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# Clinical Genetic Testing in Endocrinology: Current Concepts and Contemporary Challenges

**Short Title:** Genetic Testing in Endocrinology

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**SUMMARY**

Recent advances in DNA sequencing technology have led to an unprecedented period of disease-gene discovery offering many new opportunities for genetic testing in the clinical setting. Endocrinology has seen a rapid expansion in the taxonomy of monogenic disorders, which can be detected by an expanding portfolio of genetic tests in both diagnostic and predictive settings. Successful testing relies on many factors including the ability to identify those at increased risk of genetic disease in the busy clinic as well as a working knowledge of the various testing platforms and their limitations. The clinical utility of a given test is dependent upon many factors, which include the reliability of the genetic testing platform, the accuracy of the test result interpretation and knowledge of disease penetrance and expression. The increasing adoption of 'high-content' genetic testing based on next-generation sequencing (NGS) to diagnose hereditary endocrine disorders brings a number of challenges including the potential for uncertain test results and/or genetic findings unrelated to the indication for testing. Therefore, it is increasingly important that the clinician is aware of the current evolution in genetic testing, and understands the different settings in which it may be employed. This review provides an overview of the genetic testing workflow, focusing on each of the major components required for successful testing in adult and paediatric endocrine settings. In addition, the challenges of variant interpretation are highlighted, as are issues related to informed consent, prenatal diagnosis and predictive testing. Finally, the future directions of genetic testing relevant to endocrinology are discussed.

## INTRODUCTION

Establishing a genetic diagnosis may have many potential benefits for the patient and wider family. Until recently, genetic testing in the endocrine clinic was typically reserved for the diagnosis of a limited number of hereditary monogenic syndromes (e.g. Multiple Endocrine Neoplasia Type 1 (MEN1) or Type 2 (MEN2)) or disorders associated with an abnormal complement of sex chromosomes (e.g. Turner syndrome, Klinefelter syndrome). However, recent advances in DNA sequencing technologies (i.e. 'next-generation sequencing' (NGS)) have resulted in an intense period of disease-gene discovery, and a greater insight into the molecular-genetic basis of many endocrine disorders.[1,2] These advances now offer many new opportunities for genetic testing in the clinical setting and the potential for improved health outcomes. At the same time, the increased use of genetic testing brings many challenges, not least those associated with accurate test interpretation and deciphering the impact of genetic variation on human health.[3-5] Therefore, prior to the widespread adoption of new testing strategies it is important to consider both the potential utility and validity of any given test, as well as evaluating any harm that may arise. For example, factors that influence the clinical utility and validity of a genetic test include: the genetic heterogeneity of the disorder; the reliability of the test result (i.e. accuracy of variant interpretation); and knowledge regarding disease penetrance and expressivity. In contrast, potential harm may arise from variant misclassification (e.g. benign variants reported as pathogenic), uncertain test results or the identification of incidental findings (i.e. genetic changes unrelated to the indication for testing), as well as psychological impacts from testing. It is increasingly important that the clinician has an awareness of these considerations when deciding whether genetic testing is indicated, as injudicious use may result in clinical uncertainty and/or unfavourable outcomes. This review provides an overview of each component of the genetic testing workflow, focusing on how such testing may be integrated into the overall clinical evaluation of the patient. In addition, it highlights several of the current challenges, before discussing possible future directions of genetic testing relevant to the endocrine field.

## GENETIC BASIS OF ENDOCRINE DISEASE

The successful application of genetic testing in the clinical setting relies upon an accurate understanding of the genetic architecture of the disease or phenotype in question[6]. In the broadest sense, genetic architecture refers to the landscape of heritable genetic variation that contributes to a given phenotype, and includes the number of variants influencing a phenotype their effect size and frequency in the population, as well their interaction with each other and the environment.[6] Although the contribution of genetic variation to many phenotypes frequently represents a continuum, in the clinical context, genetic architecture is frequently divided into single-gene monogenic disorders and oligogenic/polygenic complex traits. Currently, the majority of genetic testing employed in the endocrine clinic focuses on the detection of monogenic 'Mendelian' disorders and these are the predominant focus of this review. A smaller number of endocrine disorders resulting from large structural genetic changes (i.e. chromosomal abnormalities) may also be amenable to genetic testing and are briefly discussed.

### Monogenic Endocrine Disease

Monogenic endocrine disorders most frequently result from germline mutations affecting the coding sequence of the responsible gene. The successful recognition and diagnosis of these disorders relies on knowledge of: the mode of inheritance; the types of genetic abnormality responsible for disease; the disease penetrance and clinical expressivity; and the degree of genetic heterogeneity.

***Mode of inheritance***: Monogenic disorders may be inherited in one of six patterns (Table 1); autosomal dominant (e.g. MEN1 and MEN2 due to mutations in the *MEN1* and *RET* genes, respectively); autosomal recessive (e.g. 21-hydroxylase deficiency due to mutation in

the *CYP21A2* gene*)*; X-linked dominant (e.g. X-linked hypophosphataemic rickets due to mutation in the *PHEX* gene), X-linked recessive (e.g. Kallman Syndrome due to *ANOS1*/*KAL1* mutation); Y-linked (e.g. azoospermia and oligospermia due to deletions of part of the Y-chromosome (e.g. *USP9Y* gene)); and non-Mendelian mitochondrial inheritance (e.g. hypogonadotrophic hypogonadism and hypoparathyroidism associated with Kearns-Sayre syndrome due to mitochondrial DNA (mtDNA) deletion)).[7] In addition, germline mosaicism, in which a post-zygotic mutation occurs in the germ cells of a parent, may result in an apparent autosomal recessive pattern of inheritance, with multiple affected offspring of unaffected parents (e.g. observed for **Carney complex and** osteogenesis imperfecta type II).[8,9] Parent-of-origin effects may also be observed due to genomic imprinting. The clinical effects of pathogenic *GNAS*, *SDHD* and *SDHAF2* variants are determined by their parental origin. Maternally-inherited inactivating *GNAS* mutations give rise to Albright hereditary osteodystrophy (AHO) with resistance to multiple hormones (i.e. pseudohypoparathyroidism type 1a), whilst the equivalent paternally-inherited mutation gives rise to AHO without accompanying endocrine problems (pseudopseudohypoparathyroidism).[10] The development of phaeochromocytoma/paraganglioma (PPGL) in patients harbouring *SDHD* or *SDHAF2* mutations is usually limited to those with paternally-inherited variants.[11-14] Monogenic endocrine syndromes may also occur in the absence of an inherited defect due to *de novo* mutations (i.e. mutations arising during parental gametogenesis, or post-zygotically in the developing embryo). **For example, the majority of cases of MEN2B and X-linked Acrogigantism (XLAG) result from such *de novo* genetic events (Table 1).[15-17]**

***Genetic abnormalities leading to monogenic disease***: The most common genetic abnormalities associated with monogenic endocrine disorders are either single nucleotide variants (SNVs) (substitution of one nucleotide by another) or small insertions or deletions (indels) affecting the coding-region or splice sites of the associated gene. Disease-associated SNVs typically result in missense amino acid substitutions, nonsense mutations,

or defects in splicing, whilst in-frame indels typically disrupt protein function through gains or losses of one or more amino acids, amino acid substitutions, generation of stop codons (i.e. nonsense mutations) or defects in splicing (for descriptions see Glossary of Terms). SNVs and out-of-frame indels (frameshift changes) that predict a premature truncation of amino acid sequence frequently result in a loss-of-function (LOF) of the gene. In addition to SNVs and indels, monogenic disorders may also result from larger structural genetic defects (e.g. copy number variations (CNVs)) including partial or whole gene deletions (e.g. VHL, MEN1, Carney Complex) **as well as gene duplications (e.g. XLAG).**

***Disease penetrance and expressivity****:* Penetrance refers to the likelihood that an individual carrying a disease-causing variant will manifest the disorder (Figure 1).[18] Many monogenic disorders are highly penetrant, such that close to 100% of variant carriers manifest disease (e.g. MEN1). In contrast, several display reduced penetrance; for example, mutations in *AIP* are reported to be associated with a **~20-25%** risk of pituitary tumour development;[19-21] whilst the risk of PPGL in non-proband *SDHB* and paternally-inherited *SDHD* mutation carriers is reported to be ~22% and ~44% at 60 years of age, respectively.[11,12] Furthermore, penetrance may vary substantially between alleles in the same gene.[22] For example, population-based studies indicate that the p.Val804Met *RET* variant confers a much lower lifetime risk of medullary thyroid cancer (MTC) than other MEN2A-associated *RET* mutations.[23,24] For variants with reduced penetrance, the additional genetic and/or environmental factors contributing to disease expression remain to be defined. Disease expressivity refers to the range of disease phenotypes observed between individuals harbouring the same variant. For some monogenic disorders, individuals carrying the same variant, even within the same kindred, may manifest markedly different phenotypes (e.g. Neurofibromatosis Type 1 (NF1), MEN1).[25,26] **In contrast, some disorders display a strong genotype-phenotype correlation. For example, in MEN2, clinical expression and disease severity are predicted by the specific *RET* mutation,[27] whilst in Carney complex, large-scale deletions of the *PRKAR1A* locus typically result in**

**more severe phenotypes than those associated with *PRKAR1A* SNVs or indels, presumably due to the loss of additional genes in adjacent genomic regions.[28,29]**

*Genetic heterogeneity*. Genetic heterogeneity describes the situation in which similar clinical phenotypes may result from different genetic abnormalities. For example, germline mutations in >15 different genes have been reported in patients with hereditary PPGL,[30] whilst genetic defects in >30 genes have been implicated in autosomal forms of hypogonadotrophic hypogonadism.[31,32] In some instances, different variants in the same gene may give rise to different clinical phenotypes (e.g. loss- and gain-of-function *RET* mutations associated with Hirschsprung disease, and MEN2, respectively), whilst in other situations the severity of disease is determined by whether there is a dominant or recessive pattern of inheritance (e.g. severe perinatal and infantile forms of hypophosphatasia result from autosomal recessive *TNSALP* mutations, whilst later-onset forms are typically inherited in an autosomal dominant manner).[33]

**Chromosomal Disorders**

Large-scale abnormalities affecting all or part of one or more chromosomes may account for several endocrine genetic disorders. These include conditions associated with an abnormal number of sex chromosomes (i.e. aneuploidy) such as Klinefelter (47, XXY) and Turner (45,X0) syndromes. Alternatively, there may be gains or losses of chromosomal material (e.g. 22q11.2 deletion syndrome, or Prader-Willi syndrome (Table 1), as well as translocations and inversions. Typically these genetic abnormalities are not inherited, instead arising from errors in cell division during gametogenesis or early embryonic development, although autosomal patterns of inheritance may occur if fertility is preserved (e.g. ~15% of cases of 22q11 deletion syndrome are inherited from an affected parent). The phenotype is associated with the gains or losses of genetic material and there be overlap

with monogenic disorders (e.g. 17q24.2-q24.3 deletions which include the *PRKAR1A* gene demonstrate features of Carney complex).[29] **Several chromosomal disorders may be associated with mosaicism (e.g. Turner and Klinefelter syndrome), in which the error in mitosis arises post-fertilization (i.e. during early embryonic development), and patients frequently manifest milder phenotypes.[8]**

## APPLICATION OF GENETIC TESTING

### Potential Value of genetic testing

The decision to undertake genetic testing should be driven by the likelihood of it contributing to improved health outcomes in the patient or wider family. In some instances the benefits may be clear (e.g. guiding specific treatment or intervention), whilst in other situations, the benefits (and conversely, the potential harms) may be harder to quantify. The potential value of a given test is dependent on the clinical utility and validity in relation to the specific clinical context.[34]

***Clinical utility:*** Establishing the clinical utility of a test can be challenging, as this may not only concern the patient, but also the wider family. For the patient, a genetic diagnosis may have important diagnostic, prognostic and therapeutic implications. For example, a diagnosis of MEN1 in a patient may lead to the early detection of otherwise asymptomatic tumours through the implementation of surveillance screening programmes.[26] Genetic testing may guide patient follow up; for example, patients with PPGL due to *SDHB* mutations are at a higher risk of metastatic or recurrent disease.[11,35] In some instances a genetic diagnosis may guide therapy; for example, children with infantile hypophosphatasia (i.e. due to bi-allelic *TNSALP* mutations) may benefit from enzyme replacement therapy with alfotase-

alpha.[33,36,37] Genetic testing may also resolve diagnostic uncertainty, thereby avoiding additional downstream investigations and/or treatment,[38,39] whilst negative genetic testing may alleviate anxiety and/or uncertainty for the patient. Often, establishing a genetic diagnosis in a patient has benefit to family members by enabling predictive testing of 'at-risk' individuals (e.g. first-degree relatives). In this setting, family members identified to carry the mutation can receive appropriate clinical care (e.g. surveillance and/or treatment), whilst relatives not harbouring the mutation can be reassured. For example, the detection of MEN2-associated *RET* mutations in patients with MTC, may facilitate 'prophylactic' thyroidectomy in 'at-risk' children (i.e. individuals harbouring high-risk *RET* mutations) prior to the onset of advanced disease.[27] Establishing a genetic diagnosis may also provide valuable information in the pre-pregnancy or antenatal settings, facilitating genetic counselling, pre-conception and/or pre-natal genetic testing.[40] Finally, in some instances the clinical value of genetic testing may only emerge over time through clinical research initiatives. For example, the collection and sharing of detailed phenotypic and genetic data from patients with ultra-rare disorders may facilitate novel disease gene discovery.[4,39] Any potential clinical benefits of testing must be counterbalanced against any harm that might arise, which could include; negative psychological impacts of being labelled with a genetic diagnosis; guilt over transmission to family members; and the potential for stigmatisation and/or negative discrimination.

