetic Testing Registry (GTR), MedGen, Gene, OMIM, PubMed, and more, which can be accessed through hyperlinks within the records.

- https://www.ncbi.nlm.nih.gov/clinvar/

Orphanet

Orphanet is portal of rare diseases.

- https://www.orpha.net/consor/cgi-bin/index.php

PharmGKB® (a pharmacogenomics knowledge resource)

Clinical information including dosing guidelines and drug labels, potentially clinically actionable gene-drug associations and genotype-phenotype relationships. PharmGKB disseminates knowledge about the impact of human genetic variation on drug responses. HHS and is financially supported by NIH/NIGMS.

- https://www.ncbi.nlm.nih.gov/guide/all/

Further Reading

- https://www.ncbi.nlm.nih.gov/guide/all/
- https://www.nature.com/scitable/topicpage/genomic-data-resources-challenges-and-promises-743721/

COMMON MODIFYING FACTORS

25.1. THROMBOPHILIA

Thrombophilia comprises a group of predisposing disorders linked to various forms of blood clotting issues, including recurrent pregnancy loss, and is relatively common in society. The debate over whether testing for these conditions is beneficial, particularly in cases of pregnancy loss, has persisted in various publications. These alterations should be regarded as risk factors. In essence, thrombosis may occur when several risk factors converge or when lifestyle and environmental factors, though individually benign, are introduced. The likelihood of thrombosis increases when individuals with positive genetic risk factors are combined with factors such as physical inactivity, obesity, dehydration, and oral contraceptive use. Notably, variants like factor V Leiden and prothrombin 20210 play a significant role in various thrombosis-related diseases, while conflicting publications exist for other factors. There is a pressing need for comprehensive studies that incorporate multiple factors. In counseling patients, the elimination of easily treatable factors, such as inactivity, inadequate fluid intake, and oral contraceptive use, proves to be one of the most effective treatment approaches for multifactorial diseases. Thrombosis emerged as a significant complication during the COVID-19 pandemic, and scientific research indicates the influence of various thrombosis predispositions. The absence of thrombosis in all COVID-19-infected individuals underscores the significant role played by personal predispositions. It functions as a modifying factor, adding to the risk of death in individuals already experiencing a severe clinical profile due to COVID-19.

25.2. AUTOIMMUNE DISEASES

Autoimmune diseases are linked to a variety of skin conditions, rheumatic disorders, thyroid problems, joint issues, pregnancy complications, infertility, hepatitis, and numerous neurological ailments, primarily due to the inflammation they trigger in bodily tissues. Among the most prevalent autoimmune-related conditions are inflammatory bowel diseases. These diseases, commonly found in society and varying in severity, tend to worsen when multiple causative factors converge. In clinical practice, it's evident that very few patients neatly fit textbook definitions and often exhibit symptoms that extend beyond the confines of a specific disorder. Complaints tend to escalate with age due to cumulative tissue damage over time. Addressing even a few underlying factors, as discussed in multifactorial diseases, can significantly enhance a patient's quality of life.

One crucial aspect of these diseases is their capacity to alter the clinical course of many conditions that medical professionals encounter regularly. Gluten sensitivity is prevalent in society and can exacerbate various rheumatic ailments due to intestinal absorption issues and tissue inflammation. It may also intensify the severity of inflammatory bowel diseases. With its potential to induce neurological symptoms like gluten ataxia, restless legs syndrome, and polyneuropathies, it can mimic or accelerate the onset of similar neurological disorders, significantly impacting their severity and trajectory, particularly when coexisting with common autoimmune and auto-inflammatory diseases. Therefore, healthcare providers should inquire about this group of diseases in every outpatient clinic evaluation.

25.3. VARIANTS IN THE SAME PATHWAY

In our everyday medical practice, we frequently come across situations where the clinical presentation varies in severity among patients. Additional factors can contribute to this variability. An illustrative example of this is Spinal Muscular Atrophy (SMA) Disease. The primary causative factor in this disorder is homozygous or compound heterozygous mutations in the SMN1 gene. Another factor influencing the disease's course is the SMN2 gene, which closely resembles the SMN1 gene with only a few nucleotide differences (high homology). An increased copy number of the SMN2 gene can lead to a milder disease course, resulting in type 2 or type 3 clinical presentations. Additionally, certain genetic variants within the SMN2 gene have been identified to ameliorate the disease's progression. Similarly, the impact of genes operating within the same pathway or encoding proteins that collaborate is crucial in shaping the overall clinical profile of the disease. This aligns with the concept of personalized medicine, with pathway diseases being a central focus. Conversely, in some diseases, heterozygous changes in multiple genes within the same pathway can produce identical clinical features. The interaction of two genes may play a role in inheritance (Digenic inheritance), observed in conditions like ciliopathies and ciliary dyskinesias. Identifying and understanding these modifying factors is becoming increasingly important in disease treatment. Notably, a drug called Nusinersen, which boosts the SMN2 gene production and yields a product resembling that of the SMN1 gene, has proven effective in treating SMA Disease.

25.4. MODIFYING EFFECTS OF IMMUNODEFICIENCIES

During the COVID-19 pandemic, it became evident that some patients experience a significantly more severe clinical course than others. One crucial factor identified in these cases is the presence of immune system disorders. Failure to recognize such underlying conditions has posed significant challenges in devising effective treatment strategies for the disease. In some instances, the detection of a severe infection during adulthood may be the initial symptom of these disorders. Immune system diseases can have a far-reaching impact on various medical issues, including wound healing, cancer management, and susceptibility to cancer development. Moreover, individuals with immune system disorders are more susceptible to nosocomial infections during their hospital stays.

Conditions like common variable immunodeficiency, immune dysregulation syndromes manifesting in adulthood, and immunodeficiencies that render individuals susceptible to specific microorganisms or multiple microorganisms are more prone to being overlooked, potentially altering the clinical presentation observed at the time of diagnosis. Additionally, these disorders can manifest as symptoms like recurrent pregnancy loss, infertility, and rheumatic diseases. Some patients may even develop certain malignancies like lymphoma at a later stage in life. Therefore, it is prudent to consider the possibility of these conditions, as early diagnosis can help prevent such situations.

25.5. GENETICS IN TRAUMAS

Why doesn't everyone experience a brain hemorrhage? Why doesn't the same trauma result in fatalities for everyone? Why do some people encounter side effects that others don't? Why doesn't every individual develop acute renal failure following trauma? Could the patient's microhematuria be due to being a carrier of Alport syndrome or kidney trauma? Is the patient's aggressiveness related to a potential uric acid metabolism disorder?

Genetic and immunological factors play a significant role in acute and subacute emergency cases. Some important observations include:

- Fracture risk from simple trauma increases with mutations in the COL1A1 and COL1A2 genes and certain polymorphisms.
- Carriers of VWF deficiency in the population, which affects menstrual periods and elevates the risk of brain hemorrhage after head trauma, are relatively common (1 in 200).
- Patients with muscular and neurological disorders have a higher incidence of accidents.
- Muscle diseases caused by pathogenic variants of the RYR1 gene may lead to malignant hyperthermia during anesthesia.
- Trauma can exacerbate muscle diseases that progress with rhabdomyolysis.
- Severe tissue damage can accelerate inflammatory processes in autoimmune diseases.
- Patients with thrombophilia are at an increased risk of thrombosis when tissue thromboplastin is released after trauma.

