Review Article

Genetic Testing in Endocrinology

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Abstract

The recent genomic revolution, characterised by surges in the number of available genetic tests and known genetic associations, calls for improved genetic literacy amongst medical scientists and clinicians. This has been driven by next generation sequencing, a technology allowing multiple genes to be sequenced in parallel, thereby reducing the time and financial costs associated with genetic testing in both research and clinical settings. Endocrinology is an intuitive setting in which to consider the principles of genetic testing because endocrine disorders are due to defects in circumscribed pathways, providing clues to candidate genes. This article discusses genetic testing in contemporary endocrine practice with reference to examples of endocrine genetic disorders or multisystem genetic disorders with endocrine manifestations. Monogenic disorders are prioritised as these form the bulk of endocrine genetic disorders and the associated genetic testing is readily understandable, clinically available and practice-changing. Although it remains true that genetic testing should be embarked upon only if the result will alter management, the clinical utility of genetic testing is often underestimated and there are expanding indications for genetic testing across all areas of endocrinology.

Introduction

Cytogenetic testing in the late 1950s led to the discovery of relatively common chromosomal disorders such as Turner's[1] and Klinefelter's syndromes.[2] In the 1980s and 1990s, a combination of linkage and positional cloning followed by Sanger sequencing identified the causative mutations in many recognised familial disorders, including: tumour disorders such as multiple endocrine neoplasia (MEN) type 1 (MEN1),[3] MEN2A,[4] MEN2B (also known as MEN3)[5] and von Hippel-Lindau (vHL) syndrome;[6] hormone biosynthetic defect disorders such as the congenital adrenal hyperplasias (CAH)[7] and glucocorticoid suppressible hyperaldosteronism (familial hyperaldosteronism type 1);[8] autoimmunity through defective self-recognition by T cells in autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy (APECED) syndrome;[9] and hormone receptor defects such as familial hypocalciuric hypercalcaemia (FHH)[10] and thyroid hormone resistance.[11] Completion of the human genome project, astute phenotyping of familial disorders and the introduction of next generation sequencing (NGS; also known

as massively parallel sequencing) then unravelled numerous less common endocrine disorders, including some where a familial basis was not initially recognised, such as many of the familial phaeochromocytoma-paraganglioma (PPGL) syndromes,[12] *AIP*-related familial isolated pituitary adenomas (FIPA),[13] and *ARMC5*-related bilateral macronodular adrenal hyperplasia (BMAH).[14]

This review provides a contemporary clinical and laboratory framework for genetic testing in endocrinology. We focus on monogenic disorders as these disorders are suitable for assessment by current genetic testing methodologies; specifically, cytogenetic tests such as karyotype and single nucleotide polymorphism (SNP) array looking for genetic variation at the chromosomal level, and molecular tests including Sanger sequencing of single genes and NGS of multiple or all genes. Although individually uncommon, endocrine genetic disorders together constitute a significant proportion of endocrinology. More common endocrine disorders such as type 2 diabetes and osteoporosis are

polyallelic but testing is not feasible for clinical purposes yet.

Clinical Evaluation

Genetic testing should be preceded by comprehensive phenotyping to determine the likelihood of finding a genetic disorder and to identify candidate genes. This staged form of patient evaluation is critical in avoiding variants of unknown significance which may be regarded as the 'incidentalomas' of genetic testing.

The decision to pursue genetic testing should be an index of how likely a disorder is to be genetic multiplied by how likely a genetic result would be to alter management of the individual or their family (Table 1). For example, germline *RET* mutations are found in only 7% of patients with sporadic medullary thyroid cancer and yet testing should be considered in all affected individuals as finding a mutation in the *RET* proto-oncogene, and thereby diagnosing MEN2, allows for testing of family members and lifesaving prophylactic thyroidectomy in those who have inherited the mutation.[15]