***Clinical Validity*:** For optimal clinical application, a genetic test should have high clinical sensitivity, maximal specificity and be cost effective. Clinical validity defines the accuracy with which the test predicts a particular disease or phenotype and is determined by several factors including: the certainty with which a given genetic variant is associated with the disorder; the likelihood that a pathogenic variant carrier will develop disease (i.e. penetrance); the degree of genetic heterogeneity; and the extent of clinical expressivity. It is also important to consider the clinical setting of the test. For example, uncertainty over

variant pathogenicity and/or penetrance will substantially reduce the validity of testing in the predictive setting.

## IDENTIFYING PATIENTS WITH GENETIC DISEASE IN THE CLINICAL SETTING

Genetic testing is typically requested following the identification of a particular clinical phenotype and/or establishing a relevant family history. Several features in the clinical presentation may alert the clinician to the possibility of a genetic disorder (Figure 2), and include: the identification of specific clinical features either individually or in combination (e.g. marfanoid habitus with multiple mucosal neuromas in MEN2B, café-au-lait patches and axillary freckling in Neurofibromatosis Type 1); an early age of disease-onset (e.g. ~50% of young children and adolescents with primary hyperparathyroidism are reported to have an underlying monogenic disorder);[41] 'severe' clinical features (e.g. parathyroid carcinoma in the Hyperparathyroidism-Jaw Tumour Syndrome); and tumour multiplicity, including combinations of both endocrine and non-endocrine tumours (Table 1).

The patient's family history is paramount in establishing a genetic diagnosis (and in determining the likely mode of inheritance). However, often limited information is available due to: medical histories not being shared between family members; geographical or social separation of the kindred; relatives dying prematurely of causes unrelated to the disorder; incomplete or variable disease penetrance; small kindred size with insufficient family members to establish clear pedigrees; or instances of non-paternity. Knowledge of the geographical origin of the kindred may also be important, and indicate the presence of a known founder mutation (e.g. acromegaly/gigantism due to the Arg304Ter *AIP* variant in Ireland,[42] MEN2A due to Cys611Tyr *RET* mutation in Denmark).[43] A relevant family history will be absent in instances of *de novo* mutation **(e.g. the majority of patients with MEN2B,**

**female cases with XLAG),** disorders associated with somatic mosaicism (e.g. McCune-Albright syndrome, **sporadic male cases with XLAG**), and is often absent for autosomal recessive disorders and disorders with reduced disease penetrance.

The recognition and diagnosis of genetic disease in the paediatric setting requires specialist expertise. Several monogenic endocrine disorders present in the neonatal period or early childhood (e.g. disorders/differences of sexual development (DSDs), congenital hyperinsulinism due to *ABCC8* or *KNJ11C* mutations, neonatal severe hyperparathyroidism due to homozygous *CASR* mutations, or diabetic phenotypes due to mutations in genes involved in pancreatic development or β-cell function).[44-48] Furthermore, endocrine manifestations may be a component of complex paediatric phenotypes due to structural genetic abnormalities.[49] Again, establishing relevant family history is essential. A history of parental consanguinity significantly increases the likelihood of autosomal recessive disease (e.g. congenital hypothyroidism/thyroid dysgenesis due to homozygous *TSHR* mutations).[50] Establishing a genetic diagnosis in infants and children may have several benefits including: establishing appropriate treatment (e.g. early thyroidectomy in patients with MEN2B); alerting the clinician to the possibility of co-existing and/or future health problems; and facilitating appropriate genetic counselling to parents with regard risks to future offspring.

***Predictive genetic testing in asymptomatic individuals:*** The testing of individuals at increased risk of genetic disease (e.g. first-degree relatives of patients harbouring pathogenic variants) is typically the remit of the clinical genetics team, requiring appropriate genetic counselling. The utility of predictive testing relies on accurate estimates of variant pathogenicity and penetrance as well as the evidence base supporting downstream clinical interventions based on screening results (e.g. surveillance imaging for hereditary tumour syndromes). Where the meaning of prior genetic testing results in the family is uncertain

(e.g. identification of a variant of uncertain significance (VUS)), predictive testing is not usually recommended, although support for pathogenicity may evolve if the variant is subsequently observed in additional family members who later develop the disease, **or is reported in affected individuals from other kindreds.** Where first-degree relatives already manifest clinical features suggestive of the disorder, genetic testing is still recommended as such individuals may represent phenocopies (i.e. appearing to have a trait or disease associated with a particular genotype, who do not carry that genotype).[51] For example, a first-degree relative of a patient with a known *MEN1* mutation, who has a pituitary adenoma, may not harbour the familial *MEN1* mutation but instead have a sporadic pituitary tumour.[51] Predictive testing in the paediatric setting is usually only appropriate for disorders, which are likely to have a childhood-onset, and for young children will require authorization from a parent or guardian. Predictive testing in children for adult-onset conditions is not usually recommended and is typically deferred until an age where the individual can make their own decisions regarding testing.[52]

**Ethical Considerations and Informed Consent**

Obtaining informed consent is an essential component of the genetic testing workflow (Figure 2) and should be tailored to the individual and the test in question. Pre-test counselling should aim to address patient concerns and frequently covered issues include the implications of positive test results for family members and the risk of transmission to children. Whilst the majority of patients are willing to share information with family members, occasional conflicts may arise. Different viewpoints have been expressed regarding the obligation of affected individuals to share information with 'at-risk' first-degree relatives, although appropriate genetic counselling often resolves such issues. In the paediatric setting, conflicts may arise if parents are reluctant to give consent for diagnostic or predictive testing of their children, whilst the inclusion of parental DNA samples to improve diagnostic

yield (e.g. identification of *de novo* mutations through 'trio' testing), has the potential to reveal genetic findings relevant to the parents' health (e.g. *BRCA* mutations). Specific policy statements on genetic testing of children have been published to cover some of the complexities that may arise.[52] A further common concern of patients undergoing genetic testing is whether there will be implications for future employment or ability obtaining health-related insurance. Many countries have legislation in place to protect individuals from such discrimination (e.g. the Genetic Information Non-discrimination Act (GINA) in the United States). In the United Kingdom, a voluntary moratorium exists between the government and insurance industry, which aims to avoid such discrimination. High-content genetic testing (i.e. gene-panel, **whole exome sequencing (WES), whole genome sequencing (WGS))** raises many additional ethical issues including: whether samples should be analysed for the presence of 'actionable' variants in genes unrelated to the disease phenotype (**i.e. incidental findings**); how the large-data files generated can be stored safely whilst protecting patient privacy; and the extent to which genetic and phenotypic data should shared with the wider scientific community.

## GERMLINE GENETIC TESTING – SELECTING THE OPTIMAL TEST

The selection of the most appropriate test is determined by a number of factors including the likely genetic abnormality present (e.g. SNV, indel or structural abnormality), the genetic heterogeneity of the disorder, the availability of samples from additional family members, and whether testing is being undertaken in the diagnostic or predictive setting. In most instances, the detection of monogenic endocrine disorders genetic testing requires high-resolution DNA sequencing, whilst **the** diagnosis of disorders associated with larger chromosomal abnormalities requires alternate methods.

## Tests to detect Chromosomal Abnormalities

*Detection of aneuploidy and large chromosomal abnormalities:* Conventional karyotyping detects abnormalities of chromosome number (e.g. aneuploidy) or structure (e.g. CNVs, inversions, translocations) with limited genomic resolution (e.g. 3-10Mb). In the endocrine clinic, karyotyping is frequently requested to detect Turner or Klinefelter syndrome, although is increasingly replaced with array comparative genomic hybridisation (aCGH), which has improved resolution (typically 100-400kb), **and may also detect a proportion of those with mosaicism**. aCGH detects the majority of structural abnormalities (with the potential exception of balanced translocations) and is commonly used as the first-line genetic investigation for the children with neurodevelopmental delay or congenital abnormalities,[49] and includes evaluation for the 22q11.2 deletion syndrome and Prader-Willi syndrome (Table 1). Additional methods to detect structural changes include: fluorescence in situ hybridization (FISH), which can be designed to identify abnormalities located to specific chromosomal regions (e.g. translocations); whole-genome SNP array **and droplet digital PCR (ddPCR) to detect CNVs;**[53] and multiplex-ligation dependent probe amplification (MLPA), which is commonly used to detect partial or whole gene deletions associated with several monogenic disorders (e.g. MEN1, VHL) (Table 1).

## DNA sequencing

High-resolution DNA sequencing is required for the diagnosis of the majority of monogenic disorders, although the content of available tests varies by several orders of magnitude (Figure 3)[54]. Whilst the sequencing of individual genes by Sanger-based methods has been the mainstay of genetic testing over the past decades, NGS-based methods are increasingly employed, providing a highly cost-effective and time-efficient method for genetic diagnosis.[4,54] Determining the optimal strategy for DNA sequencing is dependent on the

clinical setting and in particular the degree of genetic heterogeneity associated with the specific phenotype or disorder (Figure 3).

*Low content genetic testing: single-variant, single-gene or pauci-gene testing:* Where a disease phenotype is highly likely to be caused by a mutation in a single gene (i.e. disorders with low genetic heterogeneity), limiting the DNA sequence analysis to the relevant gene provides an effective strategy for diagnosis. Sanger sequencing is usually employed for such single-gene tests, in which the coding exons (and splice junctions) are amplified using the polymerase chain reaction (PCR) and sequenced with a high-fidelity DNA-polymerase (Figure 3). Although Sanger sequencing provides the "gold-standard' for DNA sequencing, such an approach becomes inefficient if multiple genes require evaluation and in this setting NGS approaches offer many advantages (Figure 3). Sanger sequencing retains an important role in the context of predictive genetic testing, in which DNA sequence analysis is limited to confirming the presence or absence of a specific pathogenic variant (i.e. in first-degree relatives of a known mutation carrier). Sanger sequencing is also often used to confirm the presence of likely pathogenic variants detected by clinical NGS methods.

*High Content Genetic Testing and NGS methods*

The fundamental advance of any of the NGS methods is the concept of 'massive parallel sequencing', which describes the simultaneous automated acquisition of DNA sequence of millions of short DNA fragments, which are subsequently assembled into meaningful data by aligning to a reference genome.[4,54,55] The genomic content of the test is determined by the degree of enrichment of specific DNA regions prior to sequencing. Whilst highly cost-effective for the acquisition of raw DNA sequence data, NGS platforms typically require bioinformatic support, and variant interpretation is often challenging (Figure 3).