Some individuals have an elevated risk of inflammation and related complications after trauma. Autoinflammatory and autoimmune diseases can result in complex situations when patients present with multiple conditions simultaneously. For example, the Mediterranean region has a high frequency of heterozygous FMF variants (1 in 20) and a general frequency of autoinflammatory diseases (1 in 200).

In the emergency room, healthcare providers strive to identify a person's chronic diseases and risk factors after traffic accidents or major traumas. Many of these conditions are rare and genetic, making early identification a significant advantage. Research has shown that some genetic diseases influence post-traumatic tissue damage, inflammation responses, bleeding, wound healing, and the development of post-traumatic renal failure. While some of these conditions can be detected before a trauma occurs, others may not exhibit symptoms until then. The concept of population screening for these diseases, which will play a crucial role in reducing and managing risks, is expected to be a significant topic in healthcare decisions in the coming years.

25.6. COEXISTENCE OF TWO DISORDERS

Multifactorial diseases are commonly encountered in society, and the simultaneous presence of two prevalent conditions can result in patients exhibiting a blend of clinical symptoms, making diagnosis challenging. Additionally, the coexistence of two distinct single-gene disorders is a frequent clinical issue. This is especially notable when both disorders affect the same organ system, and it is a prevalent concern in regions with a high rate of consanguinity.

Further Reading

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7140819/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4448705/
- https://www.nature.com/scitable/topicpage/same-genetic-mutation-different-genetic-disease-phenotype-938/

25.7. INFECTIOUS DISEASES

The increased opportunities for travel, both for tourism and commercial purposes, have brought about the possibility of encountering infections or parasitic diseases that are rarely seen in one's home country. Diagnosing such diseases can be challenging as experience plays a crucial role, and they might be easily overlooked. Even though there are guidelines for taking precautions before specific trips, healthcare professionals may face difficulties in diagnosing these conditions. Furthermore, certain infectious diseases, like Lyme disease, which can mimic other illnesses, pose a diagnostic challenge, and available diagnostic methods may not always yield 100% accuracy.

Another significant factor in infectious diseases is the individual's immune system. Problems with the immune system or the presence of chronic systemic diseases can substantially alter the disease's course. Individuals who are particularly sensitive to specific microorganisms may experience clinical presentations that deviate from the expected norm. For instance, individuals with pathogenic mutations in the RANBP2 gene have a higher risk of developing encephalopathy following a sudden febrile illness and may face life-threatening complications. While infectious agents such as influenza A, influenza B, Parainfluenza, and Mycoplasma pneumonia are known culprits, a significant portion of patients may have unidentified pathogens causing their illnesses.

Infections like tuberculosis don't affect everyone, even among individuals living in the same household. In such cases, it's important to identify individual-specific immune factors and screen other at-risk individuals within the same family. Typically, those with predispositions may experience more severe forms of the illnesses.

25.8. TOXICOLOGY

Toxins and the field of toxicology present a subject that requires examination from two distinct perspectives. While acute cases of exposure to environmental toxins are generally easier to diagnose, chronic toxin exposure often carries a higher risk of being overlooked. Clinical manifestations resulting from toxins have the potential to mimic numerous other diseases, including rare conditions. Therefore, information about an individual's living environment, occupation, contact with toxins, hobbies, and proximity to workplaces (even if they don't work there) can be invaluable when diagnosing diseases that defy clinical diagnosis. Particularly, any changes in these factors that occurred before the onset of the illness should prompt further exploration into toxicological aspects. The importance of screening for toxic factors should not be underestimated in cases where patients cannot be diagnosed through conventional means.

25.9. TERATOLOGY

During pregnancies, when various ultrasound anomalies or pregnancy-related issues arise, considerations often turn towards factors such as the TORCH group of infections, radiation exposure, and medication usage. However, this line of questioning can sometimes be overlooked, and assessing these factors can be exceptionally challenging. Evaluating the impact of radiation exposure and medication use during pregnancy is particularly difficult because there are no definitive tests that can prove their effects. Medications that come into contact with the body during pregnancy fall into different subclasses. While it's possible to assess the potential risks associated with a drug class, the effects of medications depend on various factors, including the timing of exposure during pregnancy, the developmental stage of the embryo affected, the dosage, and individual pharmacokinetic factors. Consequently, even drugs with a high potential for risk may be used in certain cases. Some studies on adverse effects rates can be found in databases for many drugs. Additionally, data on numerous drugs have been gathered through animal experiments and observations related to drug use during pregnancy by people. Conducting experiments involving pregnant women is not feasible, which makes collecting human data challenging.

Given the scarcity of data in this field, it's crucial to consult with experienced professionals before making any decisions. Nowadays, communication is more accessible, and if personal experience falls short when dealing with such patients, it's essential to reach out to experts and seek their opinions to make informed decisions. Moreover, when deciding whether to continue or terminate a pregnancy, it's imperative to provide comprehensive information to both the mother and father, making them aware that reaching a definitive decision in many cases, except for very few patients, can be extremely challenging.

25.10. PHENOCOPY

The term "phenocopy" refers to a situation where a disease, caused by a non-genetic mechanism, mimics the clinical characteristics of a genetic disease. A common example is when a woman in a family with a history of familial breast cancer develops sporadic breast cancer, even though she doesn't carry the same genetic mutation that runs in her family.

Further Reading

- https://www.share4rare.org/news/toxic-agents-radiation-and-microbes-non-genetic-factors-can-cause-rare-disease
- https://www.infectioncontroltoday.com/view/rare-infectious-diseases-a-tutorial

EMERGENCIES AND RARE DISORDERS

Emergency departments encounter rare diseases through various avenues. Individuals who have already received a diagnosis of a rare disease may find themselves in the emergency room for various reasons. In such situations, emergency room physicians may hesitate to administer conventional treatments due to their limited knowledge and experience with these rare conditions. This uncertainty can contribute to anxiety about potentially making incorrect decisions. One way to address this is by seeking consultation with a specialist in the relevant field. However, this may not always be feasible, which is why Orphanet publishes emergency guides. The number of these guides is steadily increasing, providing valuable resources for emergency care.

Another critical scenario involves patients who were previously undiagnosed or diagnosed but unconscious when brought to the emergency room. While it may not be possible to diagnose many rare diseases in the emergency setting, healthcare providers should remain vigilant for atypical clinical features in every case. Indications that should raise concern include excessive bleeding or tissue damage following minor trauma, inadequate response to standard drug treatment protocols, and the development of side effects or complications during treatments. Consulting clinical databases like OMIM can be a helpful resource in such situations. However, as previously discussed, population screening for rare disease possibilities remains the most effective solution for emergency services. Additionally, educating individuals around previously diagnosed patients and providing them with information about the disease can significantly aid in achieving accurate diagnoses and appropriate treatment in the emergency department.

Further Reading

- https://www.orpha.net/consor/cgi-bin/Disease_Emergency.php?lng=EN

PRINCIPLES OF REQUESTING GENETIC TESTING

One of the most critical aspects for physicians to understand about genetic tests is the limited information available for many patients regarding the genetic variants discovered. When assessing these variants, detailed clinical information is paramount. Preliminary diagnoses and comprehensive clinical summaries, if available, significantly influence the pathogenicity classification of the identified variant. The ACMG criteria emphasize the role of clinical compatibility in variant classification. Given that many diseases can present with similar conditions, clinical details play a crucial role in variant assessment. Another important consideration is the age of onset. While the current clinical presentation may resemble various diseases, the age at which symptoms first appear and the gradual development of these symptoms are essential in investigating and evaluating the genetic variant obtained. In cases where multiple diseases occur simultaneously, such as contiguous gene syndromes, it's necessary to evaluate whether some of the patient's symptoms are attributed to one disease and others to a different condition. In such situations, collaboration between the geneticist evaluating the laboratory results and the physician overseeing the patient is vital. It's also important to explore whether there is a second variant in addition to the genetic variant that fails to explain all of the patient's symptoms. Furthermore, when a disease appears milder than expected, investigating modifying factors is essential for planning the patient's treatment. In summary, providing detailed information and contact information for the physician following the patient is crucial for geneticists in making their evaluations through forms prepared by geneticists.

