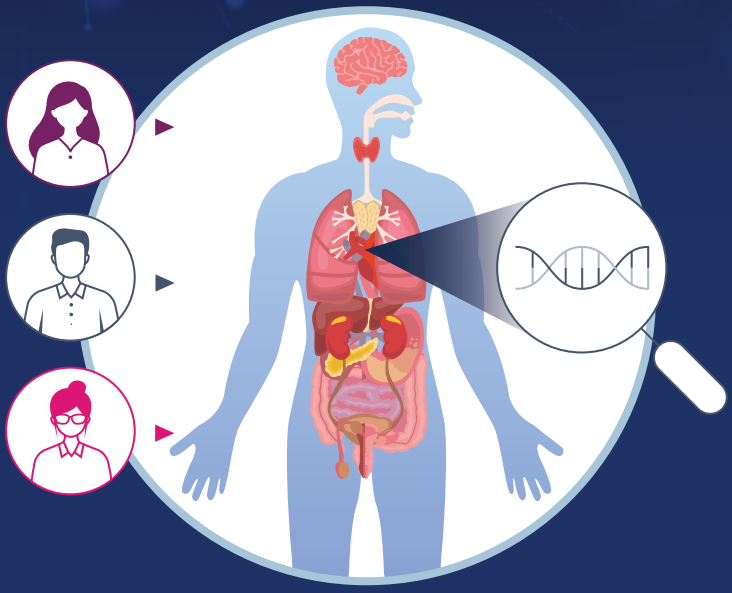
GENETICS and GENOMICS:
Applications to Medicine and Health



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The aim of this booklet is to provide an introduction to genetics for the non-geneticist, and a rationale for the early utilisation of genetic testing in the differential diagnosis of rare genetic childhood diseases at presentation.
This booklet is not intended to be an exhaustive reference text on genetics and genetic testing.

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Foreword

The success of the Human Genome Project has catapulted medicine and life sciences forward in a way that only happens once in a generation. It has resulted in the potential to analyse all (ome) units of heredity (genes) and inherited variations within a short timeframe, allowing genomic information to be applied in the clinical setting. It has also given rise to the “multiomic sandwich” where genomics, alongside epigenomics, transcriptomics, proteomics, metabolomics and other -omic technologies, has already spawned new laboratory and clinical specialties and will continue to drive their evolution.

A literate workforce, equipped to fully harness and utilise the promises of this exciting area for the benefit of patients and their families, is being developed worldwide through multiple education platforms and pathways. This publication “Genetics and Genomics: Applications to Medicine and Health” aims to provide the non-expert user of genomics important first principle teachings and starter points that can be used on a day-to-day basis as they take their first steps into the exciting world of genomics and precision medicine.

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Section 1: Introduction

The application of genomics into health and medicine has changed dramatically over the last decade, ushered in by the development of **next-generation sequencing (NGS)** technologies. It has never been more accessible for healthcare professionals to employ genetic/genomic testing to aid rapid diagnosis or identify people at risk of developing disease, which allows for the proactive treatment of the condition, sometimes before clinical features have manifested.

In order to utilise medical genomics in an effective, efficient and safe way, professionals in this area will have to become literate in the latest technologies. This booklet aims to provide non-expert healthcare providers with introductory information about genomics that will increase their understanding of how to apply genomics in the healthcare setting.

Section 2: Basic concepts of genetics and genomics

2.1 What are genes?

A **gene** is a defined segment of **DNA** that contributes to a cellular function or physical characteristic (**phenotype**).[1] It may be characterised by its sequence, transcriptional behaviour, similarity (homology) to other known genes, or function. The smallest human genes are only a few hundred base pairs in length, while the largest is the gene that encodes the muscle protein dystrophin, which has 2.4 million base pairs.[2,3]

The portions of a gene that contain regions which code for protein products are called **exons**. The intervening, generally non-coding sequences between exons are called **introns**. **Regulatory regions** govern the activity **(transcription)** of a gene.[4-6]

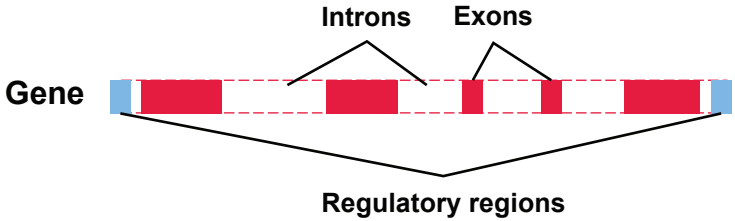

Figure 1. Basic structure of a gene, including protein-coding exons, non-coding introns, and regulatory regions.

2.2 What is a genome?

A **genome** represents the sum of all **DNA** in an organism, including its genes and non-coding regions. Most of the genome is located within the cell **nucleus**, organised into linear pieces called **chromosomes** (the human genome has 23 chromosome pairs, while the fruit fly has only four chromosome pairs).[7]

Nuclear DNA carries the genetic information used to synthesise the majority of cellular proteins that determine an individual's phenotype. Each human chromosome contains a different number of genes (from >200 genes on the Y chromosome to >3000 genes on the largest chromosome).[8] Genes occupy specific regions on the chromosomes, known as **loci** (traits like hair texture and colour are determined by genes that occupy different loci, often on different chromosomes).[9]

The genetic information contained within a chromosomal gene is first converted into RNA, which leaves the nucleus and directs protein synthesis in the cytoplasm of the cell.

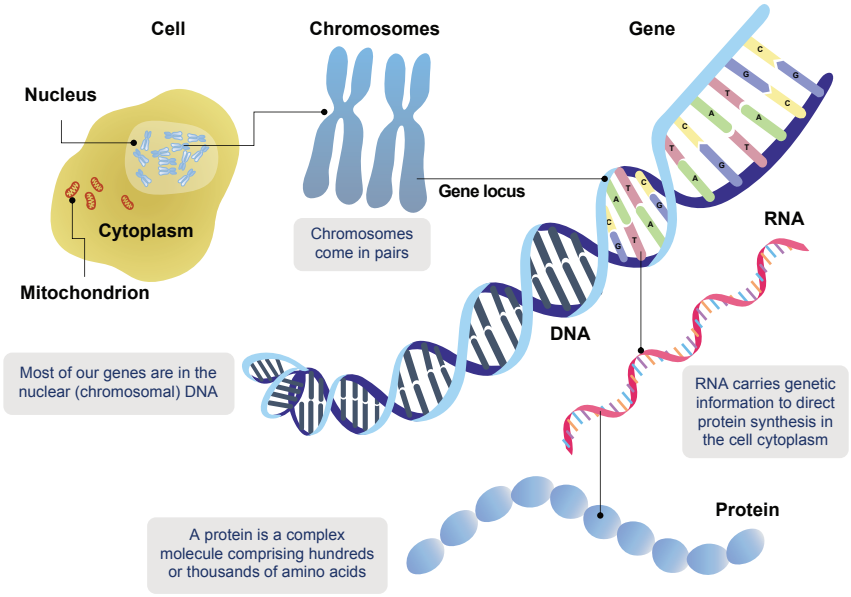

Figure 2. Genetic components of the cell.

Mitochondria are small bodies in the cytoplasm that drive cellular metabolism—the generation and processing of cellular energy. Mitochondria contain multiple copies of a small genome (37 genes) that codes for proteins involved in cellular metabolism. Mitochondrial DNA can only be inherited from the mother.[10]

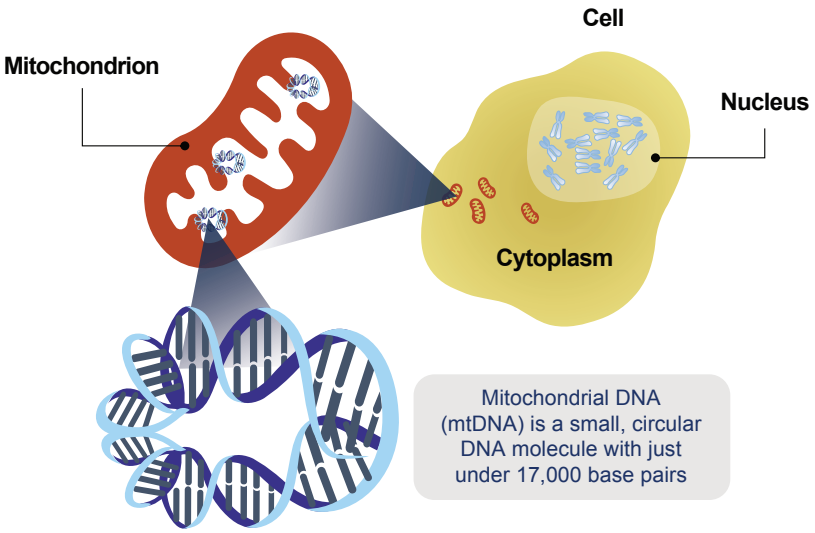

Figure 3. Mitochondria and mitochondrial DNA.[10]

2.3 The DNA-RNA-protein paradigm

The process of **gene expression** occurs through the generation of RNA (**transcription**), and subsequent protein synthesis (**translation**). Referred to as the *central dogma of molecular biology*, these processes link the genetic information contained within genes to their functions within the cell.[11]

Transcription is the process where DNA is used to generate a messenger RNA (mRNA) molecule, which carries the genetic code from the nucleus to the cytoplasm.