*Disease-targeted Gene Panels*

The current major application of NGS methods in the clinical setting involves the use of disease-targeted gene panels. This approach is suited to disorders associated with locus heterogeneity in which variants in one of many potential genes may be responsible for disease. In this method custom-designed oligonucleotide probes or primer-sets are designed to capture and amplify regions of interest (i.e. exonic regions of relevant genes), prior to sequencing with an appropriate NGS platform. Compared with more comprehensive NGS methods (e.g. WES/WGS), the bioinformatic/variant interpretation pipeline is usually more straightforward (Figure 3). Disease-targeted gene panels are increasingly employed in the endocrine setting and may be used to evaluate patients with a range phenotypes including: disorders of calcium homeostasis; endocrine tumours (e.g. PPGL); pituitary disorders (e.g. combined pituitary hormone deficiency (CPHD), isolated GnRH deficiency); **monogenic/oligogenic forms of idiopathic hypogonadotrophic hypogonadism (IHH);** DSDs; and monogenic neonatal and infancy-onset diabetes (Table 1).[30,48,56] As the content of the genetic test increases the potential for identifying variants of uncertain significance (VUSs) increases and may lead to diagnostic uncertainty. In addition, the potential clinical utility of gene-panel testing is likely to be reduced by variability in disease expression and penetrance. In some clinical settings, gene panel testing is used in combination with other platforms including aCGH. For example, DSDs may arise from either single gene defects (>50 genes implicated) or from larger chromosomal abnormalities and combining genetic testing approaches can establish a genetic diagnosis in 40-60% of cases.[47,57]

*Whole Exome Sequencing (WES)/ Whole-Genome Sequencing (WGS)*

In contrast to disease-targeted gene panels, where prior knowledge of the likely causative gene is required, WES or WGS are suited to the investigation of individuals or kindreds suspected of genetic disease of unknown aetiology (i.e. ‘gene-discovery’ approach). In this

setting, both WES and WGS have been transformative in establishing the genetic aetiology of many monogenic endocrine disorders,[1] and in contrast to earlier gene-discovery studies, have often facilitated successful gene discovery with small numbers of patients (i.e. without the need for extensive pedigrees).[1,38,58-60]

WES limits analysis to the exome (i.e. the ~1-2% of the genome that comprises exons), which harbours the vast majority of variants responsible for Mendelian disease. **In addition, WES sequences intron-exon boundaries and can therefore detects DNA variants affecting splice junctions**. In WES, prior to sequencing, the DNA sample undergoes a 'capture' step in which oligonucleotide 'baits' (typically 60-100 nucleotides in length) are used to isolate the **exonic regions (and intron-exon boundaries)**, whilst allowing the remainder of the genomic DNA to be discarded. Thus, the accuracy of this approach is dependent on the reliability and efficiency of the capture step, which may be incomplete (i.e. leading to missing data). Following sequencing, bioinformatic tools are used to align the sequence reads to a reference genome, thereby allowing variant annotation. For clinical utility, the potential limitations of the approach need to be defined including the extent of any missing data (Figure 3). Clinical exome panels represent a modification of WES in which analysis is limited to genes with known disease associations (e.g. 4000-7000 genes). WGS represents the most comprehensive sequencing platform, providing unbiased coverage all coding and non-coding genomic regions with the potential to identify most types of genetic abnormality. The increased content results in the acquisition of very large data files and downstream bioinformatic analysis and interpretation is complex due to the huge numbers of variants identified.

Currently, the use of WES and WGS to evaluate patients or kindreds with endocrine disease is largely limited to the discovery setting although has been employed on clinical grounds as part of national sequencing efforts (e.g. Genomics England 100,000 Genome Project). More broadly, WES/WGS have particular clinical utility in the evaluation of patients with severe undiagnosed phenotypes in which a genetic aetiology in suspected.[2,38,61] For example, both WES and WGS have resulted in high diagnostic yields in neonatal and paediatric populations with suspected monogenic disease.[2,62,63] In this setting the availability of parental DNA samples may improve diagnostic yield, allowing the recognition of *de novo* genetic variants, and/or clarifying modes of inheritance.[38] More recent studies confirm the clinical utility of WES/WGS in evaluating children and adults with complex undiagnosed disorders (including those with endocrine manifestations), enabling a genetic diagnosis to be established in ~35-40% of patients.[39,64]

**Challenges of Variant Interpretation**

The diagnosis of monogenic endocrine disorders relies on the accurate molecular and clinical interpretation of DNA sequence variants. However, recent large-scale sequencing projects (e.g. 1000 Genome Project,[65,66] Exome Sequencing Project,[67] ExAC,[68] GnomAD, DiscoverEHR[69]) have revealed unexpectedly high rates of rare variation occurring in the background population, which hampers such interpretation.[23] For example, each individual harbours ~3 million SNVs within their genome, and of these 1000-5000 will occur in the coding-region of known disease genes.[5] In addition, most individuals harbour rare CNVs whose relevance to a given phenotype may be difficult to ascertain.[70] Whilst variant filtering and bioinformatic tools enable the majority of genetic variants to be discounted from analysis on the grounds that they are unlikely to be disease-causing (e.g. based on population frequency and *in silico* predictions), NGS testing platforms will frequently identify a subset of potentially relevant variants that require more detailed evaluation.

Currently, the majority of Molecular Genetic Laboratories adopt the American College of Medical Genetics and Genomics (ACMG) guidelines to assess variant pathogenicity.[18,71] These guidelines are intended for the evaluation of penetrant monogenic disorders with well-established disease-gene associations, and employ a multi-tiered approach, which considers a number of variant- and gene-specific factors (Table 2).[18,71] The individual pieces of evidence are then considered in combination, and using pre-determined criteria, enable the classification of variant into one of five groups; 'benign', 'likely benign', 'uncertain significance', 'likely pathogenic' or 'pathogenic' (although these categories are based on estimated probabilities and are not absolute)[3]. Although the ACMG guidelines provide a standardised approach to variant interpretation, there is considerable scope for variability and/or ambiguity in how specific criteria are applied, such that individual users may report different interpretations of the same variant.[71] In addition, the appropriate application of individual criteria may be dependent on the availability of accurate information regarding disease prevalence and penetrance, the degree of genetic heterogeneity, knowledge of protein structure/function, and/or prior reports of variant classifications (e.g. from the literature or disease-specific variant databases), and in many instances **these** data may be absent or unreliable.[71,73]

If a variant fails to meet the criteria for either a pathogenic/likely pathogenic or benign/likely benign categorization, it is stated to be of 'uncertain significance' (i.e. VUS). Variants in this category arise when there are either conflicting levels of evidence or incomplete/inadequate information, and may be associated with a continuum of risk spanning a range of probabilities. The challenge associated with the VUS category is highlighted by the observation that ~40% of all variants in the ClinVar database are reported to have this designation.[74,75] Thus, the clinician needs to be aware of the potential for such ambiguous test results, and to appreciate that not all VUSs confer equal 'risk'. In addition, it is important

to consider how VUS variants are communicated to the patient and whether individuals harbouring such variants require clinical follow up.

Given the current limitations of variant interpretation, a number of initiatives have been established to enhance the reliability and/or application of existing ACMG guidelines. For example, the ClinGen consortium (www.clinicalgenome.org) has established processes to standardise the implementation of variant interpretation criteria for specific monogenic disease genes.[76] Variant repositories such as ClinVar, which have previously been reported to have high rates of conflicting interpretations and an inflated number of pathogenic variants,[72] allow continual variant re-evaluation by applying a rating system to the evidence supporting each submission.[75] At the same time, high-throughput functional assays are being developed that allow the evaluation of large numbers of variants. **For example, *in vitro* studies using saturation gene-editing, in which virtually all possible coding-region SNVs are systematically engineered into the relevant gene to assess their functional impact, were recently employed to characterise ~4000 possible *BRCA1* SNVs,** offering the potential for immediate downstream clinical utility.[77] There has also been a continual evolution of computational tools used for *in silico* variant prediction. **For example, the rare exome variant ensemble learner (REVEL) tool combines 13 individual prediction algorithms into a single score, resulting in improved reliability.**[78,79] Similarly, statistical modelling, employing large population datasets, coupled with disease-specific information (e.g. penetrance, genetic heterogeneity, mode of inheritance) enable more stringent allele frequency cut-offs to be used in variant filtering, whilst Bayesian modelling has also been used in the application of components of the ACMG recommendations to enable a more quantitative framework for analysis.[71,80-83]

Although the clinician does not require a detailed knowledge of the methods employed for variant classification, it is important to recognise the limitations of such analysis and in particular, that an assertion of variant pathogenicity arising from molecular classification does not equate to a clinical diagnosis for the patient[3]. Instead, the genetic test result should be integrated into the overall clinical assessment (i.e. as a probabilistic biomarker), which accounts for the patient's phenotype together with all other relevant information (e.g. family history), to allow a clinical-molecular diagnosis.[3,71,74,84] Such 'case-level' interpretation may be hampered by incomplete disease penetrance, variable disease expressivity and incomplete phenotypic information and where diagnostic uncertainty exists, communication between the clinician and molecular genetics team (as well as the patient) is essential.[85] Furthermore, the interpretation of some variants may change as new information becomes available (e.g. large-scale population datasets, high-throughput functional assays, new disease-gene associations). Thus, for variants where initial evaluation is uncertain, periodic re-evaluation should be considered. Likewise, as new disease-gene associations emerge, repeat genetic testing may be appropriate for those in whom prior testing has not yielded a diagnosis.

**Incidental Findings**

A further problem arising from the application of WES and WGS approaches in the clinical setting is the possibility of identifying pathogenic variants associated with disorders other than those for which the test was originally performed.[86,87] Several studies have estimated that 1-4% of the population harbour a pathogenic variant in 'actionable' disease genes as defined by ACMG,[5,69,87] although the disease risks associated with many such variants may have been inflated (e.g. due to inaccurate estimates of penetrance or pathogenicity due to ascertainment and reporting biases). There is controversy as to whether all clinical WES/WGS data should be analysed for such incidental findings and whether these variants

should always be reported back to the patient.[85-89] Attempts to quantify the potential value of reporting actionable incidental findings have been made, taking into account disease 'severity' and penetrance, as well as the potential efficacy and burden of clinical interventions resulting from a positive test, although there is currently a paucity of evidence to support a particular approach.[85,90] For the endocrine field, the identification of asymptomatic individuals with pathogenic/likely pathogenic incidental findings in endocrine tumour genes (i.e. *SDHB, SDHC, SDHD, SDHAF2, MEN1, RET,* and *VHL* in current ACMG gene list) will likely result in increased demand for tumour surveillance programs.[23] Guidelines are required for the appropriate management of such individuals and until then patients will be subject to variable practice.

## SPECIAL SITUATIONS FOR TESTING

### Pre-implantation and Pre-natal genetic testing

Pre-conception genetic counselling together with associated genetic testing provides an important component of clinical care for prospective (or existing) parents at risk of transmitting genetic disorders to their offspring. This includes individuals with a pre-existing genetic diagnosis, carriers of disease-associated mutations, or parents in whom previous pregnancies or offspring have been affected by genetic disease. Increasingly, pre-implantation genetic diagnosis (PGD) may be offered in situations where parents are at risk of transmitting severe monogenic disorders[40,91] and has been successfully employed in the context of MEN1,[92] MEN2,[93] and CAH.[94] PGD is only possible in the context of *in vitro fertilization* (IVF), and involves the sequencing of DNA isolated from a small number of early blastocyst cells.[40,91] Knowledge of the genetic status of each pair of parental alleles is required to ensure that both maternal and paternal alleles are adequately sequenced in the

blastocyst cells. Having established the genetic status of a set of blastocysts, a 'low risk' embryo (e.g. without the mutation) is transferred to the mother. For autosomal recessive disorders, if no mutation-free blastocysts are identified, transfer of an embryo carrying one defective allele may be considered, in the knowledge that the offspring will be a mutation carrier. Due to the potential for false negative results in PGD (e.g. **due to allele 'drop-out' in which only one of the two target alleles is successfully amplified during sequencing**), pre-natal genetic testing is recommended once pregnancy is established.[40,91] In principle, similar methods can be used for genetic disorders associated with structural chromosomal abnormalities,[91] **whilst PGD can also be employed in specific settings to reduce the risk of transmitting disorders associated with mitochondrial DNA mutations (i.e. by identifying embryos with a low mutation load).**[95]

In contrast to PGD, pre-natal genetic testing evaluates the genetic status of the foetus once pregnancy is established and can be used to detect aneuploidy, chromosomal abnormalities or single gene defects. Indications for testing include scenarios in which there is an increased risk of transmission of a hereditary disease, or where antenatal screening has identified features suggestive of a genetic diagnosis. Typically, pre-natal genetic testing involves invasive approaches to acquire foetal DNA for evaluation (i.e. chorionic villous sampling, amniocentesis), which carry a 0.5-1% miscarriage rate. Non-invasive prenatal genetic diagnosis (NIPD) and/or testing (NIPT) avoids such risks and relies on the analysis of cell-free circulating foetal DNA, which is released into the maternal circulation from the placenta, and is detectable from ~5-6 weeks gestation. NIPD is most widely used for the detection of aneuploidy and foetal sex determination, which may be important for X-linked disorders or where the severity of the clinical phenotype is sex-dependent (e.g. congenital adrenal hyperplasia (CAH)).[46] For monogenic disorders, paternally-inherited or *de novo* mutations can be identified by NIPT, whilst the identification of maternally-inherited

mutations is more challenging and requires highly sensitive methods to differentiate foetal from maternal DNA.