Another significant consideration in selecting genetic tests is choosing the appropriate test for a specific disease. Some diseases are best diagnosed using particular genetic tests. For example, Duchenne Muscular Dystrophy is most commonly caused by deletions, and combined with duplications, copy number-based analysis can diagnose nearly sixty percent of patients. In contrast, many other diseases typically start with sequence analysis, but in this disease, prioritizing copy number analysis is essential. In some instances, tests not typically included in routine planning may be useful for diagnosis in special cases. If a variant cannot be found in a disease that is usually diagnosed almost 100% by sequence analysis methods, investigating structural chromosomal changes that may disrupt that gene due to chromosomal breaks can unexpectedly lead to a diagnosis of a single gene disease through chromosome analysis. Particularly in cancer and leukemia cases, detailed communication about treatment challenges is essential in test planning. Therefore, clinician collaboration with the laboratory during test planning and the subsequent discussions and decision-making processes contribute to accurate planning.

Pre- and post-test counseling is of utmost importance in genetic testing. Genetic tests performed for chronic or life-threatening diseases often place families under stress. Providing inaccurate information to family members can heighten their stress levels, undermine their trust in the test, lead to unexpected reactions, and result in erroneous decisions. Hence, it is crucial to work in harmony and provide support throughout the counseling process before and after the test, particularly for rare diseases. Additionally, patients and their relatives should be informed about the reliability limits of the tests. Both insufficient and excessive information can increase family stress levels, so providing tailored information while considering their socio-cultural context is vital.

CANCER GENETICS

The landscape of cancer-related diagnosis, monitoring, and treatment planning has undergone significant transformations due to advancements in genetic technologies. Traditionally, the diagnosis and classification of cancer relied on clinical symptoms, radiological findings, and pathological examinations. However, recent years have witnessed the emergence of new approaches that integrate genetic testing with these traditional methods. These changes initially began in hematologic cancers and gradually extended to solid tissue tumors. With time, the use of more comprehensive genetic test panels has become increasingly common, allowing for a broader understanding of the disease. A key driving force behind this shift is the enhancement of cancer treatment success through personalized medicine practices.

Genetic tests are utilized in various aspects of cancer care, including:

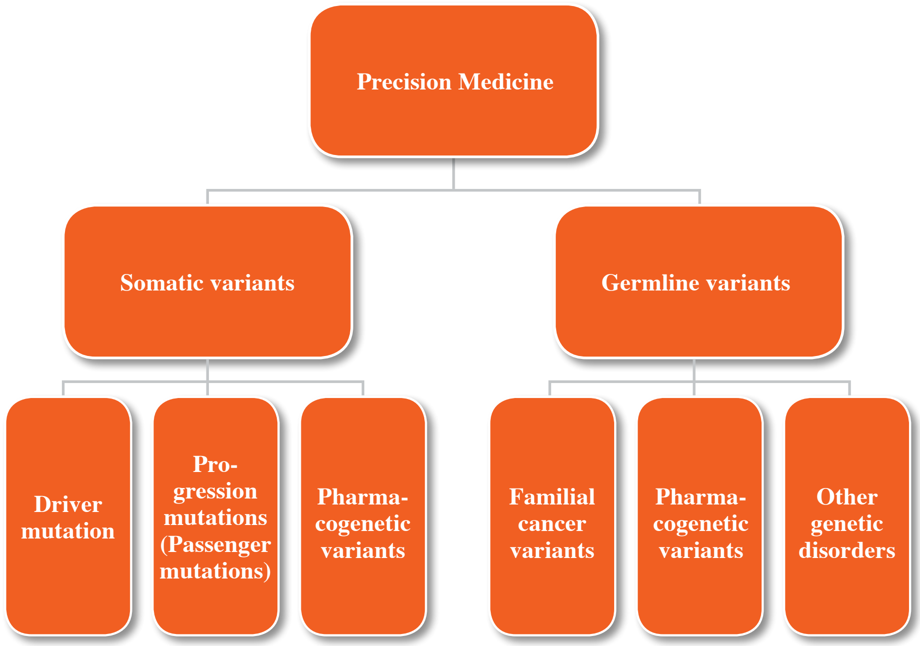
1. **Differential Diagnosis:** Distinguishing between different types of cancer based on genetic markers.
2. **Prognosis Assessment:** Predicting the likely course and outcome of the disease.
3. **Sensitivity and Resistance Detection:** Identifying whether a patient is likely to respond to specific therapies or develop resistance to them.
4. **Follow-up:** Monitoring the progression of the disease and treatment effectiveness.

At the core of cancer development lies DNA damage, with the most common mechanisms of DNA damage including:

- Mechanisms that regulate or reduce the rate of cell reproduction.
- Apoptosis, a programmed cell death process.
- DNA repair mechanisms, responsible for fixing damaged DNA.

Further Reading

- https://www.cancer.gov/publications/pdq/information-summaries/genetics/overview-hp-pdq
- https://www.ncbi.nlm.nih.gov/books/NBK65761/



28.1. SPORADIC CANCERS

Cancer can be categorized into two groups: familial cancers and sporadic cancers. Even in familial cancers, somatic mutations can occur, as cancer is a rapidly progressing disease that can disable certain control mechanisms, leading to the emergence of new genetic mutations. These mutations may drive the disease to advance to the next stage or cause resistance to treatment. Over time, a drug-resistant cell clone may become dominant, even if the initial treatment was effective against the original mutation.

Before delving into cancer genetics, some important definitions are necessary. A "driver mutation," also known as a triggering mutation, initiates the disease. Typically, an oncogene is activated, or a tumor suppressor gene loses function, leading to rapid cell division, increased metastatic potential, and resistance to treatment over time. Identifying the driver mutation is crucial for understanding the cause of any changes affecting treatment.

The concept of driver mutations is also vital for classifying the disease. For example, a mutation causing breast cancer may also contribute to stomach cancer in some patients. If a drug is effective against this driver variant, it may also be useful for treating other organ cancers associated with this mutation. Passenger mutations, which occur alongside the driver mutation but generally affect fewer cells, play a secondary role.

The selection of cancer treatment depends on various factors, including driver mutations and resistance mutations. Some variants are drug-specific, while others, like Multidrug Resistance Protein 1 (MDR1) gene variants (ABCB1 gene), are non-specific and impact drug resistance.

While treating cancers localized to a specific part of the body, it's essential not to overlook genetic characteristics present throughout the body. Familial cancers, accounting for about 10% of all cancers, are worth considering. With the advent of cheaper and more accessible genetic screening tests, it's debatable whether these tests should be conducted for all cancer patients, especially in certain types like breast cancer, renal clear cell carcinoma, and rare cancers.

In addition to genetic variants, there are genetic traits that influence an individual's drug metabolism rate. These genes, along with any undiagnosed chronic conditions, are critical for determining drug treatments. Investigating the potential for chronic diseases through genetic screening when treatment begins is advisable.