Translation occurs in the cytoplasm, where the genetic code within the mRNA is used to synthesise proteins.

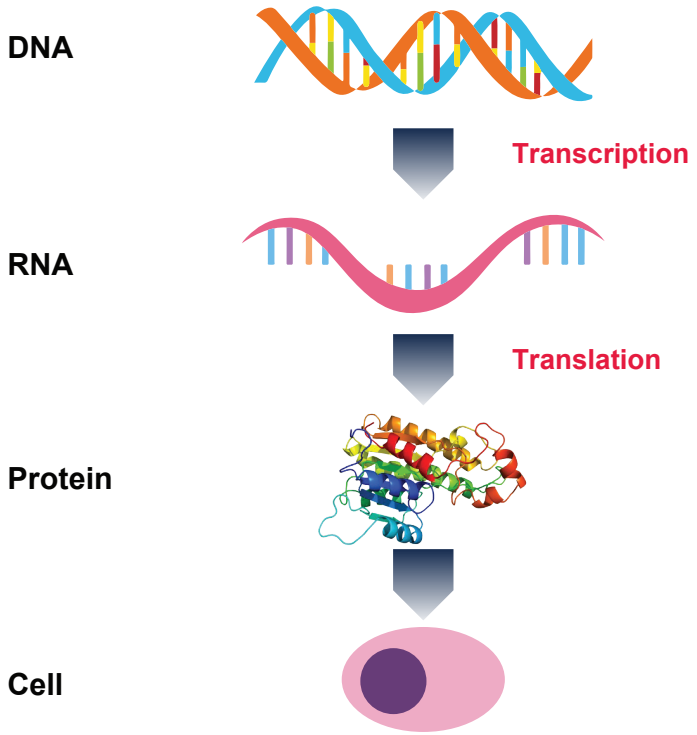

Figure 4. The DNA-RNA-protein paradigm connects the genetic information encoded in the DNA to the function of cellular proteins.[11]

The entire gene, including **introns** and **exons**, is initially transcribed, but the introns are subsequently removed by another specialised RNA-protein complex—the spliceosome. After splicing, mature mRNAs consist of the continuous protein-coding sequence and regulatory sequences that coordinate translation.[12]

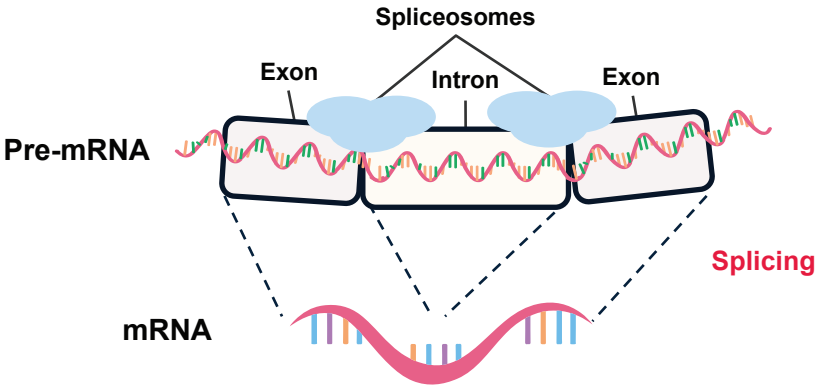

Figure 5. RNA molecules are processed by removing introns through RNA splicing.[12]

Section 3: The application of genetics and genomics to medicine and health

3.1 Medical genomics

The DNA of every human on the planet is very similar. In fact, >99% of the human genome sequence is the same in any two individuals, whether they are related or not.[13] Physical differences between individuals (**phenotypes**), such as eye colour and height, can occur as a result of changes (**variants**) in the DNA. Changes in DNA occur all the time, both spontaneously and randomly, and the collective characteristics of an individual's DNA is known as a **genotype**.

Genetic alterations can occur at the level of the chromosome, gene, or gene sequence, and are found in both coding and non-coding regions of the genome. Quantitative genomic variations, including losses (deletions) and gains (duplications) of chromosomal segments, are referred to as **copy number variations (CNVs)**, whereas sequence-level variants include **insertions, deletions**, and **substitutions** of one or more **nucleotides**.

In some cases, variants can lead to defective cellular functions and cause disease. Medical genomics refers to the analysis and utilisation of genetic information or data in healthcare. Evolving technologies have facilitated a cost-effective integration of genomic tests into clinical practice. Growing knowledge of molecular genetics has increased understanding of the relationships between genes and diseases.

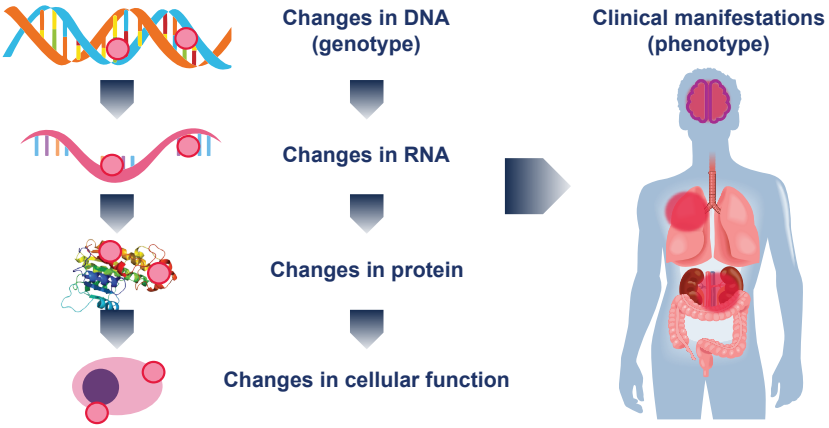

Figure 6. Changes in an individual's DNA can lead to physical changes, including disease.

3.2 Using genetics and genomics to establish diagnoses

In the past, genetic/genomic analysis was usually considered a second- or third-line investigation to establish a disease diagnosis, owing to high costs, long turnaround times, and the limitations of available methods. Recent advancements in technology, along with increased availability of genetic testing platforms, means that genetic/genomic analysis has now become a first-line diagnostic investigation for many diseases, complementing initial clinical investigations and biochemical analyses.[14-16]

Diagnostic pipelines are being developed that use genomics along with medical literature and standardised terms (such as the Human Genome Variation Society [HGVS] nomenclature and human phenotype ontology [HPO] terms), which ensure accurate, deep, consistent, and structured characterisation of an individual's phenotype. This approach can reduce the time to diagnosis, enabling early intervention and disease management.[16,17]

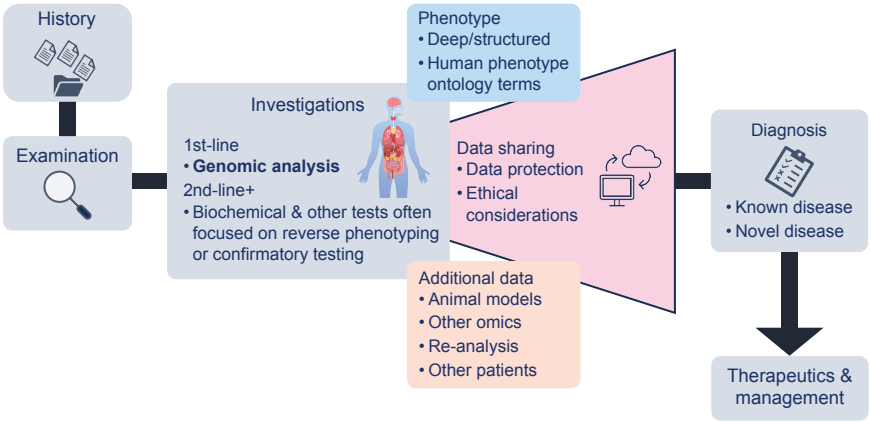

Figure 7. The role of genetic testing in disease diagnosis.[16]

3.3 Overview of genomic analysis technologies

A genetic disorder may have a chromosomal, single gene (monogenic), or polygenic/multifactorial aetiology. About half of rare genetic disorders do not have any clear cause, and most are isolated or nonsyndromic.[18] Patients and their families are often keen to understand the origin of a disorder, and accurate aetiological determination can have specific implications for treatment, prognosis, assessment of recurrence risk, and counselling of families.

Many methods are available for genomic analysis, and some of the major methods are outlined on the following pages.

3.3.1 Chromosomal G-banded analysis (standard karyotyping)

Standard chromosomal G-banded (or Giemsa-banded) analysis (often referred to as karyotyping) is performed if signs suggestive of a known chromosome disorder (such as Down's, Turner, or Klinefelter syndromes) are observed. A staining technique is used to visualise and pair chromosomes based on their length, banding pattern, and the location of the **centromere**, which separates the chromosome into a shorter p arm and a longer q arm.[19] Using this technique, abnormalities of chromosome number or structure can be broadly classified.