**Somatic Genetic Testing for Endocrine Cancers**

Evaluating tumour samples for recurrent somatic (i.e. non-germline) 'driver' mutations may have diagnostic, prognostic and therapeutic value and has become part of standard clinical care for several cancer (e.g. *EGFR* and *ALK* mutation testing in non-small cell lung cancer; *BRAF* Val600 mutations in malignant melanoma)[96]. **Currently, such approaches have limited clinical utility in the endocrine field, although are gaining traction in specific settings. For example, BRAF inhibitors (e.g. vemurafenib, dabrafenib) have shown promise in the treatment of papillary craniopharyngiomas harbouring the somatic *BRAF* (Val600Glu) mutation,[97,98] whilst somatic mutation testing of MTC may have future clinical value in guiding therapy with selective RET kinase inhibitors.**[99] In the research setting, the genetic profiling of endocrine tumours with WES/WGS has been transformative in defining the mutational landscape of such tumours frequently leading to the identification of many new 'driver' genes (e.g. *USP8* mutations in corticotroph adenomas;[100,101] *ATRX/DAXX* mutations in pancreatic neuroendocrine tumours;[102,103] *KCNJ5* mutations in aldosterone-producing adenomas).[104] A current challenge is translating these advances into clinical benefit and in this regard many potential applications are emerging, including several related to thyroid cancer. For example, the utility of a NGS gene panel to improve the diagnostic accuracy associated with indeterminate cytology thyroid nodules has been assessed using the ThyroSeq v2.1 panel,[105] which combines the detection of somatic mutations in thyroid cancer-associated genes (e.g. *BRAF*, *HRAS, NRAS, KRAS*), with gene expression data, and was reported to have a sensitivity and specificity for diagnosing thyroid cancer of 91% and 92%, respectively.[106] Establishing how tumour genomics and molecular profiling can be exploited to identify novel therapeutic targets and/or ensuring the optimal

use existing targeted therapies is a major priority, particularly for cancers for which current treatments are limited (e.g. adrenocortical carcinoma, metastatic PPGL, pancreatic NETs).[107] In this regard novel precision oncology approaches combining tumour transcriptome profiles with complex bioinformatic algorithms have been reported to hold promise.[108] A final potential application of clinical genetic testing related to cancer is the emerging use of circulating cell-free DNA (cfDNA) for diagnosis, disease stratification and/or disease monitoring (i.e. the 'liquid-biopsy').[109] These methods exploit the release of tumour DNA into the circulation (i.e. circulating-tumour DNA (ctDNA)), which may be amplified or captured prior to NGS sequencing, allowing the detection of DNA sequence alterations arising from the cancer. Currently, these methods are not in clinical practice for endocrine cancers, but preliminary studies indicate potential utility in specific settings including the monitoring of advanced thyroid cancer.[110]

## FUTURE DIRECTIONS & CONCLUDING REMARKS

The recent advances in DNA sequencing offer unparalleled opportunities for genetic testing in mainstream clinical practice with costs of high-content tests (i.e. gene panel, WES/WGS) now falling to those of other routinely employed diagnostic tests (e.g. cross-sectional imaging). However, the advances in sequencing technology have not been paralleled by similar progress in understanding the biological and/or clinical relevance of the huge diversity of genomic variation evident at both the individual and population level.[54] As a consequence, the current interpretation of high-content tests, including assessments of variant pathogenicity, remains extremely challenging. On-going large-scale sequencing projects, which simultaneously collect detailed clinical information (e.g. utilising electronic health records) aim to address some of these deficiencies (e.g. NIH-funded eMERGE and IGNITE networks),[4] as do several laboratory- and bioinformatic-based studies. Despite the improved knowledge that such studies bring, there also remains a need to understand how genetic variants interact with other genes, the environment and/or additional modifying

factors (e.g. epigenetic modifications) to influence disease expressivity and/or disease penetrance.[74]

Looking forward, it is likely that genetic testing will move into other settings. For example, there is a growing debate on the use of germline genetic testing for primary screening at a population-level to identify individuals at risk of serious disease, including monogenic cancer syndromes.[111-115] Testing in this setting could include the detection of hereditary endocrine tumour syndromes (e.g. MEN1, MEN2, VHL, PPGL due to *SDHX* mutations) and a predictable outcome of this approach would be the identification of large numbers of asymptomatic individuals harbouring pathogenic/likely pathogenic or VUS variants, which in turn will likely place an increased burden on endocrine services due to an expansion in tumour surveillance programs. In this setting, variant interpretation is more challenging, given the lack of clinical context to the molecular classification.[71,84] Prior to the adoption of any such programs, improved probabilistic models are required to estimate more accurately the likelihood that an individual harbouring a given variant will develop disease.[71] However, the recent FDA first marketing authorization for direct-to-consumer genetic testing for specific *BRCA* mutations indicates the likely direction of travel for population-based testing.[116] Other emerging areas for genetic testing include pharmacogenomic assays to identify individuals with enhanced, reduced or adverse responses to specific therapies **(e.g. association of aminoglycoside ototoxicity with a mitochondrial DNA mutation)**.[117] Furthermore, recent studies indicate considerable genetic variation in drug-targeting sites of many G-protein coupled receptors (GPCRs), which may provide an area of furtive research for the endocrine field.[118] Likewise, another recent study demonstrated asthma patients carrying specific genetic variants in the *PDGFR* gene were at increased risk of steroid-induced adrenal suppression.[119] Thus, it is possible that genetic testing may not only be used to guide therapy but also to predict those at increased risk of adverse endocrine outcomes.

Finally, the increased use of genetic testing in the clinical settings requires enhanced communication between the physician and clinical geneticist, as well as transparency and openness with the patient (and wider family). However, many questions remain unresolved, including; how best to obtain genuinely informed consent; how to effectively communicate ambiguous or uncertain test results; should incidental findings always be evaluated and reported; how can genetic data be shared effectively whilst preserving patient privacy? These issues apply to the whole medical community and are not unique to endocrinology. Ultimately, despite the rapid progressing made, it will likely take many years before the potential health benefits associated with increased genetic testing can be fully assessed.

**REFERENCES**


1. de Bruin C, Dauber A. Insights from exome sequencing for endocrine disorders. *Nat Rev Endocrinol*. 2015; 11:455-464

2. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med*. 2013; 369:1502-1511

3. Biesecker LG, Nussbaum RL, Rehm HL. Distinguishing variant pathogenicity from genetic diagnosis: How to know whether a variant causes a condition. *JAMA*. 2018; 320:1929-1930

4. Adams DR, Eng CM. Next-generation sequencing to diagnose suspected genetic disorders. *N Engl J Med*. 2018; 379:1353-1362

5. Evans JP, Powell BC, Berg JS. Finding the rare pathogenic variants in a human genome. *JAMA*. 2017; 317:1904-1905

6. Timpson NJ, Greenwood CMT, Soranzo N, Lawson DJ, Richards JB. Genetic architecture: The shape of the genetic contribution to human traits and disease. *Nat Rev Genet*. 2018; 19:110-124

7. Chow J, Rahman J, Achermann JC, Dattani MT, Rahman S. Mitochondrial disease and endocrine dysfunction. *Nat Rev Endocrinol*. 2017; 13:92-104

8. Biesecker LG, Spinner NB. A genomic view of mosaicism and human disease. *Nat Rev Genet*. 2013; 14:307-320

9. Stelmachowska-Banas M, Zgliczynski W, Tutka P, Carney JA, Korbonits M. Fatal carney complex in siblings due to de novo large gene deletion. *J Clin Endocrinol Metab*. 2017; 102:3924-3927

10. Tafaj O, Juppner H. Pseudohypoparathyroidism: One gene, several syndromes. *J Endocrinol Invest*. 2017; 40:347-356

11. Tufton N, Sahdev A, Drake WM, Akker SA. Can subunit specific phenotypes guide surveillance imaging decisions in asymptomatic SDH mutation carriers? *Clin Endocrinol (Oxf)*. 2018; PMID: 30934121:Epub ahead of print

12. Andrews KA, Ascher DB, Pires DEV, et al. Tumour risks and genotype-phenotype correlations associated with germline variants in succinate dehydrogenase subunit genes SDHB, SDHC and SDHD. *J Med Genet*. 2018; 55:384-394

13. Favier J, Amar L, Gimenez-Roqueplo AP. Paraganglioma and phaeochromocytoma: From genetics to personalized medicine. *Nat Rev Endocrinol*. 2015; 11:101-111

14. Benn DE, Robinson BG, Clifton-Bligh RJ. 15 years of paraganglioma: Clinical manifestations of paraganglioma syndromes types 1-5. *Endocr Relat Cancer*. 2015; 22:T91-103

15. Acuna-Hidalgo R, Veltman JA, Hoischen A. New insights into the generation and role of *de novo* mutations in health and disease. *Genome Biol*. 2016; 17:241

16. Pepe S, Korbonits M, Iacovazzo D. Germline and mosaic mutations causing pituitary tumours: Genetic and molecular aspects. *J Endocrinol*. 2019; 240:R21-R45

17. Trivellin G, Daly AF, Faucz FR, et al. Gigantism and acromegaly due to Xq26 microduplications and *GPR101* mutation. *N Engl J Med*. 2014; 371:2363-2374

18. Richards S, Aziz N, Bale S, et al. 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. *Genet Med*. 2015; 17:405-424

19. Beckers A, Aaltonen LA, Daly AF, Karhu A. Familial isolated pituitary adenomas (FIPA) and the pituitary adenoma predisposition due to mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. *Endocr Rev*. 2013; 34:239-277

20. Hernandez-Ramirez LC, Gabrovska P, Denes J, et al. Landscape of familial isolated and young-onset pituitary adenomas: Prospective diagnosis in AIP mutation carriers. *J Clin Endocrinol Metab*. 2015; 100:E1242-1254

21. Williams F, Hunter S, Bradley L, et al. Clinical experience in the screening and management of a large kindred with familial isolated pituitary adenoma due to an aryl hydrocarbon receptor interacting protein (AIP) mutation. *J Clin Endocrinol Metab*. 2014; 99:1122-1131

22. Minikel EV, Vallabh SM, Lek M, et al. Quantifying prion disease penetrance using large population control cohorts. *Sci Transl Med*. 2016; 8:322ra329

23. Newey PJ, Berg JN, Zhou K, Palmer CNA, Thakker RV. Utility of population-level DNA sequence data in the diagnosis of hereditary endocrine disease. *J Endocr Soc*. 2017; 1:1507-1526

24. Loveday C, Josephs K, Chubb D, et al. p.Val804Met, the most frequent pathogenic mutation in RET, confers a very low lifetime risk of medullary thyroid cancer. *J Clin Endocrinol Metab*. 2018; 103:4275-4282

25. Pasmant E, Vidaud M, Vidaud D, Wolkenstein P. Neurofibromatosis type 1: From genotype to phenotype. *J Med Genet*. 2012; 49:483-489

26. Thakker RV, Newey PJ, Walls GV, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab*. 2012; 97:2990-3011

27. Wells SA, Jr., Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*. 2015; 25:567-610

28. Correa R, Salpea P, Stratakis CA. Carney complex: An update. *Eur J Endocrinol*. 2015; 173:M85-97

29. Salpea P, Horvath A, London E, et al. Deletions of the *PRKAR1A* locus at 17q24.2-q24.3 in Carney complex: Genotype-phenotype correlations and implications for genetic testing. *J Clin Endocrinol Metab*. 2014; 99:E183-188

30. Group NGSiPS, Toledo RA, Burnichon N, et al. Consensus statement on next-generation-sequencing-based diagnostic testing of hereditary phaeochromocytomas and paragangliomas. *Nat Rev Endocrinol*. 2017; 13:233-247

31. Wang Y, Gong C, Qin M, Liu Y, Tian Y. Clinical and genetic features of 64 young male paediatric patients with congenital hypogonadotropic hypogonadism. *Clin Endocrinol (Oxf)*. 2017; 87:757-766

32. Stamou MI, Cox KH, Crowley WF, Jr. Discovering genes essential to the hypothalamic regulation of human reproduction using a human disease model: Adjusting to life in the "-omics" era. *Endocr Rev*. 2016; 2016:4-22

33. Whyte MP. Hypophosphatasia: Enzyme replacement therapy brings new opportunities and new challenges. *J Bone Miner Res*. 2017; 32:667-675

34. Burke W. Genetic tests: Clinical validity and clinical utility. *Curr Protoc Hum Genet*. 2014; 81:9 15 11-18

35. van Hulsteijn LT, Dekkers OM, Hes FJ, Smit JW, Corssmit EP. Risk of malignant paraganglioma in SDHB-mutation and SDHD-mutation carriers: A systematic review and meta-analysis. *J Med Genet*. 2012; 49:768-776

36. Whyte MP, Madson KL, Phillips D, et al. Asfotase alfa therapy for children with hypophosphatasia. *JCI Insight*. 2016; 1:e85971

37. Whyte MP, Greenberg CR, Salman NJ, et al. Enzyme-replacement therapy in life-threatening hypophosphatasia. *N Engl J Med*. 2012; 366:904-913

38. Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. *Nat Genet*. 2015; 47:717-726

39. Splinter K, Adams DR, Bacino CA, et al. Effect of genetic diagnosis on patients with previously undiagnosed disease. *N Engl J Med*. 2018; 379:2131-2139