In summary, personalized medicine aims to apply the fundamental principles of medicine to predict and address potential issues before patients experience problems. Germline variants lead to familial hereditary cancer, while somatic variants cause sporadic cancer. Interestingly, both types of cancer often result from mutations in the same genes, such as BRCA1-2, MSH2, APC, TP53, etc.

Proto-oncogenes are genes responsible for regulating cell division as needed, while tumor suppressor genes halt cell division when it's no longer required. Mutations in these genes can either increase (gain-of-function variants) or decrease (loss-of-function variants) their normal function, contributing to cancer development.

In familial cancers, variants in tumor suppressor genes can lead to the development of cancer when a second variant occurs in one of the cells, a concept known as the "second hit hypothesis." In other words, while the inheritance of these diseases is dominant, at the tissue level, it behaves recessively. Therefore, both germline and somatic variants are required for cancer development, with the first variant inherited from the parents and the second often triggered by environmental factors.

The "Tumor Agnostic Approach" prioritizes the biological mechanisms of cancer development over the tumor's location or histopathological characteristics. This approach is particularly significant in planning treatment for rare cancers and cancers of unknown origin and is likely to gain prominence in cancer diagnosis and treatment in the future.

28.2. RED FLAGS OF FAMILIAL CANCERS

Several factors should raise suspicion of a genetic predisposition to cancer, including:

1. **Early Onset:** The development of cancer at a young age, such as breast cancer before age 40 or early menopause.
2. **Positive Family History:** A family history of the same type of cancer can indicate a genetic link.
3. **Aggregated Family History:** The occurrence of different types of cancer within a family, like osteosarcoma, brain, breast, leukemia, and adreno-

cortical tumors in Li-Fraumeni syndrome linked to pathogenic variants of the TP53 gene.

4. **Bilateral or Multifocal Tumors:** The development of tumors in both sides or multiple locations.
5. **Gender-Incompatible and Rare Cancers:** Uncommon cancers, such as breast cancer in men, may suggest a genetic factor.
6. **Additional Non-Cancerous Findings:** The presence of non-cancerous symptoms or conditions indicative of a specific syndrome, like macrocephaly, oral papilloma, scoliosis, and mental retardation in autosomal dominant Cowden disease associated with pathogenic variants of the PTEN gene.

28.3. OVERVIEW OF CANCER TESTS

We can categorize the tests planned for cancer patients into two main types: somatic variant tests and germline variant tests.

Somatic variant tests involve analyzing DNA from tumor tissue or liquid biopsy. Tumor tissue can be obtained from the tumor itself, lymph nodes, or metastatic sites. In some cases where surgical procedures are not feasible or the cancer mass is small, liquid biopsy can be used. Liquid biopsy examines DNA released from apoptotic cancer cells into the plasma, primarily detecting driver variants. It can also be employed during treatment follow-up to identify new passenger mutations if repeated biopsies are not possible. However, liquid biopsy may not yield results in all cancer types or situations.

Germline testing for familial cancers typically employs methods like exome sequencing or whole-genome sequencing. Whole-genome sequencing offers advantages, including germline pharmacogenetic analysis. Additionally, targeted panels for pharmacogenetic analysis can complement familial cancer gene panel testing.

Next-generation sequencing (NGS) has gained prominence for initial cancer diagnosis, as it allows cost-effective analysis of multiple genes from tumor tissue and liquid biopsy. If a driver variant is identified in initial screening, more precise methods like real-time PCR or digital PCR may be utilized for minimal residual disease monitoring during treatment follow-up. However, these tests require specific designs for individual or grouped mutations, and NGS methods with higher resolution can also be considered.

RNA-based analysis methods are preferred for diseases like leukemia and sarcoma, where fusion variants are common. Real-time PCR and FISH are suitable for a small number of fusion studies, while RNAseq should be considered for a larger scale. RNA-based methods can also monitor disease progression over time by measuring gene expression levels.

Immunohistochemistry, performed on pathology preparations, assesses the expression levels of gene products and contributes to evaluating overexpression or low expression.

After the initial molecular analysis, regular patient follow-up should include re-biopsy or liquid biopsy in case of drug resistance development.

28.4. RARE CANCERS

Approximately 5.1 million individuals in the European Union (EU) and the United Kingdom are afflicted by rare cancers, accounting for roughly 25% of all cancer cases. Rare cancers often demand unique and tailored approaches in their diagnosis and management. They tend to manifest at unexpected ages, necessitating specialized clinical and organizational strategies.

Some distinctive challenges posed by rare cancers include:

- Delayed or incorrect diagnosis.
- Limited access to suitable treatments and specialized expertise.
- Commercial impracticality in developing novel therapies.
- Challenges in conducting sufficiently powered clinical studies.
- Limited availability of tissue banks for research purposes.

References

- Secretariat of the Special Committee on Beating Cancer. Background Note on Paediatric and Rare Cancers. European Parliament, 15 Feb. 2021.
- https://www.esmo.org/policy/rare-cancers-working-group/what-are-rare-cancers/specific-challenges-of-rare-cancers

MANAGEMENT IN RARE DISEASE APPROACH

29.1. PHARMACOGENETICS

Pharmacogenetic evaluations are essential in tailoring drug treatments to individual patients based on their genetic makeup. Here are some key points to consider in pharmacogenetic assessments:

1. **Drug Metabolism System:** Understanding the patient's drug metabolism system is crucial. This often involves examining genes, primarily the cytochrome P450 (CYP) enzymes, responsible for metabolizing drugs. Different genetic variants can influence how these enzymes function, leading to variations in drug metabolism.
2. **Organ Function:** It's essential to consider the health of organs involved in drug metabolism, such as the liver and kidneys. Diseases affecting these organs can alter drug metabolism and require different dosages or medications.
3. **Genetic Variability:** Genetic variants affecting drug metabolism can vary among populations and ethnicities. Therefore, it's important to consider regional differences and rely on common databases and literature reviews for interpretation.
4. **Targeted Studies:** In some cases, targeted genetic studies are conducted when a specific drug with known pharmacogenetic implications is prescribed. These studies often focus on single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) in genes associated with that drug's metabolism.
5. **Panel Examinations:** For patients with chronic conditions or those taking multiple medications, panel examinations are useful. These panels analyze multiple genes involved in drug metabolism to provide a broader understanding of how various drugs may be affected.
6. **Haplotypes:** Haplotypes are groups of polymorphisms located on the same allele and can act collectively. Sometimes, a single variant may result in a loss of function, but when combined in a haplotype with other variants, it may lead to a gain of function.
7. **Copy Number Analysis:** Copy number variations involve deletions or duplications of gene copies. These variations can significantly impact enzyme function and may require additional genetic analysis to determine their effect.
8. **Databases:** Several databases are available for interpreting genetic data in pharmacogenetics. The Pharmacogenomics Knowledgebase (PharmGKB) is a widely used resource that provides valuable information for interpreting genetic variations and their impact on drug responses.
9. **Online Software:** Many online software tools are available to assist in analyzing genomic data for pharmacogenetic purposes. These tools simplify data interpretation and help guide treatment decisions based on genetic information.

Overall, pharmacogenetic assessments are becoming increasingly important in optimizing drug treatments, reducing adverse reactions, and improving patient outcomes. They require a multidisciplinary approach, combining genetics, medicine, and pharmacology to make informed decisions about drug selection and dosing.