Some endocrine genetic disorders have well known, characteristic patterns, whilst others are exceedingly rare. It is impossible to know all genetic disorders but generic clues may be useful, such as young-onset and/or aggressive disease or the development of multiple rare disorders within an individual, or one or more rare disorders within multiple family members. An autosomal dominant family history of sulphonylurea-responsive diabetes mellitus in a lean individual with glycosuria immediately brings to mind *HNF1A*-related monogenic diabetes. *GATA6*-related monogenic diabetes is little known by comparison but the phenotype of pancreatic agenesis, congenital heart disease and other congenital malformations should raise suspicion for a genetic aetiology worthy of evaluation. Skin manifestations may hint at heritable syndromes in patients with apparently sporadic tumours. For instance, the classic interscapular rash of cutaneous lichen amyloidosis in a patient with apparently sporadic medullary thyroid cancer, phaeochromocytoma or primary hyperparathyroidism should prompt consideration of MEN2.[16]

The primary aim of the family history is to determine whether the disorder in the presenting patient (the 'proband') is likely to be genetic. Broad questions may be illuminating. For example, a family history of primary hyperparathyroidism may be represented only by affected members having fractures and renal calculi. Extending to a three-generation pedigree is critical in identifying disorders with reduced penetrance. For example, affected relatives with familial isolated pituitary adenoma syndrome are not uncommonly separated by unaffected relatives.[17] Consanguinity between the parents of an affected individual is an important clue to autosomal recessive disorders. If genetic testing has been deemed to be useful, a secondary aim of the family history is to determine which family member should be tested first. Environmental risk factors should be sought in each individual to identify alternative explanations for disease predisposition. In a family suspected to have monogenic diabetes, an obese individual may be the presenting patient and they may ultimately be found to carry the genetic mutation responsible for the family's diabetes, but the patient may alternatively transpire to be a 'phenocopy' with hyperglycaemia due to sporadically occurring type 2 diabetes related to their obesity rather than the familial mutation. Testing a lean family member would be more worthwhile because of their greater likelihood of having the family's genetic disorder rather than type 2 diabetes.

Non-genetic investigations may guide genetic testing. Hypercalcaemia, suggesting concomitant primary hyperparathyroidism, should prompt consideration of MEN2 in patients with phaeochromocytoma or medullary thyroid cancer, or MEN1 in patients with pituitary adenoma or gastroenteropancreatic neuroendocrine tumours. Negative succinate dehydrogenase subunit B (SDHB) immunohistochemical staining in PPGL specimens suggests a loss-of-function mutation in an *SDHx* gene (*SDHA*, *SDHB*, *SDHC* or *SDHD*),[18] whilst negative parafibromin immunohistochemical staining in parathyroid adenomas suggests a loss-of-function mutation in *CDC73* (formerly *HRPT2*) which may cause familial hyperparathyroidism or hyperparathyroidism-jaw tumour syndrome. Phenotypic tests may at other times be sufficient for diagnosis. For example, classical or non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency may be unequivocally diagnosed using basal or ACTH-stimulated 17-hydroxyprogesterone levels, respectively; however, subsequent sequencing of the *CYP21A2* gene may still be indicated to guide reproductive planning.

Genetic Counselling

Genetic disorders may be associated with feelings of fear, despair and guilt which should be addressed in an empathetic, non-directive manner before and after genetic testing. Pretest counselling should address the utility of genetic testing specific to the patient and their disorder. Detecting a 'premutation' (55-199 CGG repeats) in *FMR1* in a woman with premature ovarian insufficiency not only provides an aetiological answer to her presentation, but also allows screening of at-risk family members and informs reproductive planning. The latter is pertinent as the trinucleotide repeat region is unstable and prone to inter-generation expansion, particularly during maternal inheritance, whereby expansion to a full mutation (>200 CGG repeats) in male offspring produces fragile X

Table 1. Examples of indications for genetic testing in endocrinology.