40. Dolan SM, Goldwaser TH, Jindal SK. Preimplantation genetic diagnosis for mendelian conditions. *JAMA*. 2017; 318:859-860

41. Alagaratnam S, Kurzawinski TR. Aetiology, diagnosis and surgical treatment of primary hyperparathyroidism in children: New trends. *Horm Res Paediatr*. 2015; 83:365-375

42. Radian S, Diekmann Y, Gabrovska P, et al. Increased population risk of AIP-related acromegaly and gigantism in ireland. *Hum Mutat*. 2017; 38:78-85

43. Mathiesen JS, Kroustrup JP, Vestergaard P, et al. Founder effect of the RET(C611Y) mutation in multiple endocrine neoplasia 2a in Denmark: A nationwide study. *Thyroid*. 2017; 27:1505-1510

44. Stanley CA. Perspective on the genetics and diagnosis of congenital hyperinsulinism disorders. *J Clin Endocrinol Metab*. 2016; 101:815-826

45. Hannan FM, Thakker RV. Calcium-sensing receptor (CASR) mutations and disorders of calcium, electrolyte and water metabolism. *Best Pract Res Clin Endocrinol Metab*. 2013; 27:359-371

46. El-Maouche D, Arlt W, Merke DP. Congenital adrenal hyperplasia. *Lancet*. 2017; 390:2194-2210

47. Eggers S, Sadedin S, van den Bergen JA, et al. Disorders of sex development: Insights from targeted gene sequencing of a large international patient cohort. *Genome Biol*. 2016; 17:243

48. Hattersley AT, Greeley SAW, Polak M, et al. ISPAD clinical practice consensus guidelines 2018: The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*. 2018; 19 Suppl 27:47-63

49. Martin CL, Ledbetter DH. Chromosomal microarray testing for children with unexplained neurodevelopmental disorders. *JAMA*. 2017; 317:2545-2546

50. Cangul H, Aycan Z, Saglam H, et al. TSHR is the main causative locus in autosomal recessively inherited thyroid dysgenesis. *J Pediatr Endocrinol Metab*. 2012; 25:419-426

51. Turner JJ, Christie PT, Pearce SH, Turnpenny PD, Thakker RV. Diagnostic challenges due to phenocopies: Lessons from multiple endocrine neoplasia type1 (MEN1). *Hum Mutat*. 2010; 31:E1089-1101

52. Committee On Bioethics, Committee On Genetics and American College Of Medical Genetics and, Genomics Social, Ethical, Legal Issues Committee. Ethical and policy issues in genetic testing and screening of children. *Pediatrics*. 2013; 131:620-622

53. Iacovazzo D, Caswell R, Bunce B, et al. Germline or somatic GPR101 duplication leads to X-linked acrogigantism: A clinico-pathological and genetic study. *Acta Neuropathol Commun*. 2016; 4:56

54. Kuo FC, Mar BG, Lindsley RC, Lindeman NI. The relative utilities of genome-wide, gene panel, and individual gene sequencing in clinical practice. *Blood*. 2017; 130:433-439

55. Forlenza GP, Calhoun A, Beckman KB, et al. Next generation sequencing in endocrine practice. *Mol Genet Metab*. 2015; 115:61-71

56. Fang Q, George AS, Brinkmeier ML, et al. Genetics of combined pituitary hormone deficiency: Roadmap into the genome era. *Endocr Rev*. 2016; 37:636-675

57. Nixon R, Cerqueira V, Kyriakou A, et al. Prevalence of endocrine and genetic abnormalities in boys evaluated systematically for a disorder of sex development. *Hum Reprod*. 2017; 32:2130-2137

58. Bamshad MJ, Ng SB, Bigham AW, et al. Exome sequencing as a tool for mendelian disease gene discovery. *Nat Rev Genet*. 2011; 12:745-755

59. Chong JX, Buckingham KJ, Jhangiani SN, et al. The genetic basis of mendelian phenotypes: Discoveries, challenges, and opportunities. *Am J Hum Genet*. 2015; 97:199-215

60. Guo MH, Dauber A, Lippincott MF, Chan YM, Salem RM, Hirschhorn JN. Determinants of power in gene-based burden testing for monogenic disorders. *Am J Hum Genet*. 2016; 99:527-539

61. Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA*. 2014; 312:1870-1879

62. Tan TY, Dillon OJ, Stark Z, et al. Diagnostic impact and cost-effectiveness of whole-exome sequencing for ambulant children with suspected monogenic conditions. *JAMA Pediatr*. 2017; 171:855-862

63. Meng L, Pammi M, Saronwala A, et al. Use of exome sequencing for infants in intensive care units: Ascertainment of severe single-gene disorders and effect on medical management. *JAMA Pediatr*. 2017; 171:e173438

64. Lionel AC, Costain G, Monfared N, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. *Genet Med*. 2017; 20:435-443

65. Genomes Project C, Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature*. 2015; 526:68-74

66. Fu W, O'Connor TD, Jun G, et al. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. *Nature*. 2013; 493:216-220

67. Tennessen JA, Bigham AW, O'Connor TD, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science*. 2012; 337:64-69

68. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016; 536:285-291

69. Dewey FE, Murray MF, Overton JD, et al. Distribution and clinical impact of functional variants in 50,726 whole-exome sequences from the discovEHR study. *Science*. 2016; 354

70. Ruderfer DM, Hamamsy T, Lek M, et al. Patterns of genic intolerance of rare copy number variation in 59,898 human exomes. *Nat Genet*. 2016; 48:1107-1111

71. Strande NT, Brnich SE, Roman TS, Berg JS. Navigating the nuances of clinical sequence variant interpretation in mendelian disease. *Genet Med*. 2018; 20:918-926

72. Romanet P, Odou MF, North MO, et al. Proposition of adjustments to the ACMG-AMP framework for the interpretation of MEN1 missense variants. *Hum Mutat*. 2019; PMID: 30869828:Epub ahead of print

73. Shah N, Hou YC, Yu HC, et al. Identification of misclassified ClinVar variants via disease population prevalence. *Am J Hum Genet*. 2018; 102:609-619

74. Manolio TA, Fowler DM, Starita LM, et al. Bedside back to bench: Building bridges between basic and clinical genomic research. *Cell*. 2017; 169:6-12

75. Landrum MJ, Lee JM, Benson M, et al. Clinvar: Improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 2018; 46:D1062-D1067

76. Strande NT, Riggs ER, Buchanan AH, et al. Evaluating the clinical validity of gene-disease associations: An evidence-based framework developed by the clinical genome resource. *Am J Hum Genet*. 2017; 100:895-906

77. Findlay GM, Daza RM, Martin B, et al. Accurate classification of BRCA1 variants with saturation genome editing. *Nature*. 2018; 562:217-222

78. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: An ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet*. 2016; 99:877-885

79. Li J, Zhao T, Zhang Y, et al. Performance evaluation of pathogenicity-computation methods for missense variants. *Nucleic Acids Res*. 2018; 46:7793-7804

80. Whiffin N, Minikel E, Walsh R, et al. Using high-resolution variant frequencies to empower clinical genome interpretation. *Genet Med*. 2017; 19:1151-1158

81. Kobayashi Y, Yang S, Nykamp K, Garcia J, Lincoln SE, Topper SE. Pathogenic variant burden in the exac database: An empirical approach to evaluating population data for clinical variant interpretation. *Genome Med*. 2017; 9:13

82. Tavtigian SV, Greenblatt MS, Harrison SM, et al. Modeling the ACMG/AMP variant classification guidelines as a bayesian classification framework. *Genet Med*. 2018; 20:1054-1060

83. Brnich SE, Rivera-Munoz EA, Berg JS. Quantifying the potential of functional evidence to reclassify variants of uncertain significance in the categorical and bayesian interpretation frameworks. *Hum Mutat*. 2018; 39:1531-1541

84. Berg JS. Exploring the importance of case-level clinical information for variant interpretation. *Genet Med*. 2017; 19:3-5

85. Knoppers BM, Zawati MH, Senecal K. Return of genetic testing results in the era of whole-genome sequencing. *Nat Rev Genet*. 2015; 16:553-559

86. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013; 15:565-574

87. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG sf v2.0): A policy statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2017; 19:249-255

88. Roche MI, Berg JS. Incidental findings with genomic testing: Implications for genetic counseling practice. *Curr Genet Med Rep*. 2015; 3:166-176

89. Hehir-Kwa JY, Claustres M, Hastings RJ, et al. Towards a european consensus for reporting incidental findings during clinical NGS testing. *Eur J Hum Genet*. 2015; 23:1601-1606

90. Berg JS, Foreman AK, O'Daniel JM, et al. A semiquantitative metric for evaluating clinical actionability of incidental or secondary findings from genome-scale sequencing. *Genet Med*. 2016; 18:467-475

91. Brezina PR, Kutteh WH. Clinical applications of preimplantation genetic testing. *BMJ*. 2015; 350:g7611

92. Lima AD, Alves VR, Rocha AR, et al. Preimplantation genetic diagnosis for a patient with multiple endocrine neoplasia type 1: Case report. *JBRA Assist Reprod*. 2018; 22:67-70

93. Chen S, Li S, Zhang J, et al. Preimplantation genetic diagnosis of multiple endocrine neoplasia type 2a using informative markers identified by targeted sequencing. *Thyroid*. 2018; 28:281-287

94. Simpson JL, Rechitsky S. Preimplantation diagnosis and other modern methods for prenatal diagnosis. *J Steroid Biochem Mol Biol*. 2017; 165:124-130

95. Sallevelt S, Dreesen J, Coonen E, et al. Preimplantation genetic diagnosis for mitochondrial DNA mutations: Analysis of one blastomere suffices. *J Med Genet*. 2017; 54:693-697

96. Spencer DH, Ley TJ. Sequencing of tumor DNA to guide cancer risk assessment and therapy. *JAMA*. 2018; 319:1497-1498

97. Himes BT, Ruff MW, Van Gompel JJ, et al. Recurrent papillary craniopharyngioma with BRAF V600E mutation treated with dabrafenib: Case report. *J Neurosurg*. 2018:1-5

98. Larkin S, Karavitaki N. Recent advances in molecular pathology of craniopharyngioma. *F1000Res*. 2017; 6:1202

99. Subbiah V, Velcheti V, Tuch BB, et al. Selective RET kinase inhibition for patients with RET-altered cancers. *Ann Oncol*. 2018; 29:1869-1876

100. Faucz FR, Tirosh A, Tatsi C, et al. Somatic USP8 gene mutations are a common cause of pediatric Cushing disease. *J Clin Endocrinol Metab*. 2017; 102:2836-2843

101. Reincke M, Sbiera S, Hayakawa A, et al. Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nat Genet*. 2015; 47:31-38

102. Jiao Y, Shi C, Edil BH, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science*. 2011; 331:1199-1203

103. Scarpa A, Chang DK, Nones K, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. *Nature*. 2017; 543:65-71

104. Choi M, Scholl UI, Yue P, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*. 2011; 331:768-772

105. Yip L, Sosa JA. Molecular-directed treatment of differentiated thyroid cancer: Advances in diagnosis and treatment. *JAMA Surg*. 2016; 151:663-670

106. Nikiforov YE, Carty SE, Chiosea SI, et al. Impact of the multi-gene thyroseq next-generation sequencing assay on cancer diagnosis in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology. *Thyroid*. 2015; 25:1217-1223

107. Angelousi A, Dimitriadis GK, Zografos G, Nolting S, Kaltsas G, Grossman A. Molecular targeted therapies in adrenal, pituitary and parathyroid malignancies. *Endocr Relat Cancer*. 2017; 24:R239-R259

108. Alvarez MJ, Subramaniam PS, Tang LH, et al. A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors. *Nat Genet*. 2018; 50:979-989

109. Husain H, Velculescu VE. Cancer DNA in the circulation: The liquid biopsy. *JAMA*. 2017; 318:1272-1274

110. Cote GJ, Evers C, Hu MI, et al. Prognostic significance of circulating RET M918T mutated tumor DNA in patients with advanced medullary thyroid carcinoma. *J Clin Endocrinol Metab*. 2017; 102:3591-3599

111. Turnbull C, Sud A, Houlston RS. Cancer genetics, precision prevention and a call to action. *Nat Genet*. 2018; 50:1212-1218

112. Foulkes WD, Knoppers BM, Turnbull C. Population genetic testing for cancer susceptibility: Founder mutations to genomes. *Nat Rev Clin Oncol*. 2016; 13:41-54

113. Prince AE, Berg JS, Evans JP, Jonas DE, Henderson G. Genomic screening of the general adult population: Key concepts for assessing net benefit with systematic evidence reviews. *Genet Med*. 2015; 17:441-443