- https://www.pharmgkb.org/

While pharmacogenetics may not directly pertain to the matter at hand, it's crucial to recognize the significance of certain organ-related illnesses when it comes to drug selection and dosage determination. In the context of rare diseases and medical practice, taking a holistic approach becomes paramount. If a solution cannot be exclusively derived from genetic analysis, it becomes necessary to conduct more extensive examinations of the organ systems. Genomic tests are of immense value, particularly in predicting diseases that may not manifest any symptoms during the evaluation period.

Further Reading

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10675377/
- https://www.mdpi.com/2073-4425/11/11/1337

29.2. MAIN MECHANISMS IN THERAPIES OF GENETIC DISORDERS

Three types of treatment can be mentioned

- Symptomatic treatment (treatment of symptoms)
- Risk management (Preventive treatment)
- Etiological or specific treatment (correcting the problem or eliminating the deficiency in the mechanism of the disease)

29.3. SYMPTOMATIC THERAPIES

Medical Interventions

- Managing epilepsy with antiepileptic medications.
- Utilizing physical therapy to address movement limitations and muscle weakness.
- Providing specialized education for individuals with intellectual disabilities.
- Administering speech therapy for those with speech disorders.
- Employing implant and hearing aid treatments to address hearing disorders.
- Utilizing surgical interventions for the correction of deformities.
- Implementing risk management strategies, including preventive treatments.
- In some muscle diseases, anesthesia may trigger a hyperthermic reaction, necessitating cautious treatment.
- Delayed wound healing, as seen in conditions like Ehlers Danlos syndrome, may require delayed removal of stitches.
- Patients with undetermined disease details may encounter unforeseen risks, underscoring the importance of striving for a definitive diagnosis in all cases.

Dietary Interventions

- Managing hypercholesterolemia through dietary modifications.
- Addressing phenylketonuria through dietary adjustments.

Some Examples of Therapeutic Approaches in Genetic Disorders

Intervention level	Treatment strategy
Gene	Changing the somatic genotype ◊ Transplantation • Beta Thalassemia ◊ Gene therapy • Transfer of the gene for the cytokine receptor subunit in X-linked SCID ◊ Pharmacogenetic modulation of gene expression • Increasing the amount of HbF with Decitabine in sickle cell anemia
mRNA	Destruction of dysfunctional mRNA by RNA interference
Protein	Protein replacement • Administration of glucocerebrosidase enzyme in Gaucher disease • In case of factor 8 deficiency - giving factor 8 Increasing residual function • Pyridoxine treatment in homocystinuria
Metabolic or other biochemical dysfunction	Disease-specific compensation (Balancing) ◊ Diet • Low phenylalanine diet in phenylketonuria ◊ Pharmacological • Administration of sodium benzoate in urea cycle defects
Clinical Phenotype	Medical Intervention • Blood transfusion in thalassemia Surgical treatment • Surgery in heart diseases
Family	Genetic counseling Carrier screening Prenatal diagnosis Preimplantation genetic diagnosis

Prenatal Therapies

- Administering prenatal biotin for biotinidase deficiency.
- Providing maternal cobalamin during pregnancy for responsive Methylmalonic aciduria.
- Managing Congenital Adrenal Hyperplasia (21 Hydroxylase) with cortisol analogues.
- Administering prenatal L-Serine for phosphoglycerate dehydrogenase (PGDH) deficiency, an L-Serine synthesis disorder.

Urea Cycle Defects

- Ammonium is neurotoxic.
- Urea is a waste product that can be eliminated through urine.
- Sodium benzoate converts ammonium into urea.

Enzyme Inhibition

- Heterozygous familial hypercholesterolemia is a condition.
- Statins are inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is crucial in cholesterol synthesis.

Increasing Protein or Gene Activity

- Small molecules like vitamins, non-peptide hormones, and certain drugs can increase protein or gene activity.
- Existing libraries of known small molecules are being examined for potential drug use.

Small Molecules Targeting Stop Codons

- Different tRNA molecules correspond to each amino acid and recognize stop codons.
- Some molecules hinder the function of the tRNA recognizing stop codons.
- Ataluren has shown promise in addressing stop codon variants in CFTR and DMD.

Small Molecules Correcting Protein Folds (Pharmacological Chaperones)

- Mutations can disrupt protein folding.
- Chaperones can correct misfolding.
- Lumacaftor and Ivacaftor correct folding disorders caused by the DeltaF508 mutation, the most common mutation in cystic fibrosis, especially when used together.

Small Molecules Increasing Protein Function without Disrupting Intracellular Traffic

- Ivacaftor enhances protein activity in eight cystic fibrosis mutations (e.g., p.Gly551Asp), and the FDA has approved its use for these mutations.

Small Molecules Increasing Mutant Enzyme Activity: Vitamin-Responsive Metabolic Diseases

- Some vitamins act as cofactors for enzymes.
- High-dose vitamin supplementation can enhance the function of mutant enzymes, provided they have some residual activity.

Treatment Through Metabolic Manipulation

Type of metabolic intervention	Agent or Technique	Disease
Avoidance	Antimalarial Drugs Isoniazid	G6PD Deficiency (Favism) Slow Acetylators
Dietary Restriction	Phenylalanine Galactose	Phenylketonuria Galactosemia
Replacement	Thyroxine Biotin	Congenital Hypothyroidism Biotinidase Deficiency
Changing	Sodyum Benzoat (Ammonium→Urea)	Urea Cycle Defects
Enzyme Inhibition	Statins	Heterozygous Familial Hypercholesterolemia
Receptor Antagonist	Losertan	Marfan Syndrome
Depletion	LDL Apheresis	Homozygous Familial Hypercholesterolemia

- Vitamins have high toxic doses.
- In homocystinuria due to cystathionine synthase defect, 50% of cases respond to high doses of vitamin B6, with non-responders having no residual activity.

Increasing Protein Amount

- This involves providing the missing protein externally.
- Can be extracellular or intracellular protein replacement, or achieved through "molecular targeting."
- A well-known example is administering factor 8 in hemophilia, although antibody formation and consistent dose generation are challenges.

Extracellular Replacement of Intracellular Protein

- Adenosine Deaminase Deficiency (ADA deficiency) is an example.
- ADA deficiency disrupts the conversion of adenosine to inosine, leading to toxic purine accumulation and immunodeficiency.
- Gene transplantation is a potential treatment option.
- Polyethylene Glycol (PEG) can be added to ADA enzyme (PEG-ADA) for enhanced stability.

Advantages of PEG-ADA:

- PEG-ADA is stable and has a lasting effect for 3-6 days.
- It enables extracellular treatment of intracellular enzyme deficiencies by aiding the removal of extracellular toxic metabolites, prompting the release of intracellular ones.
- The occurrence of antibody formation against PEG-ADA is relatively rare.

Enzyme Replacement Therapy (ERT): Targeted Intracellular Enzyme Replacement:

- For ERT to be effective, externally administered enzymes must reach the right organelle within the correct cells.
- Experience gained from using ERT for Gaucher disease paved the way for its application in treating various lysosomal diseases.
- The attachment of a mannose unit to the enzyme facilitates its entry into cells via mannose receptors on macrophage surfaces, reaching lysosomes and resolving accumulation.
- ERT is effective in the non-neuronopathic group of Gaucher disease but cannot cross the blood-brain barrier for neuronopathic cases.
- Since treatment can be administered every 15 days, patients need 1-5% residual enzyme activity to bridge the gaps between treatments.
- Patients with residual enzyme activity do not typically develop antibodies due to the enzyme's structural recognition (self-tolerance).