Indication	Clinical example	Informative result	Benefit of result
Differentiation or confirmation of clinical disorders	Mild, longstanding PTH-dependent hypercalcaemia and intermediate urinary calcium creatinine clearance ratio	*CASR* mutation	Diagnosis of familial hypocalciuric hypercalcaemia which does not necessitate treatment, rather than primary hyperparathyroidism which is treated surgically
Disease monitoring	Sporadic medullary thyroid cancer	*RET* mutation	Rationale for current and ongoing assessment for other MEN2-related tumours
Therapeutic guidance	Lean individual with longstanding diabetes mellitus, glycosuria and a positive family history of diabetes	*HNF1A* mutation	Confirmation of sulphonylurea-sensitive subtype of monogenic diabetes to facilitate safe and appropriate insulin withdrawal and replacement with a sulphonylurea
Prognostication	Lean individual with longstanding mild hyperglycaemia	*GCK* mutation	Diagnosis of GCK diabetes which is non-progressive and not associated with vascular complications, therefore not requiring treatment
Family testing primarily to guide surveillance	Clinical MEN1 syndrome	*MEN1* mutation	Possibility of targeted mutation testing in presymptomatic at-risk family members to obviate the need for tumour surveillance in those who test negative
Family testing primarily to guide management	Clinical MEN2 syndrome	*RET* mutation	Possibility of targeted mutation testing in family members to facilitate prophylactic thyroidectomy
Family planning	Biochemically diagnosed non-classical congenital adrenal hyperplasia	One classic mutation and one variant allele in *CYP21A2*	Risk of classic congenital adrenal hyperplasia where the partner also carries a classic mutation in *CYP21A2* may be overcome by IVF and preimplantation genetic diagnosis

syndrome including intellectual disability.[19] By contrast, the primary aim of genetic testing in an older patient with medullary thyroid cancer may be facilitation of family testing.

These benefits must be balanced against the potential drawbacks of genetic testing, including the discovery of unexpected health risks or information on parentage. As in the identification of a *RET* mutation in an individual with medullary thyroid cancer which reclassifies their disease as MEN2, the health risks may be partly related to the genetic disorder, or they may be completely unrelated if a mutation is incidentally found in an unrelated gene such as *BRCA1/2*. Patients should be informed that results may have implications for family members; however, this is often the desired consequence of genetic testing and family members will not automatically know their own genetic status despite learning the results of their relatives. Exceptions to this include patients being informed of a germline genetic result in their monozygotic twin or in both their affected parent and their

offspring in an autosomal dominant disorder. In Australia, genetic results may impact upon certain types of insurance such as life insurance, income protection and mortgage protection. Positive screening for the familial mutation in seemingly unaffected family members may impact their ability to obtain such insurance, but for affected individuals, their history of the disorder in question likely bears greater influence on their ability to obtain insurance. Policies that are already established and not due for renewal are usually unaffected, as is private health cover. Genetic test results may also impose employment restrictions in occupations where maximum physical fitness is required or comprehensive health insurance is integrated with the employment, such as the armed services. Specimen collection, DNA storage protocols, opportunity for consent withdrawal and the protection of confidentiality should be discussed, and patients should be informed that genetic testing may not reveal the genetic cause of even the most clearly inherited disorder.

Laboratories differ but patients are usually required to sign a consent form before genetic testing can proceed. Depending on the consent form, patients may be given the option to know only results immediately relevant to their disorder or all results, including variants of unknown significance and results that are unrelated to the disorder in question. Some consent forms provide the option for patients to know all unrelated results or only medically actionable results where there are options for prevention, early diagnosis or treatment. The distress following notification of a non-actionable mutation with severe health consequences must be considered. Counselling patients for genetic tests involving multiple genes is more complex because of the increasing risk of these incidental findings. In most instances, such counselling should be performed by genetic counsellors and/or clinical geneticists, whereas limited testing of single genes or mutations may be within the scope of practice of non-geneticist clinicians.

Genetic Testing

The choice of genetic testing methodology is dictated by clinical, laboratory and funding circumstances. For example, in the current Medicare-unassisted environment, requesting clinicians may request Sanger sequencing of an individual gene or staged Sanger sequencing of multiple genes based on clinical suspicion because the initial costs are less, even though upfront NGS to analyse multiple genes simultaneously may be more informative and less expensive overall. On the other hand, laboratories may prefer NGS requests as this allows for the testing of multiple patient samples from disparate clinical settings and, potentially, economies of scale. The final decision of which test to pursue is usually made by the requesting clinician with relevant laboratory input.