114. Feero WG, Wicklund CA, Veenstra D. Precision medicine, genome sequencing, and improved population health. *JAMA*. 2018; 319:1979-1980

115. Lindor NM, Thibodeau SN, Burke W. Whole-genome sequencing in healthy people. *Mayo Clin Proc*. 2017; 92:159-172

116. Gill J, Obley AJ, Prasad V. Direct-to-consumer genetic testing: The implications of the us FDA's first marketing authorization for BRCA mutation testing. *JAMA*. 2018; 319:2377-2378

117. Lanvers-Kaminsky C, Zehnhoff-Dinnesen AA, Parfitt R, Ciarimboli G. Drug-induced ototoxicity: Mechanisms, pharmacogenetics, and protective strategies. *Clin Pharmacol Ther*. 2017; 101:491-500

118. Hauser AS, Chavali S, Masuho I, et al. Pharmacogenomics of GPCR drug targets. *Cell*. 2018; 172:41-54 e19

119. Hawcutt DB, Francis B, Carr DF, et al. Susceptibility to corticosteroid-induced adrenal suppression: A genome-wide association study. *Lancet Respir Med*. 2018; 6:442-450

120. Maniam P, Zhou K, Lonergan M, Berg JN, Goudie DR, Newey PJ. Pathogenicity and penetrance of germline SDHA variants in pheochromocytoma and paraganglioma (PPGL). *J Endocr Soc*. 2018; 2:806-816

121. Benn DE, Zhu Y, Andrews KA, et al. Bayesian approach to determining penetrance of pathogenic SDH variants. *J Med Genet*. 2018; 55:729-734

122. Huang KL, Mashl RJ, Wu Y, et al. Pathogenic germline variants in 10,389 adult cancers. *Cell*. 2018; 173:355-370 e314

123. Wasserman JD, Tomlinson GE, Druker H, et al. Multiple endocrine neoplasia and hyperparathyroid-jaw tumor syndromes: Clinical features, genetics, and surveillance recommendations in childhood. *Clin Cancer Res*. 2017; 23:e123-e132

124. Newey PJ, Bowl MR, Cranston T, Thakker RV. Cell division cycle protein 73 homolog (CDC73) mutations in the hyperparathyroidism-jaw tumor syndrome (HPT-JT) and parathyroid tumors. *Hum Mutat*. 2010; 31:295-307

125. Romanet P, Mohamed A, Giraud S, et al. UMD-MEN1 database: An overview of the 370 MEN1 variants present in 1,676 patients from the french population. *J Clin Endocrinol Metab*. 2018; 104:753-764

126. Machens A, Dralle H. Variability in penetrance of multiple endocrine neoplasia 2a with amino acid substitutions in RET codon 634. *Clin Endocrinol (Oxf)*. 2016; 84:210-215

127. Binderup ML, Galanakis M, Budtz-Jorgensen E, Kosteljanetz M, Luise Bisgaard M. Prevalence, birth incidence, and penetrance of von Hippel-Lindau disease (VHL) in Denmark. *Eur J Hum Genet*. 2017; 25:301-307

128. Alrezk R, Hannah-Shmouni F, Stratakis CA. MEN4 and CDKN1B mutations: The latest of the MEN syndromes. *Endocr Relat Cancer*. 2017; 24:T195-T208

**Figure Legends**

**Figure 1. Penetrance estimates for monogenic endocrine tumour disorders.**

For several hereditary endocrine tumour syndromes, establishing accurate estimates of disease penetrance may be challenging and in many instances prior estimates are likely to have been overstated due to ascertainment and reporting biases (e.g. resulting from the inclusion of index cases in calculations and/or the inadequate genetic investigation of control populations). A number of recent studies have demonstrated unexpectedly high frequencies of disease-associated variants in the background population, facilitating revised estimates of disease penetrance for several endocrine tumour genes.[12,23,120,121] In addition, although estimates of disease penetrance are frequently reported at the gene level, different pathogenic variants in the same gene may be associated with marked differences in penetrance. For example, although the majority of MEN2A-associated *RET* mutations are reported to have a high disease penetrance (e.g. 70-100% by the age of 70 years), recent population-level studies predict that the Val804Met variant has a much lower penetrance of 1-8% (dashed box).[24] In addition, distinguishing low penetrance disease alleles from benign variants is challenging and typically requires large disease and control cohorts to establish genuine associations. For example, a recent study demonstrated that several *AIP* missense variants previously reported to be pathogenic in individuals with pituitary adenomas were no more frequent in this setting than in control populations[23]. Germline variants with reduced penetrance may be indicated by an absence of relevant family history (i.e. occurring in apparently sporadic disease settings).[103,122] For the figure above, estimates of disease penetrance were established from: Genetic Home Reference (https://ghr.nlm.nih.gov/); Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/); and literature-based estimates. Penetrance estimates for monogenic tumour syndromes are often expressed as the likelihood of developing disease by a given age and as a consequence estimates may

not be comparable. As such the penetrance estimates above are intended to be illustrative. References relevant to specific genes include: *AIP;*[19,42] *CDC73;*[123,124] *MEN1;*[26,125] SDH complex genes;[11,12,120,121] *PRKAR1A;*[28] *RET;*[23,24,126] *VHL.*[127] Penetrance estimates for MEN4 due to *CDKN1B* mutations are not available due to low numbers of reported cases.[128] Abbreviations: FIPA, Familial isolated pituitary adenoma syndrome; HPT-JT, Hyperparathyroidism-Jaw Tumour Syndrome; PC, Parathyroid carcinoma; PPGL, phaeochromocytoma/paraganglioma.

**Figure 2. Illustrative workflow for clinical genetic testing**

The genetic testing workflow can be divided into three phases: pre-test, testing and molecular interpretation, and post-test. The pre-test phase includes the appropriate identification of individuals in whom genetic testing may be appropriate. In the diagnostic setting, this often requires a high index of suspicion and is dependent on a thorough evaluation of the patient and establishing any relevant family history. For those in whom a genetic diagnosis is suspected the decision to undertake testing should consider the potential utility of establishing a diagnosis, as well as the most appropriate testing strategy. Informed consent should address any patient concerns, and should be tailored to the testing strategy planned. Consent may also seek to gain permission for storage and future testing of DNA samples, contact with relevant family members if considered clinically indicated, and establish how ambiguous test results and/or incidental finding are dealt with. Genetic testing undertaken on a research basis requires different ethical permissions and the testing platforms may not have the same validity required for clinical testing. All testing and molecular classification should be undertaken in accredited laboratories. The interpretation of genetic variants should follow standardised processes (e.g. adhering to ACMG guidelines) that enable classification of variants into one of 5 classes (i.e. pathogenic, likely pathogenic, benign, likely benign and uncertain significance). However, the clinician needs to be aware of the limitations of such systems and to recognize that the molecular classification of

variants does not equate to a clinical diagnosis. Instead, during the post-test phase, the genetic test result should be incorporated into the overall clinical assessment of the patient. Where there is doubt, discussion with the clinical and molecular genetics team should aim to determine whether any additional support for a particular diagnosis can be established. If the clinical phenotype strongly points to a particular genetic diagnosis, but initial testing is negative, it may be appropriate to consider a different testing platform. When molecular classification identifies a variant of uncertain significance, decisions regarding how such results are communicated to the patient, and whether clinical follow-up is required often require multidisciplinary working. It is also possible that variant classification may change over time as additional information becomes available.

**Figure 3**: **DNA sequencing methods and their utility in the diagnosis of monogenic endocrine disorders**

The most appropriate DNA sequencing method to diagnose a monogenic endocrine disorder will be determined by the clinical setting, as well as the local availability of testing platforms. Although NGS-based sequencing methods have gained increased traction in the clinical setting, Sanger sequencing of one or more individual genes (either targeted to specific exons or covering the whole coding-region) retains an important role in the diagnosis of monogenic disorders with low genetic heterogeneity (e.g. *RET* mutations in MEN2A or MEN2B), and is also used for predictive genetic testing (e.g. for first-degree relatives of patients with known disease-associated mutations). In addition, Sanger sequencing is used to confirm the presence of likely pathogenic variants found by high-content NGS methods. Sanger sequencing will not reliably detect partial or whole gene deletions, which may account for a proportion of individuals with specific monogenic disorders (e.g. MEN1, Carney complex), and when this is suspected, alternate methods of detection are required (e.g. MLPA/CGH array). Disease-targeted gene panels are increasingly employed to investigate disorders with high genetic heterogeneity (e.g. PPGL, disorders of calcium homeostasis),

enabling the cost-effective sequencing of multiple genes simultaneously. The majority of panels employ NGS methods, following either bait-based capture or PCR-based amplification of the targeted exons. However, it is possible that some genomic regions will be 'missed' (e.g. due to incomplete 'capture' of target regions). In mainstream clinical practice, WES and WGS are rarely used to diagnose monogenic endocrine disorders, although each has been pivotal for endocrine disease-gene discovery in the research setting. These approaches are reported to have clinical utility in both paediatric and adult settings for the investigation of patients with severe undiagnosed phenotypes,[39] and retain a critical role in the research investigation of individuals and/or kindreds with genetic disorders of unknown aetiology. However, an important consideration prior to undertaking WES/WGS is the challenge of variant interpretation. For example, it is estimated that every individual will harbour ~1000-5000 coding-region variants in disease-associated genes, of which ~1% are likely to be categorized as of potential functional significance (i.e. VUS or likely pathogenic/pathogenic status).[5] In the absence of additional information (e.g. availability of parental samples to identify *de novo* mutations, segregation data in a kindred with multiple affected individuals) interpreting such variants remains problematic and may not inform the clinical picture. Furthermore, ~3-5% of the population are predicted to harbour incidental findings (i.e. potential