Modulation of Gene Activity:

- Increasing the activity of a mutant gene, as seen in hereditary angioedema treatment using danazol.
- Increasing the activity of another gene, such as maintaining high HbF levels in Beta Thalassemia and sickle cell anemia through gene editing or the use of decitabine.
- Inhibiting gene expression through small interfering RNAs (siRNA) to reduce the overproduction of mutant proteins, particularly applicable in transthyretin-related diseases.

Exon Skipping Therapy:

- Exon skipping therapy addresses exon deletions in diseases like Duchenne Muscular Dystrophy, allowing the formation of a shorter, functional protein.
- By using antisense oligonucleotides, it can skip exons with stop codons, transforming the clinical picture in patients.

Non-medicinal Therapies in Rare Diseases:
Gene Editing:

- Utilizing the CRISPR/Cas9 system to correct nucleotide substitution variants, as seen in the CCR5 gene for HIV transmission prevention.

Cell/Tissue/Organ Transplantation:

- Organ replacement due to damage, and transplantation of tissues capable of producing the missing protein or enzyme.
- Types include hematopoietic stem cell transplantation, corneal stem cell transplantation, and skin stem cell transplantation.
- Challenges include tissue compatibility and transplantation risks.

Autologous Transplantation:

- Correcting genetic structures in a person's own tissue, minimizing tissue compatibility issues.
- Successful in some diseases.

Gene Transplantation:

- Necessary for loss-of-function mutations.
- Gene insertion into human DNA via episomes or other methods.
- Requires knowing the gene and molecular defect, appropriate vector, and risk/benefit assessment.
- Risks include adverse reactions to the virus, disruption of functional genes, and potential cancer gene disruption.

Further Reading

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9704033/

29.4. GENETIC COUNSELING

Genetic counseling goes beyond simply informing individuals about the risk of recurrence in future pregnancies; it is a comprehensive and effective treatment approach. Its primary goal is to actively involve and support patients and their families throughout the treatment journey. This process is built on establishing mutual trust and extends far beyond a couple of meetings, often becoming a long-term commitment. Multiple trained professionals may collaborate in this effort.

The genetic counseling process begins when there is a possibility of a genetic disease, and it encompasses various stages, including:

- Informing patients and their families about available tests prior to planning these tests.
- Explaining the test procedures and what to expect during testing.
- Offering post-test counseling to discuss test results.
- Providing information about the potential disease and its implications.
- Addressing the possibility of disease recurrence in future pregnancies and involving other family members.
- Covering the disease's progression and prognosis.
- Offering information about relevant associations, patient support groups, and specialized treatment centers.

Genetic counseling also encompasses activities aimed at risk assessment before pregnancy, with the goal of preventing the occurrence or impact of genetic diseases during pregnancies. This includes explaining processes such as preimplantation genetic diagnosis and prenatal diagnosis, clarifying the nature and consequences of various tests, and alleviating any uncertainties or concerns the patient may have.

In addition to informing patients and their families, genetic counseling may involve educating healthcare professionals, such as doctors, and, when necessary, other healthcare personnel. Ultimately, genetic counseling is a holistic set of measures designed to provide support, guidance, and information to individuals and families facing genetic and hereditary challenges.

Further Reading

- https://www.ncbi.nlm.nih.gov/books/NBK236049/

29.5. PRENATAL DIAGNOSIS AND PREIMPLANTATION GENETIC DIAGNOSIS

Both preimplantation genetic diagnosis (PGD) and prenatal diagnosis are technically feasible methods for nearly all genetic diseases. However, various factors, including regional differences, societal norms, and religious beliefs, can influence preferences regarding their use. Therefore, it is crucial for individuals to receive adequate information and education before making decisions about these options.

In the realm of preimplantation genetic studies, there are some common terms to be aware of:

- PGT-A, which stands for aneuploidy detection, is primarily a screening study, not a targeted one.
- PGT-M is employed for familial variant testing in embryonic cells.
- PGT-SR refers to the preimplantation genetic test for structural chromosomal variants.

Preimplantation genetic diagnosis typically involves the biopsy of embryos between the third and fifth days of development. In recent years, fifth-day biopsy procedures have become more favored due to advances in knowledge and experience. While previous methods included Polar body Biopsy, FISH, and Array-Based techniques, contemporary approaches utilize SNP tests conducted through Sanger or next-generation sequence analysis, as well as STR-based methods.

For PGT-M studies, it is imperative to have a confirmed diagnosis of the genetic disease within the family and to demonstrate the specific variant through genetic tests before proceeding. Test plans may involve testing the mutation itself and assessing nearby STR or SNP polymorphisms to ensure the safety of the study.

In PGT-A, molecular tests are employed to investigate aneuploidies, typically in cases with indications such as advanced maternal age, recurrent pregnancy loss, recurrent in vitro fertilization failures, or a prior history of numerical chromosome disorders.

One significant advantage of PGD studies is their ability to reduce the likelihood of pregnancy termination, sparing families from the trauma associated with such processes. However, PGD also presents technical challenges and can be cost-intensive. Nevertheless, when compared to the lifelong expenses of treating and caring for genetic diseases, these techniques may ultimately prove to be cost-effective.

Prenatal diagnosis studies serve various purposes, including:

- Detecting familial diseases.
- Identifying the causes of abnormal ultrasound findings and abnormal prenatal screening results.
- Assessing the need for prenatal treatment.

Both PGD and prenatal diagnosis can play crucial roles in ensuring the well-being of both the unborn child and the family, but informed decision-making is key to their successful implementation.

Further Reading

- https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2020/03/preimplantation-genetic-testing
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5548328/
- https://www.acog.org/womens-health/faqs/prenatal-genetic-screening-tests
- https://www.gov.uk/government/publications/fetal-anomaly-screening-programme-handbook/prenatal-diagnosis

29.6. GENETIC SCREENING

Planning genetic disease screening programs has significant implications for public health policy and cost-effectiveness. Continual evaluation of the economic and social impacts of rare disorders is essential. Recent advancements in next-generation design technology have made genetic screening more cost-effective.

In the United States, a report on rare diseases highlights the following key points:

- An estimated 15.5 million children and adults had one of 379 rare diseases in 2019, resulting in $449 billion in direct medical costs (45%).
- The total economic burden was $997 billion.
- Indirect costs accounted for $437 billion (44%), non-medical costs for $73 billion (7%), and healthcare expenses not covered by insurance for $38 billion (4%).
- Hospital inpatient care and prescription medications were the main drivers of excess medical costs associated with rare diseases.
- Labor market productivity losses from absenteeism and early retirement constituted significant categories of indirect costs.
- In summary, approximately 4.5% of the total U.S. budget in 2019 was allocated to rare diseases.
- When considering screening strategies, several factors come into play:
- Cost-effectiveness in comparison to treatment expenses.
- Ease of application.
- Effectiveness in detecting and managing genetic diseases.
- Prioritizing diseases that benefit from early diagnosis, have a high financial burden, or where early detection enhances treatment effectiveness.

Types of screenings include neonatal screening, preconceptional screening, prenatal screening (including biochemical, genetic, and ultrasound-based methods), and population screening.

Some countries have taken new steps in pre-pregnancy screenings:

- Israel screens for the 2000 most common diseases in their population before marriage.
- The United States recommends carrier screening for couples planning a pregnancy, with a focus on diseases with a carrier frequency of 1/200 and above.