Detectable chromosome abnormalities include **aneuploidy**—an excess or deficiency of the normal **diploid** state (two copies of each chromosome present).[20] Each chromosome arm has a distinct banding pattern, which can help to map aberrations and identify genes that may be affected at that location on the chromosome (**locus**).[19]

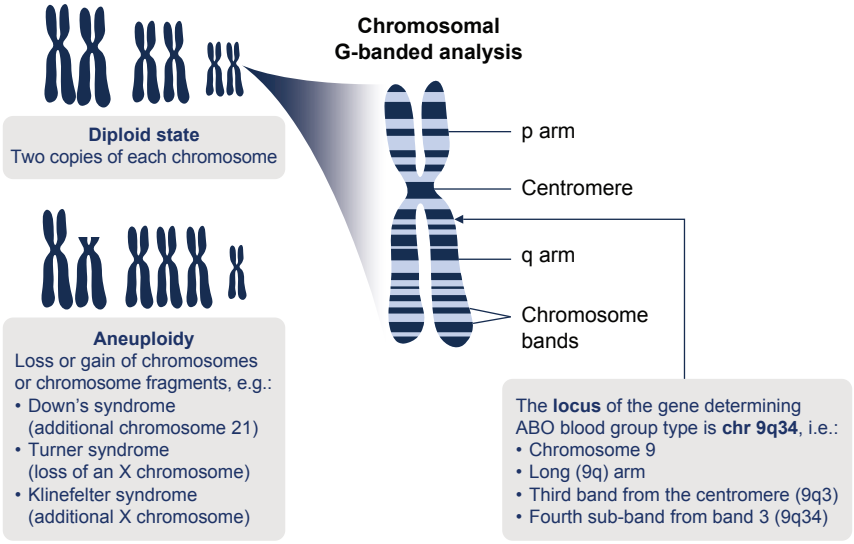

Figure 8. Chromosomal G-banded (or Giemsa-banded) analysis can be used to identify overt chromosome abnormalities such as aneuploidies. G-banded nomenclature can also help identify gene loci (the locus of the ABO gene is given as an example).[19,21]

3.3.2 Fluorescent in situ hybridisation

Fluorescent in situ hybridisation (FISH) uses specific fluorescently labelled small DNA fragments or probes, allowing for targeted analysis of specific chromosomes at higher resolution than G-banded analysis.[22] This technology is commonly used to test for submicroscopic deletions, e.g. a deletion of chromosome 22q11, which commonly results in DiGeorge syndrome.[23]

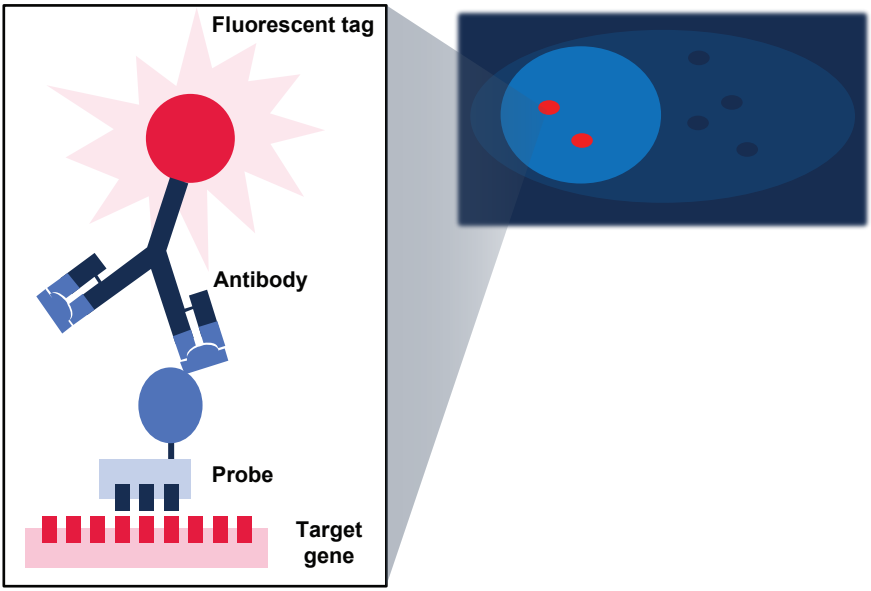

Figure 9. Fluorescent in situ hybridisation is used for targeted, high-resolution chromosome analysis.[22,24]

3.3.3 Array comparative genomic hybridisation/chromosomal microarray analysis

Array comparative genomic hybridisation (**array CGH**) is an approach that can be used to detect chromosomal **CNVs** (copy number gains or losses) at a greater resolution than conventional chromosomal G-banded analysis or FISH.[25]

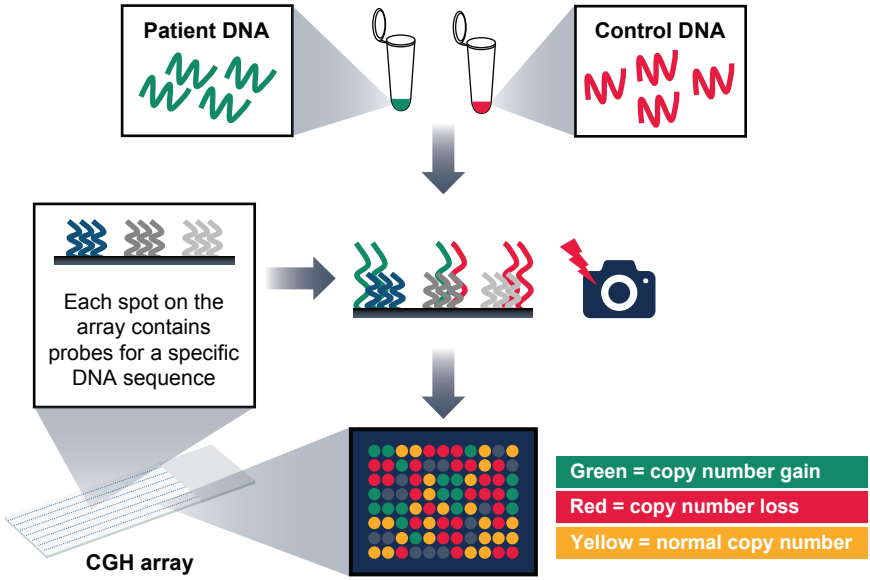

Figure 10. Array comparative genomic hybridisation. Adapted from Thiesen A, 2008.[25]

This assessment platform is often referred to as a **microarray**, but it is important to be aware that other types of microarrays exist. Array CGH and single nucleotide polymorphism (SNP) arrays, used to determine variations in individual genes, are the most commonly used microarrays in the clinical setting.[26] Other array-based platforms, used mainly in medical research, can evaluate changes in mRNA, protein, or tissues, and are used to determine variations in gene expression, assess protein–protein interactions, or examine the molecular biology or immunohistochemistry of patient samples.[27,28]

Array CGH may detect **CNVs** much more precisely than chromosomal G-banded analysis and can reveal specific genes included in a chromosomal deletion or gain.[29] However, array CGH does have limitations, and it does not replace chromosomal G-banded analysis in all situations. Array CGH will not identify balanced chromosome rearrangements (e.g. balanced translocations and inversions), point mutations (single nucleotide variants or **SNVs**), or epigenetic abnormalities, as these changes do not result in any loss or gain of chromosomal material.[30] Array CGH may not detect some types of polyploidies (e.g. triploidy—three copies of a chromosome) or low-level mosaicism (if the **CNV** is present in <20% of cells in the sample), and thus certain clinical scenarios will require chromosomal G-banded analysis (e.g. when mosaicism is suspected).[30]

The results of microarray testing are often complex and careful interpretation, often with the assistance of a clinical geneticist, is required (see **section 3.6**). Three possible results may be attained from array CGH[31]:

- A normal result with no clinically significant variation
- A definitively abnormal result with a known pathological variation
- A variant of uncertain significance (**VUS**)

CNVs often have unknown pathological significance (thus are deemed **VUS**). Furthermore, every person has approximately 100 **CNVs**, which can affect numerous genes but are only sometimes pathogenic.[32,33] It may therefore be difficult to establish the significance of some **CNVs**, and targeted array CGH of samples from the parents may be recommended in the first instance to help with interpretation.[30,31]

3.3.4 Next-generation sequencing

The advent of massively parallel sequencing, or **next-generation sequencing (NGS)**, has made it possible to sequence large sections of genomes in a short time frame.[34] Technological advances have facilitated the integration of **whole-genome sequencing** (**WGS**: sequencing of the entire genome of an individual) and **whole-exome sequencing** (**WES**: sequencing their exons) into healthcare.[35]

The continued identification of pathogenic disease-causing variants and increased understanding of genotype–phenotype correlations have led to the development of **gene panel assays** for hundreds of conditions, offering a more targeted approach to genetic testing than WGS or WES. Gene panel assays are now available for patients and their families with suspected genetic diseases with specific malformations, such as a skeletal dysplasia.[36] Overall, targeted gene panel testing, led by an experienced clinical geneticist, could be a low-cost, time-effective test, particularly if the suspected disorder has a polygenic/multifactorial origin.