Testing for monogenic disorders utilises germline DNA extracted from peripheral blood leucocytes, hair follicles or buccal swabs. Which genetic test to then perform depends on the anticipated genetic cause of the disorder in question. Online tools can assist in collating potential genetic causes (e.g. Online Mendelian Inheritance in Man database[20] and GeneReviews[21]) and clinically available tests (e.g. Genetic Testing Registry[22] and RCPA Genetic Tests and Laboratories Website[23]).

The most common cytogenetic test in the endocrine setting is karyotyping to diagnose aneuploid disorders such as Turner's syndrome (45,X) and Klinefelter's syndrome (45,XXY). Increasing the number of cells analysed may reveal mosaicism with therapeutic ramifications. The presence of the Y chromosome in all or any cells in phenotypic females poses a risk of gonadoblastoma, whilst monosomy X in mosaicism with a normal female karyotype (45,X/46,XX) is associated with milder ovarian insufficiency, sometimes allowing for menarche and even spontaneous conception. Karyotyping in disorders of sexual development may be complemented by fluorescent *in situ* hybridisation (FISH), which employs probes to identify and quantify relevant genes such as *SRY* on the Y chromosome. A positive FISH result in this case indicates the presence of the Y chromosome or translocation of the *SRY* gene onto the X chromosome, the latter being the most common cause of 46,XX complete gonadal dysgenesis. SNP array is a cytogenetic test with resolution between karyotype and sequencing methodologies, identifying copy number variants (CNVs) to an approximate resolution of 0.2 MB (200,000 bp). Endocrine disorders that are well suited to investigation by SNP array include Prader-Willi syndrome (15q11.2 microdeletion), DiGeorge syndrome (22q11.2 microdeletion) and, more recently, X-linked acrogigantism (XLAG; Xq26.3 microduplication).[24]

Molecular testing is based on nucleotide variation and typically involves gene sequencing either by Sanger sequencing, which is a sequencing-by-termination method targeting an individual gene, or NGS, usually via a sequencing-by-synthesis platform. NGS begins with DNA fragmentation and amplification of these DNA fragments into thousands of copies which can be simultaneously sequenced. NGS thus enables parallel sequencing of multiple genes in a cost-effective manner, although bioinformatic costs rise incrementally with the number of genes analysed. Sanger sequencing costs are dependent on the number of amplicons (continuous DNA segments) required. Because each exon requires one or more amplicons, sequencing costs rise with gene size and exon number. If a disorder is caused by a few known mutations, targeted mutation analysis is most cost-effective with Sanger sequencing of only the regions immediately flanking each mutation of interest. For example, the activating M918T mutation in the *RET* proto-oncogene accounts for 95% of cases of MEN2B, where patients have a Marfanoid body habitus and mucosal neuromas in addition to the typical MEN2 predisposition to medullary thyroid cancer and phaeochromocytoma.[15] Targeted mutation analysis for M918T is particularly time- and cost-efficient in this scenario as *RET* is a large gene consisting of 20 exons. MEN1, in contrast, occurs due to loss-of-function mutations which may arise anywhere along the *MEN1* gene, mandating sequencing of the entire gene. NGS may be preferred in the evaluation of MEN1 not only because the whole gene must be assessed, but also because differential diagnoses in this phenotype include *CDKN1B* mutations resulting in MEN4, which is clinically indistinguishable from MEN1, and *AIP* mutations resulting in FIPA.[17] Another use of targeted mutation analysis is in relatives at risk of a known familial mutation. Whilst it may take weeks or months to initially find the culprit mutation in a proband, the turnaround time is closer to a fortnight to

determine the status of family members subsequently tested by targeted mutation analysis using Sanger sequencing.