Australia has implemented newborn high-risk baby screening using whole-genome sequencing, which has reduced costs, hospital stays, mortality, and morbidity while contributing to diagnosis significantly.

Population screening is under discussion due to various challenges:

- The need for training physicians on rare diseases.
- Time-consuming genetic tests in emergency situations.
- The importance of genomic information in chronic disorders for identifying the exact cause of the disorder, secondary disorders, pharmacogenetics, and modifiers.

Overall, the evaluation and implementation of genetic screening programs require careful consideration of their economic and social impact, with a focus on balancing costs, effectiveness, and public health policy.

Further Reading

- https://ojrd.biomedcentral.com/articles/10.1186/s13023-022-02299-5

PROBLEMS IN RARE DISEASE

30.1. EXCESSIVE SUBSPECIALIZATION. CONTRIBUTION AND HARM TO RARE DISEASES

Subspecialties indeed play a crucial role in diagnosing and treating various diseases, as they provide in-depth knowledge and expertise in specific areas of medicine. However, it is equally vital to maintain a holistic approach to patient care and ensure that subspecialties do not overlook findings in other organ systems when evaluating patients.

In multisystemic disorders, where the disease affects multiple organ systems, it's common for different specialties to monitor and manage the associated symptoms or complications. Effective communication and coordination among these specialties are essential for providing comprehensive care to the patient.

Without a well-established communication system and a coordinating physician, the continuity and integrity of patient care can be compromised. This lack of coordination may result in the underdiagnosis or inadequate management of multisystemic diseases. Therefore, maintaining a collaborative and interdisciplinary approach to healthcare is crucial to ensure that patients with complex, multisystemic disorders receive the best possible care and attention to all their medical needs.

30.2. DEFICIENCIES IN MEDICAL GUIDELINES AND GENOMIC MEDICINE INTEGRATION

The development and use of medical guidelines are essential for ensuring that healthcare practices related to diagnosis, follow-up, and treatment of diseases are comprehensive and evidence-based. However, it's important to recognize that while guidelines provide valuable structure and guidance, they cannot encompass every possible scenario or clinical variation that may arise in medical practice.

Several challenges and considerations related to medical guidelines include:

1. **Common Events vs. Rare Occurrences:** Guidelines typically prioritize common events and problems to provide practical and widely applicable recommendations. However, this can inadvertently lead to a standardized approach that may not suit every patient's unique needs.
2. **Flexibility in Practice:** Physicians should maintain a degree of flexibility when dealing with patients whose conditions do not fit neatly into established guidelines. In such cases, guidelines should serve as a foundation but not restrict physicians from adapting their approach to meet individual patient requirements.
3. **Legal Concerns:** Concerns about legal repercussions can sometimes deter physicians from deviating from guidelines. It is essential to provide guidance on when and how to step outside of guidelines to prevent undue legal worries and foster patient-centered care.
4. **Conflicting Guidelines:** Multiple guidelines on the same subject can exist, and they may contain conflicting recommendations. Harmonizing and aligning guidelines while considering the most recent evidence is necessary to avoid confusion and ensure consistent care.
5. **Etiologic Evaluation:** Symptom-based guidelines may not always address the underlying causes of diseases or conditions. Evaluating the etiology of a patient's symptoms is crucial in comprehensive healthcare.
6. **Dynamic Updates:** Guidelines should be periodically reviewed, updated, and made accessible through dynamic online systems. This ensures that guidelines remain current and reflect the latest advancements in medical knowledge.
7. **Accessibility:** Guidelines should be designed to reach healthcare providers, including those in rural areas. Incorporating videos in addition to written documents can enhance understanding and correct implementation.

In summary, while medical guidelines serve as valuable tools for healthcare providers, they should be used as a foundation and not as rigid rules. Physicians should retain the flexibility to adapt their approaches when necessary to provide the best possible care for their patients. Ongoing efforts to update and harmonize guidelines, coupled with accessible and dynamic systems, can enhance their effectiveness and benefit patients in various healthcare settings.

30.3. PROBLEMS OF PATIENTS

Living with a rare or complex medical condition often comes with a range of challenges and difficulties. Some of the common issues faced by individuals dealing with such conditions include:

1. **Access to Expertise:** Many rare diseases require specialized knowledge and care. Patients may need to travel long distances to reach experts and expert centers for accurate diagnosis and appropriate treatment.
2. **Frequent Travel:** Long-term follow-up care, including routine procedures like vaccinations, may necessitate frequent travel to these expert centers, which can be both time-consuming and costly.
3. **Expensive Treatments:** Rare diseases often require expensive drugs and therapeutic protocols, placing a financial burden on patients and their families. The cost of these treatments can be substantial, and not all may be covered by insurance or social security programs.
4. **Social Security Issues:** Access to social security benefits and healthcare coverage may be challenging for individuals with rare diseases. Navigating the bureaucracy of social security systems can be daunting.
5. **Social and Psychological Impact:** Living with a rare disease can have profound social and psychological effects. Patients may experience isolation, alienation from society, and difficulty maintaining employment due to their health condition. These challenges can lead to high divorce rates and feelings of frustration.

Addressing these issues requires a comprehensive approach that includes improving access to expert care, financial support, and social services. Advocacy efforts and support from patient organizations can also play a crucial role in raising awareness and addressing the unique needs of individuals with rare diseases. Additionally, research and medical advancements aimed at developing more cost-effective treatments and increasing social support systems can contribute to improving the quality of life for those living with rare conditions.

30.4. PROBLEMS OF MEDICAL TEAM

- Inability to access diagnostic tests,
- Inability to reach expert consultation,
- Inexperience with rare diseases, etc.

30.5. SAMPLE ARCHIVING

Maintaining samples and records of deceased individuals with rare diseases is indeed crucial for various reasons, including understanding the etiology of these conditions and conducting further research. Here are some key points related to this important aspect:

1. **Sample Preservation:** Collecting and preserving biological samples like EDTA blood, Guthrie card samples, and buccal mucosal swabs from individuals with rare diseases can provide valuable genetic information for future investigations, especially in cases of sudden death. These samples can help in identifying genetic mutations and studying the molecular basis of these diseases.
2. **Tissue Freezing:** In addition to DNA storage, preserving tissues through freezing is essential for retaining RNA and living tissue. This allows for a more comprehensive analysis of the disease's underlying mechanisms and potential therapeutic targets. Proper storage conditions and facilities are crucial for maintaining tissue samples.
3. **Photographic Documentation:** Keeping a record of photographs of affected individuals, especially those with dysmorphic features, can be instrumental in diagnosis and research. These images can aid in identifying patterns or characteristics associated with rare diseases and contribute to better understanding and recognition.
4. **Establishing Commissions:** Creating dedicated committees or commissions within healthcare institutions to address the needs of individuals dealing with rare diseases is a proactive step. These committees can oversee the collection, storage, and utilization of samples and records, ensuring that best practices are followed.
5. **Guidelines and Updates:** Developing and regularly updating guidelines for the preservation of samples and records is essential. These guidelines

should cover the proper collection, labeling, storage, and ethical considerations related to these materials. Continuous updates ensure that institutions stay aligned with the latest standards and technologies.

6. **Collaboration and Research:** Making preserved samples and records available for research purposes can contribute to advancements in the understanding and treatment of rare diseases. Collaboration between healthcare institutions, research centers, and patient advocacy groups can facilitate such research initiatives.