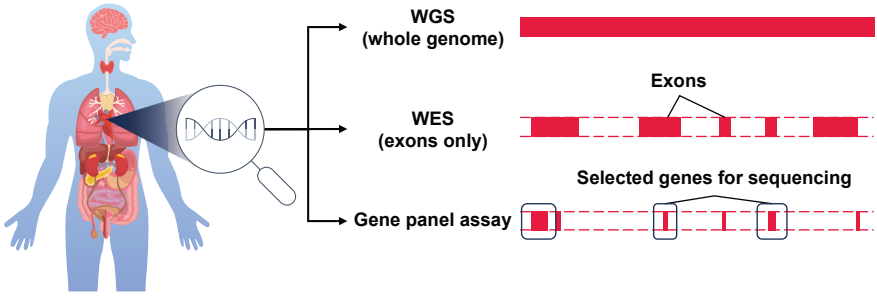

Figure 11. Approaches to next-generation sequencing analysis.

Gene panel assays are constructed, analysed, and reported with an intentional blindness to all but a specifically selected list of genes.[37] Clinicians should therefore be particularly cognizant of which genes were **not** reported when interpreting a test result. Gene panel assays may identify some genes included in a panel that are not unequivocally linked to the disease/phenotype, and for many genes the **penetrance** of a phenotype (whether a person develops any sign of a condition) is highly variable. It can therefore be challenging to translate detection of a specific variant into an absolute risk of developing a condition.[37]

3.4 Inheritance patterns

A gene **variant** can be passed onto the next generation. The new, altered version of the gene is considered a new **allele**. One chromosome within each pair is inherited from each parent, and each chromosome carries an allele for each gene. A pair of chromosomes may therefore contain different (**heterozygous**) alleles of the same gene, and all offspring may not inherit a variant allele.[38]

Over 4000 **monogenic** disorders—those that are caused by a variant in one or both alleles of a specific gene—have been described and classified by their inheritance pattern.[39] The three principal modes of inheritance are **autosomal dominant**, **autosomal recessive**, and **X-linked recessive**, while rarer patterns of inheritance include X-linked dominant, mitochondrial, triplet repeat disorders, and genetic imprinting.[40-42]

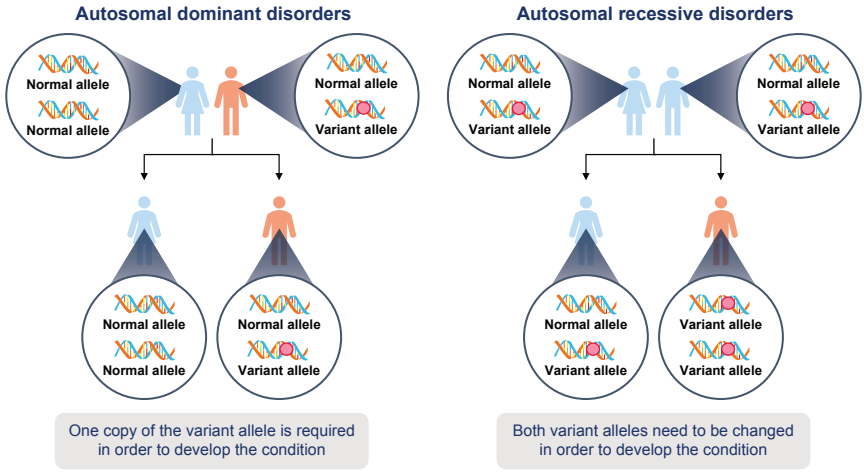

Figure 12. Autosomal dominant and autosomal recessive inheritance patterns. Individuals coloured in orange are affected by the condition, while blue individuals are unaffected.[40]

Autosomal dominant disorders are caused by a variant in one of the two alleles. Several generations are often affected within families, and usually the number of affected males and females is approximately equal. Father-to-son transmission is a hallmark of autosomal dominant inheritance. Each child born to a person with an autosomal dominant disorder has a 50% (1 in 2) chance of inheriting the **variant allele**, and consequently a predisposition to developing the condition themselves.[43] However, there is not always a family history of the condition in people with an autosomal dominant disorder, as some people can acquire a new variant by mutation of the specific gene for their condition.[44] Autosomal dominant disorders also often display variability in both **penetrance** (whether a person develops any sign of a condition) and **expression** (how the condition manifests).[45]

Autosomal recessive disorders result from variants in both alleles of a particular gene responsible for the condition. Both parents may carry a variant allele and be unaffected, as long as they also each have one normal allele.[46] In most cases, being a carrier of an autosomal recessive condition has no effect on the person. It is likely that many relatives of the patient unknowingly carry the variant. When both parents carry a variant in the same autosomal recessive gene, there is a 25% (or 1 in 4) chance of each of their children inheriting both altered variants, and probably the condition.[46] A child born to a person affected by an autosomal recessive disorder will automatically carry the condition. If one parent is a confirmed carrier of the variant and one is not, the risk to a child depends on the likelihood that the second parent carries a variant in the same gene. The frequency of autosomal recessive disorders therefore depends on the patient population, as regional populations vary in the frequency of carriers. For example, cystic fibrosis is a common autosomal recessive disorder in Western Europe, whereas the autosomal recessive disorder sickle cell anaemia is most common in West Africa.[47,48]

Sex-linked variants may result in different inheritance patterns and manifestations, depending on who carries the variant. **Y-linked** variants can only be passed from father to son, and since they one have copy of the Y-chromosome, all males who carry the variant will be affected. **X-linked** variants can be inherited by both males and females from either parent. **X-linked dominant** conditions affect all individuals who inherit the variant. **X-linked recessive** conditions, however, affect all males who inherit the variant (as they only have one X chromosome and are thus **hemizygous** for the variant), and females are only affected if they have inherited (or acquired) variants on both of their X chromosomes. Variants in **mitochondrial** genes can only be passed from mother to child, but since each cell contains many mitochondria, and some of them may not carry the variant, the condition may be expressed differently across siblings.[49]

3.5 Common challenges with genomic medicine

Physicians should be aware of a number of factors that present challenges in obtaining a reliable and informative result from genetic/genomic testing. These can occur at various points in the patient journey. Genetic counsellors and clinical geneticists can provide advice on how to navigate these potential pitfalls.[50]

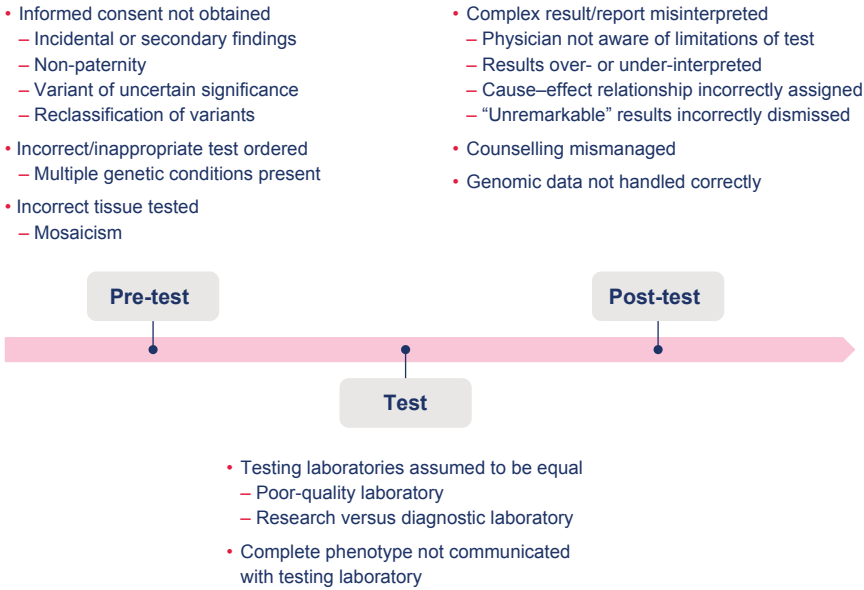

Figure 13. Pitfalls and challenges along the genetic testing journey.

3.6 Understanding gene/genome variation and establishing what is significant

The effects of sequence variation depend on where the changes occur (in a gene or in a non-coding region of the genome) and how the change affects the protein product (if located in a gene). Many sequence variants are not expected to cause disease, because they result in **neutral changes** (the protein produced from the altered gene is fully functional), or because they are exceedingly common in the population.