NGS is well suited to the investigation of disorders with multiple potential genetic causes, which now include the majority of endocrine genetic disorders, including most endocrine tumour syndromes, disorders of sexual development, congenital hypopituitarism, monogenic diabetes and hypophosphataemic rickets. Even single gene disorders may prove to have multiple genetic causes. For instance, *ARMC5* is presently the only gene implicated in familial Cushing's syndrome due to bilateral macronodular adrenal hyperplasia (BMAH) but some families do not harbour *ARMC5* sequencing variants and research is ongoing to discover other causative genes.[25] NGS may also elucidate digenic disorders where genetic mutations in two genes act synergistically, as hypothesised in cases of Kallman's syndrome, familial pituitary tumour syndrome and pituitary stalk interruption syndrome.[17,26,27] NGS may be performed in the form of a panel of genes based on a group of disorders, such as metabolic bone diseases, or a single disorder, such as pituitary adenoma. Such panels are cost- and time-efficient but they do not accommodate ongoing genetic discoveries. A carefully designed and optimised genetic panel immediately becomes outdated following the discovery of a previously unknown causative gene.[17] Virtual panels, whereby a much wider panel or all coding regions are sequenced but only the genes of interest are interrogated, may overcome this problem as it is a comparatively simpler task to return to the data and analyse a new gene of interest than to return to the patient's stored DNA to perform another genetic test.[28] An alternative to NGS standard or virtual panels is staged gene Sanger sequencing whereby the most likely culprit gene is sequenced first, followed by genes of decreasing probability, but this may ultimately be more expensive than NGS depending on when the causative mutation is found.[17]

If all genes are to be sequenced and analysed, this may either be in the form of whole genome sequencing (WGS), which covers all coding and non-coding regions, or whole exome sequencing (WES), which is restricted to coding regions. WGS is more expensive but provides insight into vast lengths of untranslated regions (UTR) and potential enhancer, promoter and repressor sites. WES is often considered more cost-effective as it is limited to the most understandable genetic data in contemporary practice and it typically provides greater depth of coverage of the coding regions of interest, though this varies by platform. These broader sequencing methodologies are advantageous where there is ongoing research into novel candidate genes in addition to multiple known susceptibility genes. WGS additionally provides information about copy number variants when analysed using appropriate bioinformatic pipelines.[29]

There are various pitfalls in sequencing (Table 2). False negative results may occur in the setting of large insertions/ deletions (referred to as 'indels'). Sanger sequencing may identify indels up to the size of an amplicon (approximately 300 bp), whilst NGS panels and standard WES may identify deletions up to approximately 400 bp and insertions up to approximately 50 bp. The precise thresholds depend on the mapper, variant caller, read length and DNA fragment in question. Larger indels may be identified by multiplex ligation-dependent probe amplification (MLPA) using commercially obtained reagents and probe-mixes to produce electrophoresis peaks which may be smaller or larger than reference samples, indicating deletions or insertions, respectively. This may be performed routinely in disorders where CNVs are a recognised cause, such as Von Hippel-Lindau syndrome where exonic or whole gene *VHL* deletions are found in 20–30% of cases,[30] or it may be done when sequencing variants are not identified despite a highly suspicious phenotype. SNP array and quantitative PCR are other methods of identifying CNVs. False negatives may also arise if a point mutation is located in an area not sequenced – for instance, in another gene when only certain genes are sequenced or in a deep intronic area in any sequencing methodology apart from WGS as Sanger sequencing, NGS panels and WES generally only sequence exons and <10 nucleotides flanking each exon. If suspected, intronic mutations may be detected by mRNA reverse transcription and amplification (RT-PCR). This is valuable in neurofibromatosis type 1, where 2–3% of cases are associated with deep intronic *NF1* variants affecting splicing with abnormal mRNA results despite normal sequencing results.[31] Both *VHL* and *NF1* are relevant in endocrinology because of their associations with phaeochromocytoma. Other than WGS, sequencing may also fail to detect promoter mutations as found in the *PTEN* gene in up to 10% of patients with Cowden syndrome, characterised by thyroid, endometrial and breast neoplasia, and hamartomas.[32]

Sequencing may produce false positive results if the bioinformatic analysis of variants is not rigorous, leading to misclassification of a benign polymorphism as a disease-causing mutation. False positives may also arise in pseudogenes. These are genes which share a similar sequence to one or more other genes. Such pseudogenes may be mistaken for one another on NGS which has lower specificity for a given nucleotide sequence than Sanger sequencing. Clinically relevant examples include *CYP21A2* which is associated with congenital adrenal hyperplasia and *PMS2* associated with Lynch syndrome where NGS may incorrectly call variants in related genes as being variants in the genes of interest. Sanger sequencing with amplicons

Table 2. Molecular genetic methodologies according to expected ability to detect abnormalities in different genetic settings.