Preserving samples and records of individuals with rare diseases not only benefits their families but also contributes to the broader scientific and medical community's knowledge. It is essential to recognize the importance of these efforts and invest in infrastructure, guidelines, and collaborations to support them effectively.

Training and Certification on Rare Diseases and UEMS MJC-RUD Studies

The assessment and certification under the European Diploma for Essentials in Rare and Undiagnosed Diseases (EC RUD) is a joint development of the UEMS Multidisciplinary Joint Committee of Rare and Undiagnosed Diseases (MJC RUD) and its collaborating partners. There are thousands of different rare diseases (RD). We support a structure in which rare disease patients are treated in complex multidisciplinary and cross-sectorial manners in multidisciplinary organizations which include also numerous highly specialized competences as well. The MJC RUD has been established to coordinate the rare disease related activities in the UEMS and to represent the UEMS towards external partners with rare disease interest. The MJC RUD acknowledges other independent efforts which have been achieved by different UEMS bodies, as some sections and boards have already had achievements in different RD topics. The MJC RUD also acknowledges that the care of some rare diseases or rare disease groups has been coordinated by a single medical specialty, or a combination of specialties. Meanwhile, it has been recognized that there is a desire from patients and their advocacy groups, policy makers, and from different medical and scientific communities to design and launch a training and assessment system with organic essentials of rare and undiagnosed disease topics, and to introduce a special training and assessment to emerge experts serving the rare disease community in a standardized level. The EC RUD is a recognized skill and knowledge-based assessment tool for medical doctors, who are clinicians practicing in situations where rare disease patients could be a recognition and management challenge. This competence includes the spectrum from the basic skill of recognition of red flag patients, who at least should be considered as suspects for specific investigations as subjects with rare and undiagnosed conditions, through the management of their diagnostics and further organization of their care pathways. Major elements are included in the ETR of Rare and Undiagnosed Diseases, approved by the UEMS Council in 2019. The competence certification is awarded to a selection of medical doctors, as described in the ETR. The aims include establishing standards in competence to world class levels and forming a community of such certified experts: those who successfully pass the exam will be honored by the designation of "Fellow of European Board of Rare Diseases". The examination is overseen and supervised by the European Board of Rare and Undiagnosed Diseases (EBRUD), mainly via the Enlarged Examination Steering Committee (EEST) and its subgroups and is also observed by representatives of the collaborating partners. The examination is also open to observers and officials of UEMS committees as appropriate. The EC RUD certification itself is valid for the awardee's lifetime as a hallmark of excellence, and the EC RUD holders qualified themselves to hold the Fellow of EC RUD. It will be considered as a license for practice only in countries where it is ratified and recognized as an official certificate for this purpose. The MJC RUD/ EC RUD also promotes continuing good medical practice through the Continuing Medical Education and Continuing Professional Development (CME/CPD) system and certification. This is strongly recommended for active practitioners every 4-5 years.

The European exam system is global and is open to any candidate regardless of nationality.

Further Readings on Genomics and Rare Diseases

In this booklet, we present you some shortcuts for easy learning and some tricky points. For further information please check the recommended websites, article and videos.

General and Clinical Information on Genetics and Rare Diseases

- https://www.genome.gov/genetics-glossary
- https://www.genomicseducation.hee.nhs.uk/
- https://irdirc.org/
- https://irdirc.org/resources-2/irdirc-recognized-resources/
- https://www.ncbi.nlm.nih.gov/books/NBK1116/
- https://www.omim.org/

E-Learning Platforms

ESHG Genetic Educational Materials and Sources;

- https://www.eurogems.org/index.html

A compendium of genetic information and resources

European School of Oncology;

- https://www.eso.net/

Organization for education and training in cancer

EURORDIS Open Academy;

- https://openacademy.eurordis.org/

Capacity-building programmes for patient advocates and mixed audiences

BBMRI.QM Academy;

- https://www.bbmri-eric.eu/services/e-learning/

E-learning resources on biobanking

Elixir Training Platform;

- https://elixir-europe.org/platforms/training

Education and training resources on life sciences

EATRIS Transmed Academy—course on translational medicine;

- https://eatris.eu/transmed-academy/

e-learning platform which hosts online courses as well as recordings of webinar series

Organizations on Rare Disorders

- https://rarediseases.org/
- https://rarediseases.org/rare-diseases/

- https://rarediseases.info.nih.gov/
- https://www.orpha.net/consor/cgi-bin/index.php
- https://health.ec.europa.eu/rare-diseases-and-european-reference-networks/rare-diseases_en
- https://www.rarediseasesinternational.org/
- https://www.udninternational.org/
- https://wilhelmfoundation.org/
- https://www.enets.org/
- https://health.ec.europa.eu/rare-diseases-and-european-reference-networks_en
- https://www.youtube.com/watch?v=vVEzzTwKUZU

Patient Organizations

- https://rarediseases.org/organizations/

National recommendations of Australia

- Links to website https://rarevoices.org.au/national-recommendations/
- PDF version of main PDF https://rarevoices.org.au/wp-content/uploads/2024/03/National-Recommendations-for-Rare-Disease-Health-Care-1.pdf

Recent Future of Rare Disease Studies

Genetic tests need to be used much more quickly in the diagnosis of rare diseases. The combination of genomic tests, toxicological screenings, infectious disease screenings, and microbiome studies should be used much more quickly in every patient who cannot be easily diagnosed. These practices are important to reduce unnecessary expenses, especially in countries with a difficult economic situation.

Carrying out genomic studies to the entire population at the neonatal stage and archiving will shorten the diagnosis process of many diseases and will prevent wasting time on generating data in emergency situations.

In addition, the use of this data to increase the chance of healthy pregnancy without taking it to the point of eugenics, the effective use of data for the correct management of chronic diseases, will contribute to the planning of more effective health approaches for the future with the widespread use of genomic data and the contribution of toxicology and infectious diseases, and will enable the rapid increase of knowledge.

Determining the etiologies of diseases will actually enable multidisciplinary clinics to become widespread and enable certain diseases to be followed in such clinics.

In addition, considering that one in every ten people has a rare disease, expanding online services to produce on-site services instead of planning to provide services only in metropolitan cities and high-level health institutions and keeping thousands of people constantly traveling, strengthening the communication of health institutions operating in small settlements with large centers, Preparing not only general guides for the follow-up of patients, but also individual guides and determining the work and services to be performed in case of emergency and assisting local health institutions in their work will be extremely important approaches to increase the comfort of life of patients.

In studies conducted in different parts of the world, studies are being carried out to speed up the process of using genomic studies and obtain results much more quickly, especially in critically ill patients and patients in need of intensive care. For this reason, the big data obtained, especially artificial intelligence technologies, can be evaluated quickly and according to the current need, and it should be structured in a way that does not require intense manpower, even if human power contributes, so robotic technologies can be included in the process in genomic, toxicological and high-tech infectious diseases studies. It is extremely important to have it.

Although patient organizations are gaining strength, we see that in a significant part of the world, patients do not reach these organizations sufficiently. These organizations are of great importance in guiding patients and ensuring that doctors and patients act in coordination. It is very important to act together for research, to create resources together, to conduct research on more patient samples, to listen to patients' problems and to ensure the production of medical devices and non-medical device daily products that increase their comfort, therefore involving industrialists and businessmen in the processes.

Apart from some drugs produced for general use, it is extremely important to hold effective discussions for facilitating studies on the application of some personal drugs and applications in the treatment of very rare diseases and to increase the number of people who think in this field.