Gene variants are classified into standard categories based on clinical and molecular evidence for their downstream effects (following recommendations from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology).[17] Those that are known or likely to cause disease are referred to as **pathogenic** or **likely pathogenic** (pathogenic meaning disease-causing). Those that are not expected to cause disease are referred to as **benign** or **likely benign**.[17] This is the equivalent of a polymorphism, and this term is generally reserved for changes in DNA sequence that do not cause a problem. A sequence variant may also be classified as a **VUS** if there is not enough evidence to definitively classify it as pathogenic or benign.[17]

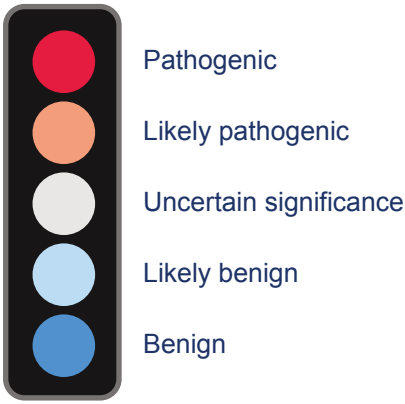

Figure 14. Classification of genetic variants.[17]

Section 4: The future of genetics and genomics

Medical genomics will go beyond DNA sequencing (**genomics**) and will incorporate assessment of additional molecular characteristics, including DNA modifications (**epigenomics**), gene expression (**transcriptomics**), protein levels and protein modifications (**proteomics**), and cellular metabolism (**metabolomics**).[51]

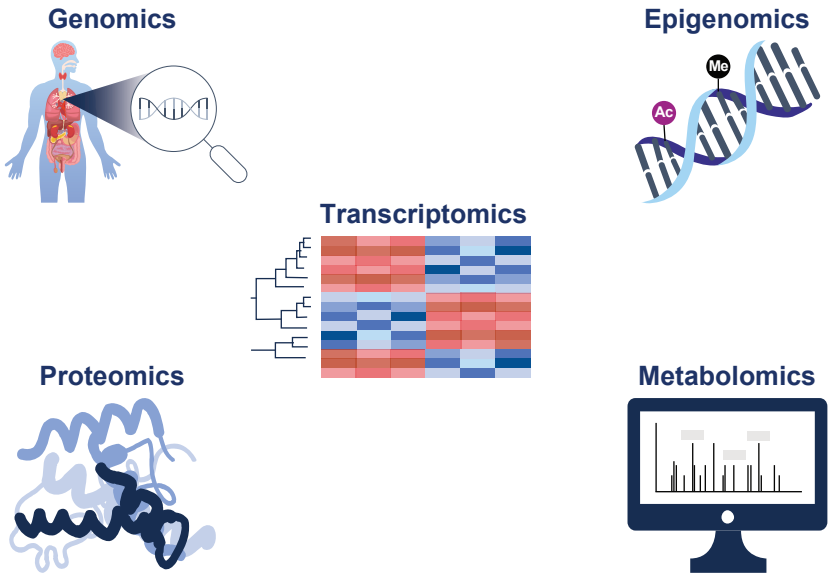

Figure 15. Technological advances will, in the future, allow deeper and more complex analysis of the molecular components of patient samples.

As the capacity for diagnosis of genetic conditions continues to increase, clinicians who may not have previously been familiar with clinical genetic terms will encounter patients with genetic diagnoses far more frequently. Clinicians and subspecialists will be required to work with clinical and lab-based geneticists to keep abreast of new developments in clinical genetics/genomics—a difficult challenge in this era of rapid technological advances.

Array CGH was added to mainstream clinical practice in the last decade, while NGS tests such as WGS, WES, and gene panel assays are fast becoming standard in clinic practice. Although exciting new technologies will increase the diagnostic rate of genetic disorders, they will also bring a new set of challenges, including an increase in the number of **VUS** and the ethical dilemma of reporting gene variants that are not associated with the phenotype under investigation.

Genetic technologies involved in non-invasive prenatal screening will likely have an increasing influence on neonatal surgical practice in the future. Genetic testing is now widely available for the investigation of trisomies 13, 18, and 21, and is being expanded to screen for many other genetic conditions.[52]

The technology to investigate the genetics/genomics of these conditions is becoming available to an increasing breadth of specialties. Close liaison between surgeons, clinical geneticists and counsellors will be essential in managing these investigations and diagnoses correctly and overcoming the associated challenges.

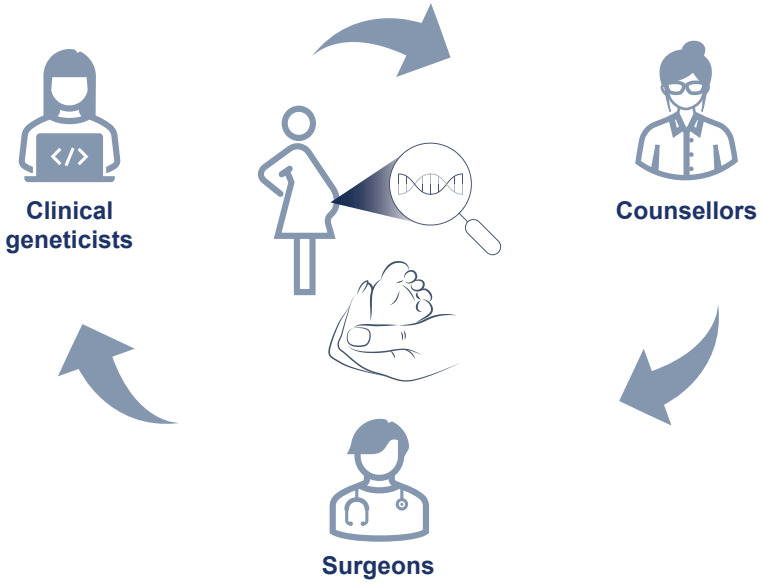

Figure 16. Genetic testing in the form of non-invasive prenatal screening is becoming increasingly important in neonatal and paediatric medicine. Communication between specialists will be essential.

Section 5: How to read a genetic test report

The genetic test report should include all the essential information that allows a healthcare provider to interpret the test result and guide disease management (**Figure 17**). As with most diagnostic tests, the report should include important clinical information from the patient, sample details, and the referring healthcare professional. A genetic test report will also include details of the assay, including the name of the assay, the **target genes** (or regions of the genome) that were assessed, and the assay **method**. The report should detail the **limitations** of the assay. Performance metrics for NGS tests may include **genome coverage** (how much of the genome was sequenced, in bases), **sequencing depth** (how many times each gene or region was sequenced), and the percentage of genes covered to a sufficient depth to ensure accurate results. The results include the variants detected, the classification of pathogenicity, along with a brief interpretation of the implications of those results, with recommendations for follow-up with the patient or family.

Figures 18–20 illustrate three example frameworks for the end user on how to approach reviewing genetic test reports. Guidance on next steps following a genetic finding are also included.

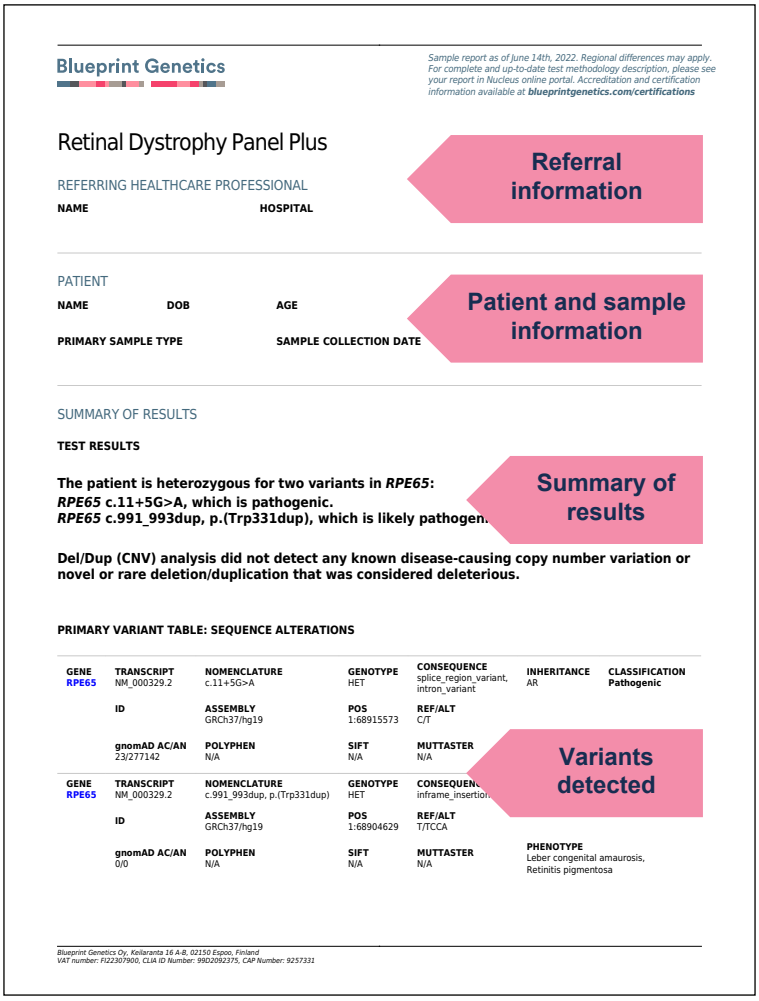

Figure 17. Sample genetic test report (available from Blueprint Genetics).[53] The report shown is for illustrative purposes only and should not be considered actual patient data. Regional differences may apply. Accreditation and certification information available at https://blueprintgenetics.com/certifications. Additional supporting information, including detailed sequencing coverage and laboratory methodology, is included in the report but not shown in this figure. (Figure continues on pages 25–27).