**Table 1. Examples of Existing and Emerging Indications for Genetic Testing in Adult and Paediatric Endocrinology**

| Disorder/Phenotype | Gene (Chromosome location) or Chromosomal abnormality | Variant Type | Inherit. Pattern | Genetic Test(s) | Clinic. Set[a] | Notes |
|---|---|---|---|---|---|---|
| ***Multi-System Monogenic Endocrine Tumour Syndromes*** | | | | | | |
| Multiple Endocrine Neoplasia Type 1 (MEN1) | *MEN1* (11q13.1*)* | SNV/Indel Gene del | AD | Single gene/Panel (MLPA/aCGH[b]) | Ad/P | *MEN1* mutations observed in ~70-90% of index cases with ≥2 clinical manifestations (parathyroid, pituitary, pancreatic endocrine tumours) and a positive family history |
| Multiple Endocrine Neoplasia Type 2A/2B (MEN2A/MEN2B) | *RET* (10q11.21) | SNV | AD | Single gene/Panel | Ad/P | Strong genotype-phenotype correlation is observed such that sequencing may be limited to specific *RET* exons |
| Multiple Endocrine Neoplasia Type 4 (MEN4) | *CDKN1B* (12p13.1) | SNV/Indel Gene del? | AD | Single gene/Panel (MLPA/aCGH[b]) | Ad/P | Only a few cases of MEN4 are reported to date such that a full clinical description is not currently available |
| Hyperparathyroidism Jaw-Tumour Syndrome (HPT-JT) | *CDC73* (1q31.2) | SNV/Indel Gene del | AD | Single gene/Panel (MLPA/aCGH[b]) | Ad/P | In addition to parathyroid tumours patients may manifest ossifying fibromas of the jaw, renal and uterine tumours |
| von Hippel Lindau Syndrome (VHL) | *VHL* (3p25.3) | SNV/Indel Gene del | AD | Single gene/Panel (MLPA/aCGH[b]) | Ad/P | Genotype-phenotype correlation observed with increased risk of PPGL in association with missense *VHL* mutations |
| Neurofibromatosis Type 1 (NF1) | *NF1* (17q11.2) | SNV/Indel Gene del | AD | Single gene/Panel (MLPA/aCGH[b]) | (Ad)/P | Endocrine manifestations may include PPGL, pancreatic NETs, GH hypersecretion, precious puberty and carcinoids |
| McCune-Albright Syndrome (MAS) | *GNAS* (20q13.32) | SNV | S.Mos[c] | Single gene/Panel | P | Somatic missense *GNAS* mutations affecting Arg201 and Gln227 residues are most commonly reported. |
| Carney Complex | *PRKAR1A* (17q24.2) | SNV/Indel Gene del | AD | Single gene/Panel (MLPA/aCGH[b]) | P | A characteristic endocrine feature is Cushing syndrome due to primary pigmented nodular adrenocortical disease |
| ***Hypothalamic /Pituitary* Disorders** | | | | | | |
| Familial Isolated Pituitary Adenoma (FIPA) | *AIP* (11q13.2*),* | SNV/Indel Gene del/ | AD/ | Single gene/Panel (MLPA[b]) | Ad/P | Founder *AIP* mutations may be prevalent in specific populations (e.g. Arg304Ter mutation in regions of Ireland). |
| X-linked Gigantism (XLAG) | *GPR101* (Xq26.3) | Gene dup | XLD *De novo* S.Mos[c] | aCGH/ ddPCR | P | Female cases typically harbour *de novo* germline *GPR101* duplications. Sporadic male cases occur due to somatic mosaicism. Mother-to-son transmission also observed. |
| Combined Pituitary Hormone Deficiency (CPHD) | *PROP1* (5q35.3), *HESX1* (3p14.3), *LHX3* (9q34.3), *LHX4* (1q25.2), *OTX2* (14q22.3), *POUF1F1* (3p11.2), *SOX2* (3q26.33) + more | SNV/Indel | AD/AR/ XLD | Gene Panel +/- CNV detection | P | Mutations in specific genes may result in additional features including intellectual disability, septo-optic dysplasia (e.g. *HESX1*, *OTX2* and *SOX2* mutations) |
| Hypogonadotrophic hypogonadism with or without anosmia | *ANOS1* (Xp22.31), *FGFR1* (8p11.23), *PROKR2* (20p12.3), *CHD7* (8q12.2), *FGF8* (10q24.32), *PROK2* (3p13) + more | SNV/Indel | AD/AR /XLR | Gene Panel | Ad/P | >20 genes implicated. Some patients may have mutations in more than one gene and inheritance patterns are dependent on the gene(s) implicated (e.g. monogenic and oligogenic) |
| **Adrenal Disorders** | | | | | | |
| Autoimmune polyglandular syndrome type 1 | *AIRE* (21q22.3) | SNV/Indel | AR (AD) | Single gene/Panel | Ad/P | Majority of kindreds demonstrate AR inheritance, although dominant-negative activity observed for specific mutations |
| Congenital Adrenal Hyperplasia | *CYP21A2* (6p21.33), *CYP11B1* (8q24.3), *CYP17A1* (10q24.32) | SNV/Indel | AR | Single gene/Panel | P | Severity of phenotype is governed by the gene involved and the degree of the respective enzyme deficiency |
| X-linked adrenoleucodystrophy (X-ALD) | *ABCD1* (Xq28) | SNV/Indel | XLD | Single gene/CNV analysis | P/Ad | Cerebral forms typically present in childhood whereas the adrenomyeloneuropathy types may manifest in adulthood. |
| Familial Glucocorticoid Deficiency | *MC2R* (18p11.21), *NNT* (5p12), *MRAP* (21q22.11), *STAR (8p1123), TXNRD2* (22q11.21) + others | SNV/Indel | AR | Single gene/Panel | P | Presents in neonatal period/early childhood with features of glucocorticoid deficiency (e.g. hypoglycaemia). STAR mutations associated with XY sex reversal |
| Hereditary Phaeochromocytoma / Paraganglioma (PPGL) | *SDHB* (1p36.13), *SDHC* (1q23.3), *SDHD* (11q23.1), *SDHAF2* (11q12.2), *MAX* (14q23.3), *RET* (10q11.21), *VHL* (3p25.3), *NF1* (17q11.2), *TMEM127* (2q11.2) + others | SNV/Indel Gene del | AD | Single gene/Panel (+CNV analysis) | P/Ad | Specific SDH genes may be associated with non-endocrine cancers (e.g. GISTs, renal cell carcinoma). Prioritised analysis of genes may be governed by clinical phenotype |

| | | | | | | |
|---|---|---|---|---|---|---|
| Adrenocortical Carcinoma (ACC) | *TP53* (17p13.1) + others (e.g. mismatch repair genes, *MEN1*, *APC*) | SNV/Indel Gene del | AD | Single gene/Panel | P/Ad | The majority of children with ACC have germline *TP53* mutations associated with Li-Fraumeni Syndromes. |
| Familial Hyperaldosteronism | *CYP11B1/2* (8q24.3), *KCNJ5* (11q24.3), *CACNA1D* (3p21.1), *CACNA1H* (16p13.3) | SNV/Gene Fusion | AD | Single gene/Panel Long-range PCR | P/Ad | Familial hyperaldosteronism type 1 results from a chimeric CYP11B1/2 enzyme. |
| ***Calcium/Phosphate Homeostasis*** | | | | | | |
| Familial Isolated Hypoparathyroidism (FIH) | *GCM2* (6p24.2), *PTH (*11p15.3*)* | SNV/Indel Gene del | AD/AR | Single gene/Panel | P/Ad | Both autosomal dominant and recessive forms reported in association with *PTH* and *GCM2* mutations. |
| Autosomal Dominant Hypocalcaemia (ADH) | *CASR* (3q13.33-q21.1), *GNA11* (19p13.3) | SNV | AD | Single gene/Panel | P/Ad | Majority of case due to activating *CASR* mutations, with a small minority due to *GNA11* mutations. |
| 22q11.2 deletion syndrome (DiGeorge) | 3Mb deletion on chromosome 22 containing 30-40 genes including *TBX1* | - | *De novo* /AD | CGH/FISH/ MLPA | Ad/P | Deletion of *TBX1* gene thought to account for majority of clinical features. Majority of cases due to *de novo* mutation |
| Pseudohypoparathyroidism type 1a / Pseudopseudohypoparathyroidism (PHP1a/PPHP) | *GNAS (*20q13.32) | SNV/Indel/ Gene del | AD | Single gene | Ad/P | Maternally and paternally inherited *GNAS* mutations are associated with PHP1a and PPHP, respectively (i.e. parent of origin effects) |
| Familial Isolated Hyperparathyroidism (FIHP) | *MEN1* (11q13.1*), CASR* (3q13.33-q21.1), *CDC73* (1q31.2*), GCM2* (6p24.2) | SNV/Indel/ Gene del | AD | Single gene/Panel | Ad/P | Before diagnosing FIHP it is important to exclude additional clinical manifestations (e.g. associated with MEN1, HPT-JT) |
| Familial Hypocalciuric Hypercalcaemia (FHH) | *CASR* (3q13.33-q21.1), *GNA11* (19p13.3) *AP2S1* (19q13.32) | SNV/Indel/ Gene del | AD | Single gene/Panel | Ad/P | Neonatal severe hyperparathyroidism (NSHPT) results from autosomal recessive inheritance of LOF *CASR* mutations |
| Hereditary Hypophosphataemic Rickets | *PHEX* (Xp22.11), *CLCN5* (Xp11.23), *FGF23* (12p13.32) + others | SNV/Indel/ Gene del | XLD/ XLR/AD/ AR | Single gene/Panel | P | XLD inheritance observed with PHEX mutations; XLR inheritance observed with *CLCN5* mutations. AD and AR forms are observed in association with other genes |
| ***Thyroid Disorders*** | | | | | | |
| Congenital Hypothyroidism | *TSHR* (14q31.1), *PAX8* (2q14.1), *DUOX2* (15q21.1), *SLC5A5 (19p13.11), TSHB* (1p13.2), *THRA* (17q21.1) + others | SNV/Indel | AR/AD | Single gene/Panel | P | *THRA* mutations are associated with Resistance to Thyroid Hormone-α with clinical features of severe hypothyroidism. *THRA* is included on many congenital hypothyroidism panels |
| Thyroid Hormone Resistance (THR) | *THRB* (3p24.2), *THRA* (17q21.1) | SNV/Indel | AD/(AR) | Single gene/Panel | P/Ad | Majority of THR cases associated with AD inheritance |
| ***Disorders of Glucose Homeostasis*** | | | | | | |
| Neonatal and Infancy-Onset Diabetes | *ABCC8* (11p15.1), *KCNJ11* (11p15.1), *GCK* (7p13), *INS* (11p15), *PDX1* (13q.12)*+ others* | SNV/Indel | AD/AR/ de novo | Single gene/Panel | P | Phenotype due to defects in pancreatic development or β-cell function/survival. Defects at 6q24 account for majority of |
| Autosomal Dominant Familial Mild Hyperglycaemia / Diabetes (MODY) | *HNF4A* (20q13.12), *GCK* (7p13), *HNF1A* (12q24.31), *HNF1B* (17q12)*+ others* | SNV/Indel | AD | Single gene/Panel | P/Ad | Mutations in GCK, HNF1A and HNF4A account for majority of cases although ≥14 genes implicated in MODY phenotype |
| Congenital Hyperinsulinism | *ABCC8* (11p15.1), *KCNJ11* (11p15.1), *GCK* (7p13) + others | SNV/Indel | AD/AR | Single gene/Panel | P | Mutations in at least 9 genes associated with phenotype. Diffuse forms usually have AR inheritance |
| **Disorders of Sex Development/ Sex Chromosome abnormalities** | | | | | | |
| 46,XY Disorder of Sex Development (DSD)/Ambiguous genitalia | *SRY (Yp11.2), AR (Xq12), NR5A1 (9q33.3), HSD17B3 (9q22.32), MAMLD1 (Xq28), SRD5A2 (2p23.1), MAP3K1 (5q11.2), NROB1 (Xp21.2), DHH (12q13.12) + others* | SNVs/ Indels/ Gene del/ CNVs | NI (AD/AR) | aCGH /FISH/Panel | P | >30 genes implicated in DSD. *De novo* genetic variants are common. Occasional Y-linked inheritance for *SRY* gene. Some genes may manifest AD or AR inheritance. |
| Turner syndrome | Aneuploidy 45 X (loss or partial deletion/ rearrangement of one X chromosome) | - | NI | Karyotype/aCGH | Ad/P | May occur in mosaic forms with potentially milder phenotype |
| Klinefelter syndrome | Aneuploidy 47 XXY (typically having one additional copy of X chromosome) | - | NI | Karyotype/aCGH | Ad/P | May occur in mosaic forms with potentially milder phenotype |
| Prader-Willi syndrome (PWS) | Paternal deletion, maternal uniparental disomy or occasional translocations involving part of chromosome 15 | - | NI | [d]Methylation specific MLPA /aCGH/ FISH | P/Ad | The genetic changes associated with PWS usually occur *de novo* but very rarely can be inherited |
| **Miscellaneous** | | | | | | |
| Multi-system complex phenotype | Unknown | SNV/Indel/ | variable | aCGH,, | P/Ad | The inclusion of parental samples may help identify *de novo* |

| | | | | | | |
|---|---|---|---|---|---|---|
| with endocrine manifestations | | CNV | | WES/WGS | | mutations (i.e. 'trio' testing) |

This table is not intended to be comprehensive but rather to highlight the diversity of conditions for which genetic testing may be considered. The information included in this table is largely derived from: Genetics Home Reference (www.ghr.nlm.nih.gov); OMIM (www.omim.org); and Genetic Testing Registry (www.ncbi.nlm.nih.gov).

[a] Describes whether genetic testing for the respective condition is typically undertaken in Paediatric (P) and/or Adult (Ad) clinical settings.

[b] Several monogenic disorders may result from partial or whole gene deletions, which may not be detected by single gene or gene panel testing. In this setting alternate methods may be required including MLPA, aCGH, or FISH. Some NGS sequencing platforms including those used in gene panel testing may also detect these large-scale deletions.

[c] Several endocrine disorders may be associated with post-zygotic somatic mosaicism (S.Mos) (e.g. XLAG, McCune Albright syndrome). Although genetic testing of blood-derived DNA frequently identifies the genetic abnormality, in some settings alternate tissue samples are required for analysis (e.g. pituitary tissue in sporadic XLAG)

[d] Methylation-specific MLPA is a modification of MLPA that can detect epigenetic alterations including those observed in imprinting disorders such as Prader-Willi syndrome.

Abbreviations: P, paediatric; Ad, adult; MOS, mosaicism; aCGH, array comparative genomic hybridization; AD, autosomal dominant; AR, autosomal recessive; XLD, X-linked dominant; XLR, X-linked recessive; CNV, copy number variant; SNV, single nucleotide variant; PCR, polymerase chain reaction; NI, not inherited; Gene del, gene deletion; Gene dup, gene duplication; PPGL, phaeochromocytoma/paraganglioma; NET, neuroendocrine tumour; S.Mos, somatic mosaicism; ddPCR, droplet digital polymerase chain reaction.