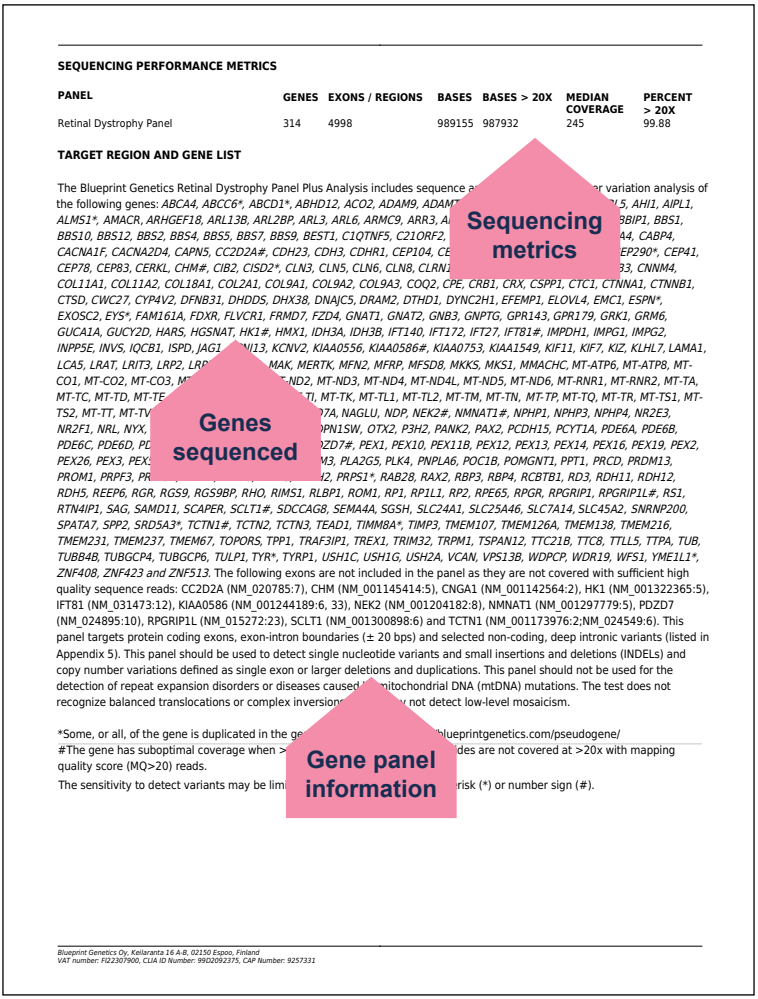

Figure 17. Sample genetic test report (continued).

STATEMENT

CLINICAL HISTORY

Patient is affected with congenital night blindness, decreased peripheral vision and pigmentary changes in the retina. Stargardt´s disease suspected. There is no family history of similar disease.

CLINICAL REPORT

Clinical information

Sequence analysis using the Blueprint Genetics (BpG) Retinal Dystrophy
an intronic splice region variant c.11+5G>A and an inframe insertion c.991
distance between these variants, NGS-based methods cannot determine whether they occur on the same (in *cis*) or different (in *trans*) parental alleles.

***RPE65* c.11+5G>A**

There are 23 individuals heterozygous for this variant in the Genome Aggregation Database population cohorts (gnomAD, n>120,000 exomes and >15,000 genomes). The variant affects the position 5 nucleotides downstream of the 5' donor splice site of intron 1 of *RPE65*. In silico splice prediction tools (SSF, MaxEnt, NNSPLICE, GeneSplicer, and HSF) predict that the variant will weaken or abolish the natural splice donor site and may therefore lead to aberrant splicing. The variant (referred to as 65+5G>A) was originally reported as either homozygous, or as heterozygous together with a frameshift variant in *RPE65*, in two patients with autosomal recessive retinitis pigmentosa (arRP) (PMID: 9326941). Subsequently, c.11+5G>A was reported in a compound heterozygous state with the disease-causing missense variant p.(Tyr368His) in two brothers with severe visual deficits and an absence of rod and cone electroretinographic responses before the age of 5 years. The patient's father was heterozygous for c.11+5G>A, and had good corrected visual acuity and normal visual fields. However, he was found to have subtle changes in his rod absolute dark-adapted threshold sensitivities and cone ERG flicker responses, and both his maculae were covered with hundreds of tiny hard drusen extending into the rod-rich retina beyond the macular arcades (PMID: 11786058). Subsequently, c.11+5G>A has been reported in multiple patients with Leber congenital amaurosis (LCA), arRP and retinal dystrophy (PMID: 17525851, 25257057, 28041643, 20683928, 29332120, 30268864, LOVD ID: RPE65_000058) as well as in a case of fund
variant p.(Ile115Thr) (PMID: 21211845).

Evidence of pathogenicity

***RPE65* c.991_993dup, p.(Trp331dup)**

This variant is absent in the gnomAD population cohorts. The variant inserts
inframe duplication of the tryptophan (Trp) amino acid at codon 331. *RPE65* c.991_993dup, p.(Trp331dup) has been reported in the literature to segregate with Leber congenital amaurosis in one family, in which the variant was detected as heterozygous together with a nonsense variant in *RPE65* in the index patient, and in a homozygous state in her affected aunt (PMID: 20683928). The variant has also been detected in the context of clinical testing and is submitted to ClinVar (Variation ID 658837). A disease-causing missense variant in the adjacent amino acid, p.(Cys330Tyr), has been shown in functional studies to result in a significant reduction in RPE65 isomerase activity, suggesting that this region of the protein may be functionally important (PMID: 16096063, 16150724, 24849605).

RPE65

RPE65, an abundant membrane-associated protein in the retinal pigment epithelium, is a key retinoid isomerase necessary for regenerating 11-*cis*-retinaldehyde in the visual cycle. Mice lacking RPE65 (*Rpe65−/−* and rd12) cannot synthesize 11-*cis*-retinoids; therefore photoreceptors in these mice lose light sensitivity. Pathogenic variants in *RPE65* are associated with retinal degenerative diseases such as autosomal recessive Leber congenital amaurosis, retinitis pigmentosa, and childhood onset retinal dystrophy (MIM *180069). Leber congenital amaurosis (LCA) is the earliest and most severe form of all inherited retinal dystrophies, which typically becomes evident in the first year of life (GeneReviews: http://www.ncbi.nlm.nih.gov/books/NBK1298/). The birth prevalence of LCA is two to three per 100,000 births. A number of genotype-phenotype correlations have emerged and it is extremely important to define the molecular diagnosis of the

Figure 17. Sample genetic test report (continued).

patients due to existing targeted therapeutic options (PMID: 27102010*)*. The prevalence of *RPE65* variants in LCA patients has been estimated at 6%-8% (PMID: 17724218, 15024725). Simonelli *et al.* showed that patients with *RPE65* variants (n=8) retain minimal visual capabilities up to 8 to 12 years and a greater integrity of retinal tissue, as shown by normal retinal thickness associated with partially preserved fundus autofluorescence. They also showed an association with night blindness. Lorenz *et al.* found that four individuals with LCA and *RPE65* pathogenic variants had measurable visual acuity at age six to ten years, despite severe visual impairment from infancy and nystagmus in three of the four (PMID: 10937591). Photophobia was not a feature and all individuals had preservation of measurable peripheral vision. Rod ERG responses were undetectable, whereas cone ERG responses were detectable in early childhood.

Thompson *et al.* screened *RPE65* in a cohort of 453 patients, of which 339 were from an unselected collection of patients with retinal dystrophy, and 114 were included on the basis of a clinical diagnosis of LCA or early-onset retinal dystrophy (PMID: 11095629). Of the latter 114 patients, 13 were found to have variants in both *RPE65* alleles (11.4%). In contrast, the *RPE65* variants accounted for 2.1% (7/339) of patients with autosomal recessive retinal dystrophy. It was concluded that *RPE65* patients share a common phenotype characterized by poor but useful visual function in early life (measurable cone ERGs) that declines dramatically throughout the school age years. In addition, a number of these patients retain residual islands of peripheral vision, although considerably compromised, into the third decade of life. Thus, the phenotype resulting from *RPE65* variants appears relatively uniform. The severity of the disease resulting from variants in *RPE65* appears to be largely independent of the variant types present in these patients.

There are 200 different disease-causing variants in the *RPE65* gene listed in the Human Gene Mutation Database (Professional 2019.3). Majority of the variants are distinct missense variants. It has been shown that the pathogenic variants eliminate the retinoid isomerase activity of RPE65. The loss of RPE65 function involves distinct mechanisms: loss of catalytic activity, a lower expression level of RPE65 or rapid degradation of the mutant protein (PMID: 16150724, 16828753).

Multiple studies have been conducted in murine and canine models of RPE65 deficiency and in human patients, in whom *RPE65* gene replacement has successfully restored cone and rod sensitivity, improved visual fields and in some cases, improved visual acuity (PMID: 27102010). The treatment is based on recombinant adeno-associated virus vector expressing RPE65 (rAAV2-CB-hRPE65). Improvement in visual function has been reported to persist for at least 3 years after treatment, however continuing retinal degeneration has been documented (PMID: 21911650, 23341635). More recent studies have reported durable benefit, but a reduction in the magnitude of improvement, after a 5- to 6-year follow-up period (PMID: 25936984). Luxturna is the first FDA approved adeno-associated virus vector-based gene therapy indicated for the treatment of patients with confirmed biallelic *RPE65* variant-associated retinal dystrophy (www.fda.gov -Luxturna).

Mutation nomenclature is based on GenBank [illegible] with nucleotide one being the first nucleotide of the translation initiation codon [illegible]

Gene information and disease association

CONCLUSION

Based on the current literature and well-established role of *RPE65* c.11+5G>A, as a disease-causing variant, we classify it as pathogenic. We classify *RPE65* c.991_993dup, p.(Trp331dup) as likely pathogenic, based on the established association between the gene and the patient's phenotype, the variant's rarity in control populations, and its detection in patients with retinal dystrophy. Disease caused by *RPE65* variants is inherited in an autosomal recessive manner. Testing of parental samples is needed to determine whether the variants occur in *cis* (on the same allele) or in *trans* (on different alleles). Compound heterozygosity of the variants (variants in *trans*) would most likely explain the patient's clinical presentation. If the patient's parents are each found to be heterozygous carriers of one of these variants, then any siblings of the patient will have a 25% chance of being compound heterozygous for these variants and thus affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier. Genetic counseling and family member testing are recommended.

Interpretation

Figure 17. Sample genetic test report (continued).

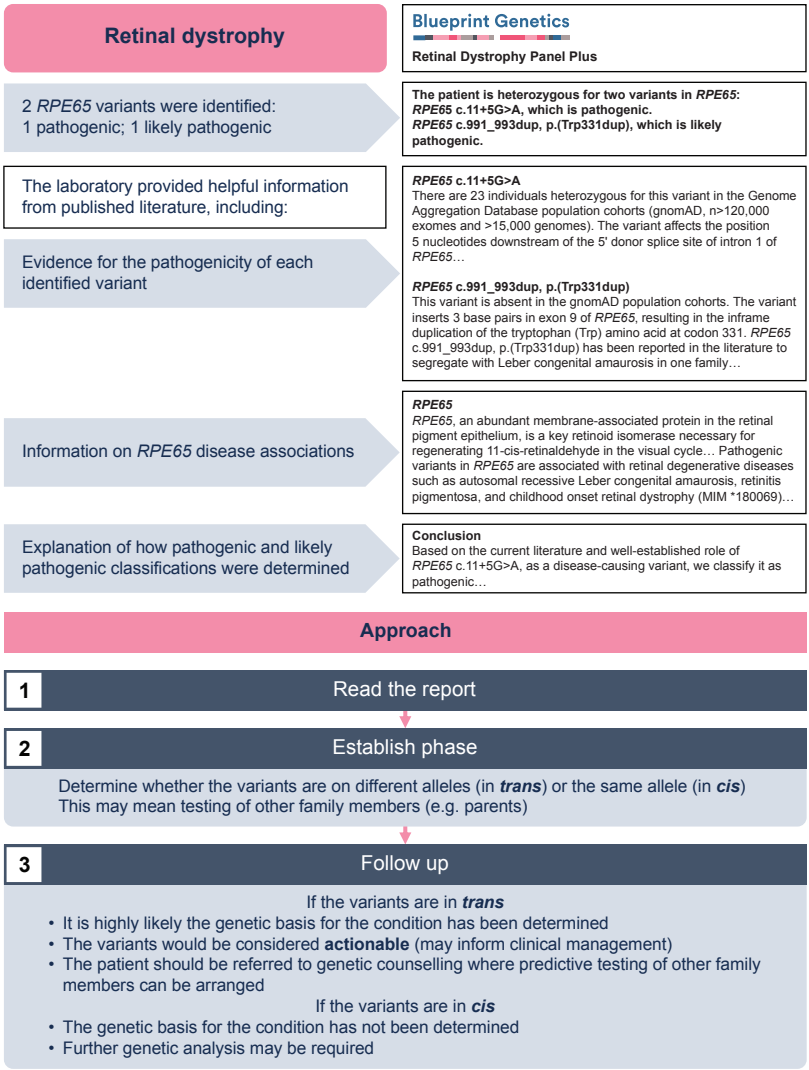

Figure 18. Example approach to reviewing a report from a genetic test for retinal dystrophy. Sample genetic test report available from Blueprint Genetics.[53] The report shown is for illustrative purposes only and should not be considered actual patient data. Regional differences may apply. Accreditation and certification information available at https://blueprintgenetics.com/certifications.

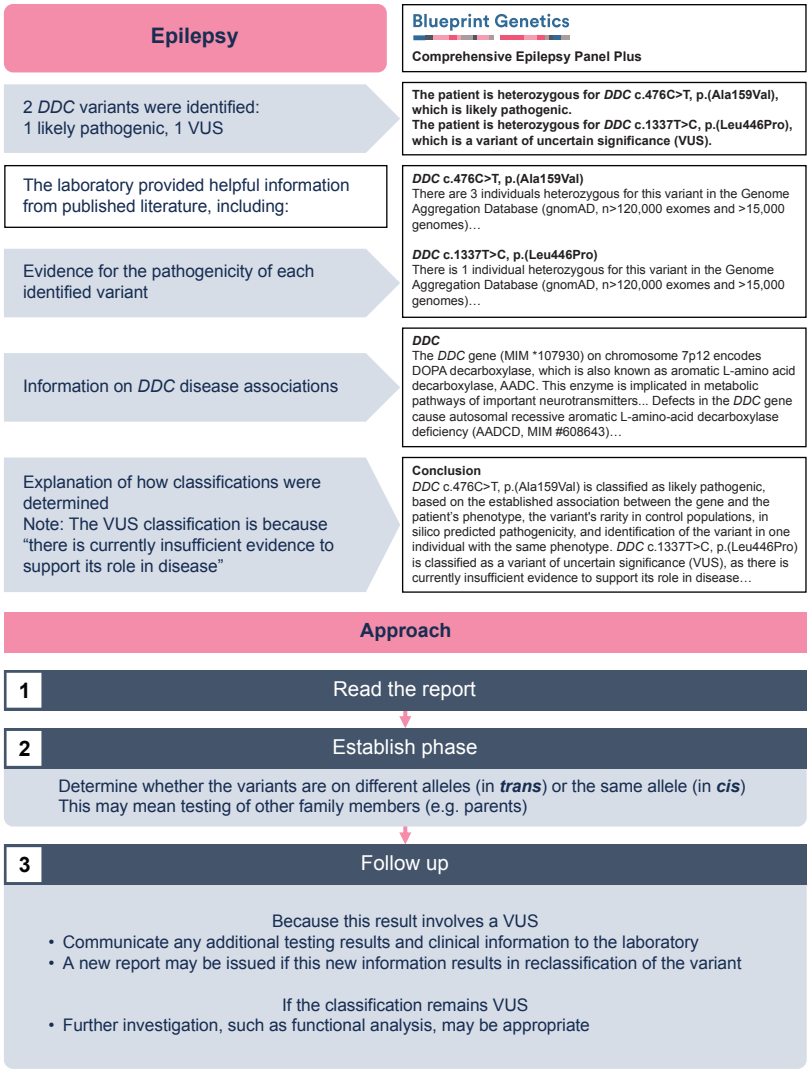

Figure 19. Example approach to reviewing a report from a genetic test for epilepsy. Sample genetic test report available from Blueprint Genetics.[54] The report shown is for illustrative purposes only and should not be considered actual patient data. Regional differences may apply. Accreditation and certification information available at https://blueprintgenetics.com/certifications.