## Table 2. Factors employed for variant interpretation by ACMG guidelines

| Variant characteristic | Evidence supporting pathogenic interpretation (strength of evidence) | Evidence supporting benign interpretation (strength of evidence) |
|---|---|---|
| **Population data**[a] | Increased prevalence of variant in affected individuals compared to controls (e.g. Odds Ratio >5) (**STR**)<br>Absence of variant in control population (e.g. GnomAD, ExAC) (**MOD**) | Minor Allele frequency >5% (**SA**)<br>Variant frequency in controls too high for disorder (**STR**)<br>Variant observed in healthy adult control and is inconsistent with known disease penetrance (i.e. fully penetrant) (**STR**) |
| **Segregation information**[b] | Co-segregation of variant with disease in multiple affected family members (**SUP-STR**) | Non-segregation of variant with disease (**STR**) |
| **Predictive data and Computational tools** | Predicted LOF/null variant in gene where LOF is a known mechanism of disease (**V.STR**)<br>Same predicted amino acid change as known pathogenic variant (**STR**)<br>Variant predicted to result in novel missense amino acid change at residue known to be affected by a different pathogenic missense mutation (**MOD**)<br>Multiple lines of computational evidence support a deleterious effect on gene function (**SUP**)<br>Variant predicted to result in protein length change as a result of an in-frame insertion/ deletion or stop-loss variant (**MOD**) | Missense mutations in which only truncating mutations cause disease (**SUP**)<br>Synonymous/silent variant with no predicted impact on splicing (**SUP**)<br>In-frame insertion/deletion in repeat region without known function (**SUP**)<br>Multiple lines of computational evidence suggest no impact on gene/gene product (**SUP**) |
| **Functional impact** | Well established functional studies show a deleterious effect (**STR**)<br>Variant occurs in mutational hotspot/ functional domain without benign variation (**MOD**)<br>Missense variant in gene in which missense variants are rare, and pathogenic missense variants occur frequently (**SUP**) | Well established functional studies show no deleterious effect (**STR**) |
| **De novo data**[c] | Confirmed *de novo* variant (i.e. maternity and paternity confirmed) in gene known to be associated with patient's phenotype (**STR**)<br>Assumed d*e novo* variant (maternity and paternity not confirmed) in gene known to be associated with patient's phenotype (**MOD**) | NA |
| **Allelic data** | For autosomal recessive disorders the variant occurs in *trans* with known pathogenic variant (**MOD**) | Variant occurs in *trans* with known pathogenic variant (for dominant disorders) or in *cis* with known pathogenic variant (for both dominant and recessive disorders) (**SUP**) |
| **Database data**[d] | Reputable source reports variant as pathogenic but evidence is not available to perform independent evaluation (**SUP**) | Reputable source reports variant as benign but evidence is not available to perform independent evaluation (**SUP**) |
| **Additional data**[e] | Patient's phenotype and family history highly specific for a disease with a single genetic aetiology (**SUP**) | Variant occurs together with a second variant in a different gene for which disease association is more compelling (**SUP**) |

**Footnotes**: The above guidance is based on the ACMG guidelines reported in Richards *et al* Genet Med 2015. These guidelines are intended for use for monogenic disorders with relatively high penetrance and for variants in genes with established disease causality. Additional details regarding the criteria for variant classification system is found in these guidelines.[18]

[a] It is Important that the control population data is matched to that of the patients under study (i.e. relevant geographical background)

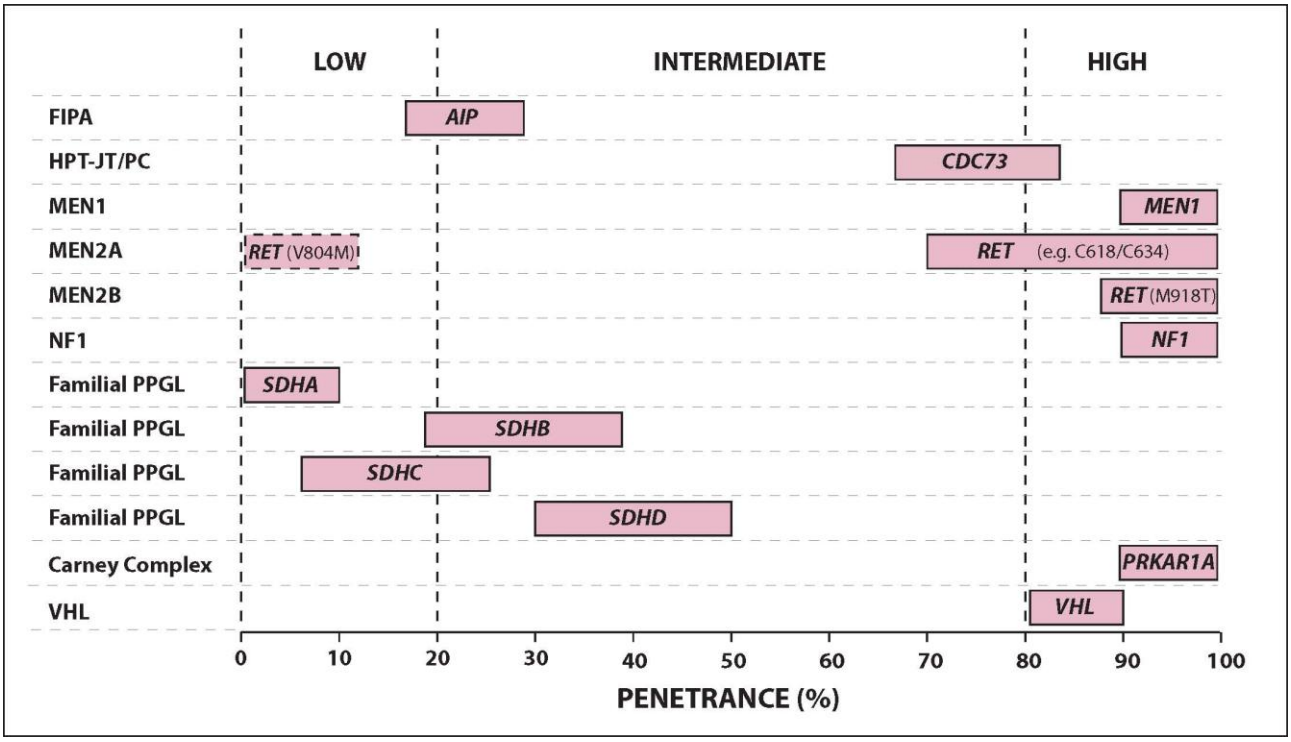
[b] The strength of evidence may vary according to the extent of the segregation data. Segregation data may be unreliable where penetrance is reduced, with late-onset disease or where clinical assessment of individuals within the kindred is incomplete. Occasionally, the variant under review may be in linkage disequilibrium with the 'true' pathogenic variant

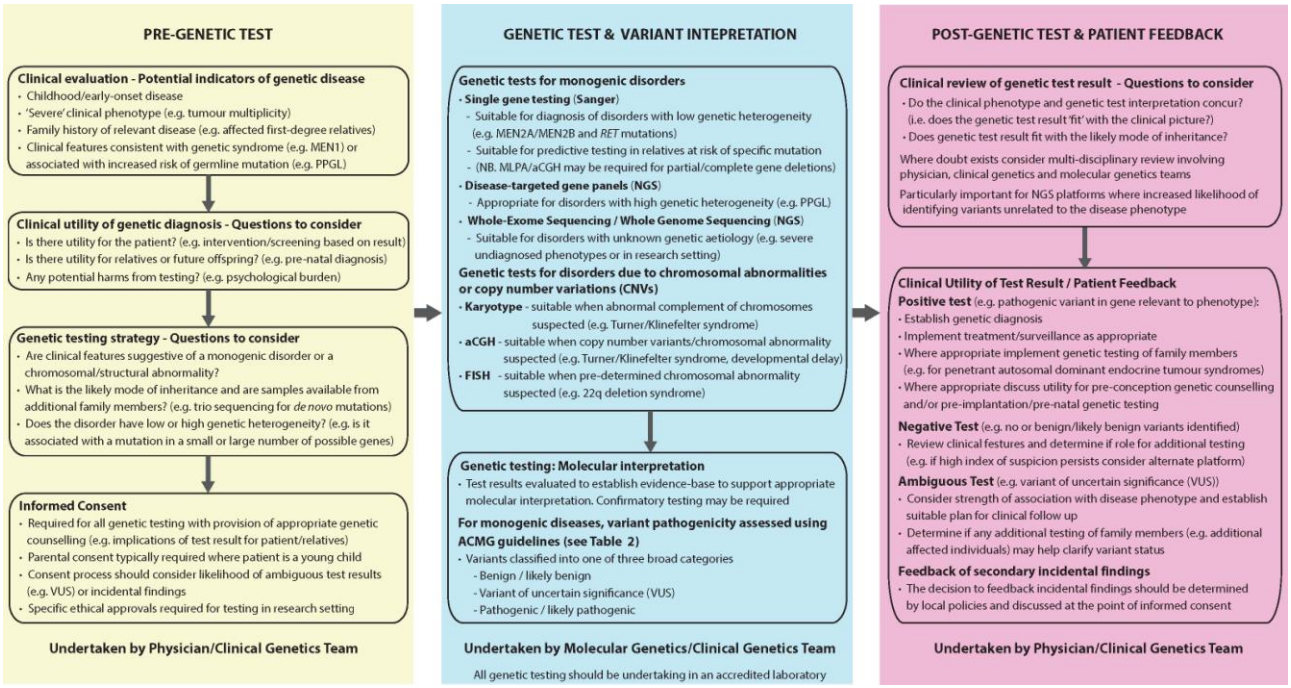
[c] *De novo* variants are observed in all individuals and cannot by themselves be taken as very strong (V.STR) or stand alone (SA) evidence of pathogenicity

[d] The reliability of variant databases may be reduced (e.g. inaccurate estimates of variant pathogenicity). Therefore, caution is required when relying on such databases unless additional supporting evidence is provided with the submission.

[e] A recent study has proposed locus-specific adjustments to the ACMG-AMP guidelines for missense variants in the *MEN1* gene which place additional weight on patient phenotype and family history (Romanet et al, Hum Mut 2019).[72]

**Abbreviations and additional terminology**; SA, Stand Alone; STR, strong; V.STR, very strong), MOD, moderate; SUP, supporting; *cis*, occurring on the same chromosome; *trans*, occurring on the opposing allele)





| | 'First" Generation (Sanger) Sequencing | Next Generation Sequencing (NGS) | | |
|---|---|---|---|---|
| | Single - Pauci Gene Test | Disease-targeted Gene Panel | Whole Exome Sequencing (WES) | Whole Genome Sequencing (WGS) |
| **No. of genes sequenced** | 1-5 | ~5-100 | >20,000 | >20,000 |
| **Typical clinical indication(s) for use** | Suspected monogenic disorders with low genetic heterogeniety | Suspected monogenic disorders with high genetic heterogeniety | Gene-discovery (i.e. suspected monogenic disorders with unknown or very wide genetic aetiology) | Gene-discovery (i.e. suspected monogenic disorders with unknown or very wide genetic aetiology) |
| **Target regions sequenced** | Exonic regions and intron/exon boundaries of gene(s) under test | Exonic regions and intron/exon boundaries of genes under test | Exonic regions and intron/exon boundaries of all known genes | Whole genome |
| **Nucleotides sequenced (content relative to single gene test)** | ~1,000-10,000 (1) | ~10,000-100,000 (~10-$10^2$) | ~30,000,000 (~3x$10^3$) | ~3000,000,000 (~3x$10^6$) |
| **Coverage of target region** | Typically excellent | Potentially reduced (some target regions may be difficult to sequence and may require Sanger sequencing to fill gaps) | Potentially reduced (a minority of target coding regions may not be adequately captured and/or sequenced) | Potentially reduced (a minority of genomic regions may not be amenable to sequencing, although more uniform coverage than WES) |
| **Genetic abnormalities detected** | Single nucleotide variants (SNVs) Small insertions/deletions (indels) | Single nucleotide variants (SNVs) Small insertions/deletions (indels) (potential to detect copy number variants (CNVs) involving coding regions) | Single nucleotide variants (SNVs) Small insertions/deletions (indels) Copy number variants (CNVs) involving coding regions | Single nucleotide variants (SNVs) Small inertions/deletions (indels) Copy number variants (CNVs) involving coding and non-coding regions Translocations/rearrangements |
| **Variant identification/ interpretation** | Fast/typically straightforward | Fast/typically straightforward (increased time with high-content panels) | Reliant on bioinformatic expertise Variant interpetation frequently challenging/time consuming | Reliant on bioinformatic expertise Variant interpetation frequently challenging/time consuming |
| **Likelihood of identifying VUS or secondary incidental findings** | Low | Low/intermediate (increased risk of VUS/incidental finding as content of panel increases) | High (3-5% risk of incidental finding) | High (3-5% risk of incidental finding) |
| **Additional comments** | Low false-negative rate for SNVs although may miss large deletions/duplications | Potential for false-positives/false-negatives Sanger sequencing often used to confirm suspected pathogenic variants | Larger indels may not be captured Sequencing of GC rich or highly homologous regions (e.g. pseudogenes) may have reduced reliability CNV analysis requires additional expertise | Interpretation of non-coding and/or structural variants limited Challenges associated with data processing and storage due to large file sizes |