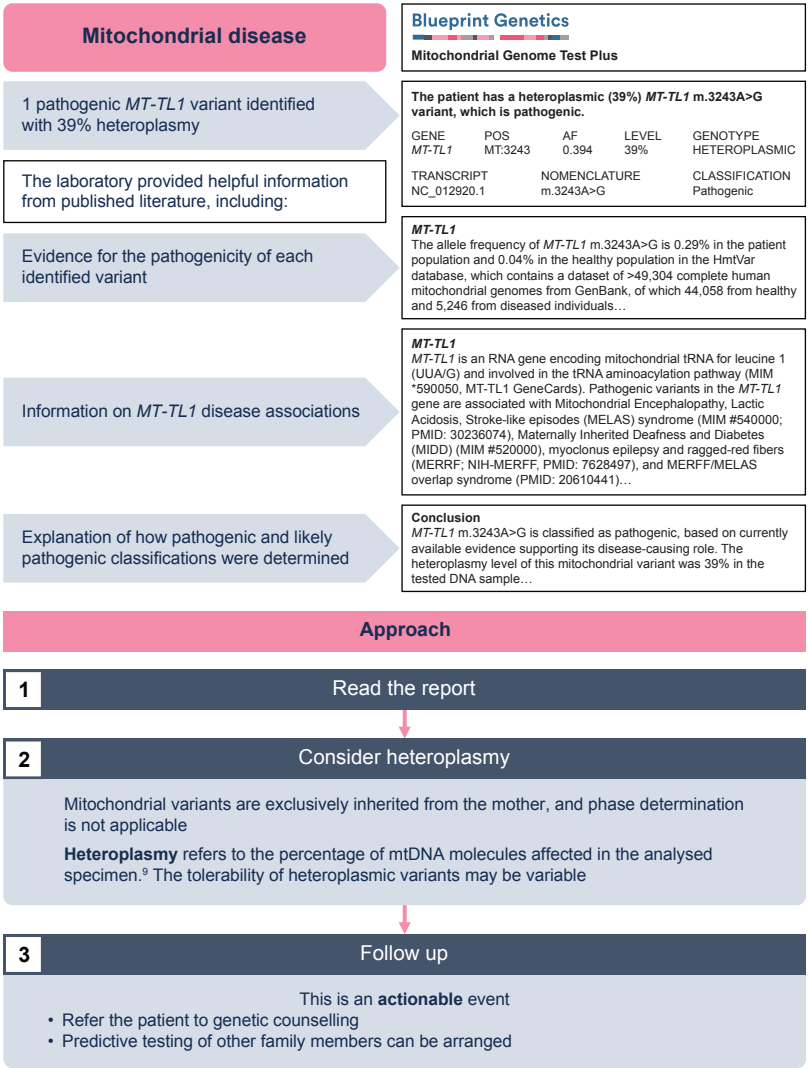

Figure 20. Example approach to reviewing a report from a genetic test for mitochondrial disease. Sample genetic test report available from Blueprint Genetics.[55] The report shown is for illustrative purposes only and should not be considered actual patient data. Regional differences may apply. Accreditation and certification information available at https://blueprintgenetics.com/certifications.

Section 6: Glossary of genetics and genomics terms

Allele: A variation of a gene occupying a given locus on a chromosome that can be acquired spontaneously or inherited from either parent. Many different alleles exist for most genes

Array comparative genomic hybridisation (CGH): A technique using fluorescently labelled reference and test DNA to identify genomic copy number imbalances (often referred to as "microarray")

Autosomal chromosome: A non-sex chromosome that is normally found in both males and females. Humans have 44 autosomal chromosomes

Autosomal dominant: An inheritance pattern characterised by transmission through several generations, male-to-male transmission, and a 50:50 risk to the children of any affected person

Autosomal recessive: An inheritance pattern characterised by several affected members of the same generation affected by a genetic condition, with carrier parents and a 1:4 recurrence risk where both parents are carriers

Base: The nitrogen-rich component of **nucleotides**, including adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). It is the sequence of bases in the nucleic acid that determines a gene product, and it is this sequence that is reported in DNA sequencing reports. Bases pair together in a specific manner (A with T; G with C) to maintain stability within the DNA molecule, which is why gene sequences are often described in **base pairs (bp)**, the unit of double-stranded DNA

Centromere: The constricted region in the chromosome that includes the site of attachment to the mitotic and meiotic spindle

Chromosome: A threadlike structure composed of a long, continuous **DNA** molecule containing an ordered sequence of linked genes organised around a protein scaffold called chromatin. Chromosomes are located in the nucleus of eukaryotic cells. All organisms have a specific number of chromosomes per cell, but this number varies from one organism to another. Chromosomes come in pairs, and the individual chromosomes of each pair are considered homologous—that is, they are identical to one another with respect to the **genes** they contain. However, a pair of chromosomes may contain different **alleles** of the same gene

Chromosomal G-banding (Geimsa-banding): A technique for analysing the overall structure of the chromosomes in a cell (often referred to as "karyotyping")

Codon: A three-base-pair element of **DNA** which encodes an amino acid

Compound heterozygote: An individual with two different variants in both alleles at the *same* locus (Note: A double heterozygote is an individual who has variants at two *different* loci)

Consanguinity: A situation where parents are related through a common ancestor and share a proportion of their genetic material. In practice, a consanguineous relationship is often considered as one between individuals who are second cousins or closer

Consultand: An individual seeking advice about a genetic disorder

Deoxyribonucleic acid (DNA): The macromolecule that stores and transmits genetic information from one generation to the next. Growing organisms use DNA as a blueprint to make all their body parts. DNA polymers are generated from thousands of **deoxyribonucleotides**, and form long, ladder-like structures, with a deoxyribose sugar-phosphate backbone and paired **bases** across the centre. DNA polymer twists to form a double helix structure that is remarkably robust and durable. This structure was described in 1953 by James Watson and Francis Crick, along with Rosalind Franklin

Exome: The sum of all protein-coding regions (**exons**) of the **genome**

Exon: A region of a gene that becomes part of the mRNA after transcription and RNA splicing. Exons include regions of a gene that code for protein products. Exons form approximately 1% of the whole genome[56]

Expression: The level of severity of the clinical manifestation caused by a gene **variant** (not to be confused with gene expression, below)

Fluorescence in situ hybridisation (FISH): A technique for studying specific chromosomes or regions of chromosomes

Gamete: A germ cell (sperm or oocyte)

Gene: A defined segment of DNA which codes for a product (protein or nucleic acid) that contributes to a cellular function or **phenotype**. It is the fundamental unit of heredity

Gene expression: The combined process of transcription, translation, protein transport, and modification that ensures conversion of the DNA genetic code into a functional protein

Genome: The sum of all **DNA** in an organism, including all of its **genes** and non-coding regions. The human genome contains 20,000–30,000 **genes** on 23 pairs of **chromosomes**, as well as a small mitochondrial chromosome[10]

Genotype: The genetic constitution (**alleles**) of an individual at one or more **genes** or **loci**. The genotype may be used to predict an individual's **phenotype**

Hemizygous: The presence of only one copy of a gene. Males are hemizygous for most genes on the X chromosome

Heterozygous: The presence of two different alleles at a specific **locus**

Intron: A non-coding region of a gene that is found between **exons**. Introns are transcribed but subsequently removed from the pre-mRNA transcript by splicing together the **exons** on either side of it

Isochromosome: An abnormal chromosome made up of two long or two short arms of a normal chromosome

Karyotype: An analysis of the number and structure of an individual's chromosomes (determined by **chromosomal G-banding**)

Locus: A specific, unique chromosomal region that corresponds to a gene or other DNA sequence (plural is **loci**)

Nucleotide: The building blocks of nucleic acids (DNA and RNA)

- **DNA** is made from polymers of **deoxyribonucleotides**, comprising a base (A, G, C, or T), a deoxyribose sugar, and phosphate molecules
- **RNA** is made from polymers of **ribonucleotides**, which comprise a base (A, G, C, or U), a ribose sugar, and phosphate molecules

Penetrance: The probability of the carrier of a variant showing signs of the condition, from the most trivial to the most severe

Phenocopy: The mimicking of a disorder usually caused by a variant in a given gene by variants in a different gene or by environmental factors

Phenotype: The appearance, physical traits, or other characteristics of the patient resulting from the interaction of the genotype and environment

Pleiotropy: The production by a single gene of two or more apparently unrelated diseases

Polymerase chain reaction (PCR): A technique used to generate large amounts of specific DNA from a small amount of target sequence

Preimplantation genetic diagnosis (PGD): A genetic analysis of an *in vitro* fertilised embryo to test for genetic disorders with the intention of implanting an embryo that is not predicted to develop the condition

Ribonucleic acid (RNA): A nucleic acid polymer composed of thousands of **ribonucleotides**. In contrast to **DNA**, single-stranded RNA molecules can be very unstable and decompose rapidly

- Messenger RNA (**mRNA**) is a short-lived molecule that is used by the cell to make proteins as part of gene expression. mRNA carries the code out of the nucleus to be translated into a protein in the cytoplasm
- Ribosomal RNAs (**rRNA**s) are components of the ribosome, which generates chains of protein products from individual amino acids during **translation**
- Transfer RNA (**tRNA**) facilitates the transport and incorporation of individual amino acids into proteins

Segregation analysis: A means to trace a DNA change through a family to test whether the change is co-inherited with the disease state

Sex chromosomes: The chromosomes that carry sex-determining genes. Sex chromosomes are found in different numbers in males and females. Human cells contain two pairs of sex chromosomes. Females have two X chromosomes, while males have one X and one Y chromosome

Single nucleotide polymorphism (SNP): The occurrence in a population of different nucleotides at particular sites in the genome. SNPs are generally not disease causing and are present at an appreciable frequency. They can be used in association studies, and several adjacent SNPs can be combined into a haplotype

Transcription: The process of creating an **mRNA** transcript from a **DNA** sequence

Translation: The process of creating a polypeptide (a protein) from an **mRNA** transcript

Variant: A permanent change in the genetic material that can be transmitted to offspring. A pathogenic variant may result in an alteration in the function of the gene product. Two or more non-pathogenic variants (alleles, sequence variants, chromosomal variants) can co-exist in a population; those variants occurring at a frequency of >1% in a given population were previously referred to as **polymorphisms**

Variant of uncertain significance (VUS): A gene variant for which there is not enough information known to classify it as pathogenic or benign

Wild type: The term used to indicate the normal allele or the normal phenotype

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