Abnormality	Sanger sequencing	MLPA	Next generation sequencing			
			Panel testing#	Whole exome testing	Whole genome testing	Long-read sequencing
Small indels (<50 bp)	Y	Y	Y	Y	Y	Y
Medium indels (50-300 bp)	M*	Y	M*	M*	Y	Y
Large indels (>300 bp)	N	Y	N	N	Y	Y
Mutation in unsuspected gene	N	N	N	Y	Y	Y
Intronic mutation	M**	N	M**	M**	Y	Y
Promoter mutation	M**	N	M**	M**	Y	Y
Pseudogenes	M*	Y	N	N	N	M*
GC-rich genes	M*	N	N	N	N	Y
Triplet repeat disorders	M*	N	N	N	N	Y

#A selected panel of genes which are sequenced and analysed, does not include 'virtual panels' where all genes may be sequenced with only a selected panel of genes being analysed; *depends on length; **depends on location; Y, abnormality likely to be detected; M, abnormality may be detected; N, abnormality likely to be missed.

targeting areas of difference between the target gene and the respective pseudogenes usually overcomes this issue. Another indication for Sanger sequencing is guanine-cytosine (GC) rich genes as the three hydrogen bonds between these two nucleotides lead to high melting points, secondary structures and ultimately poor amplification in the automated process of NGS. One such gene is *GATA6*, which causes pancreatic agenesis/hypoplasia and congenital heart disease and should be interrogated by Sanger sequencing in preference to the usual monogenic diabetes panels.[33] False positive results may also occur with NGS of repetitive regions, making it unsuitable for the evaluation of such disorders as fragile X syndrome due to triple repeat expansion in *FMR1*.[34]

McCune-Albright syndrome is a noteworthy endocrine disorder as it is a mosaic disorder due to a heterozygous postzygotic mutation. As the mutation is not inherited, parents cannot be affected and there is no risk to offspring as the mutation is incompatible with life in the germline state – any offspring born to affected individuals will have inherited the wild-type *GNAS* allele. *GNAS* testing may still be useful in individuals with mild phenotypes like monostotic fibrous dysplasia where securing the diagnosis of McCune-Albright syndrome would prompt surveillance for the various associated endocrinopathies including precocious puberty, hyperthyroidism and growth hormone excess. If no *GNAS* mutations are found on DNA sequencing using peripheral blood leucocytes, then DNA should be extracted from affected tissues. As the classic café-au-lait skin lesions often fail to demonstrate the *GNAS* mutation due to the paucity of melanocytes, resected endocrine and other tissues are more suitable for somatic testing.[35]

Genome-wide association studies (GWAS) are limited to research purposes as they compare SNPs shared between affected versus unaffected individuals, which act as surrogate markers of disease status rather than being causative mutations. GWAS may provide insights into seemingly polygenic endocrine disorders such as osteoporosis, but they add little to risk prediction beyond that achieved by family history and traditional risk factors. Such tests should not be recommended to patients and if results are presented following direct-to-consumer testing, expert advice should be sought from clinical geneticists and genomic pathologists to determine if they contain any meaningful results, the accuracy of such results and whether accredited clinical testing is required.

Result Interpretation
NGS can yield tens to millions of variants, depending on the proportion of the genome sequenced, in an individual's genetic code compared to the human reference genome. Raw data are therefore filtered by 'bioinformatic pipelines' which use measures such as conservation of the nucleotide position

across species and biophysicochemical differences in the mutant protein to prioritise variants. As these pipelines differ by software and between laboratories, reviewing raw data in a different bioinformatic laboratory may be enlightening when the initial results are apparently negative.

Whether detected by Sanger sequencing or NGS (Figure), variants should be classified according to the American College of Medical Genetics and Genomics (ACMG) categories: 1) benign, 2) likely benign, 3) of uncertain significance, 4) likely pathogenic, and 5) pathogenic.[36] Classification is based on features incorporated into most bioinformatic pipelines. For instance, nonsense variants (premature termination codon due to nucleotide substitution) and frameshift variants (altered nucleotide reading frame due to nucleotide insertion/deletion) argue for pathogenicity whilst silent variants (nucleotide substitution with unaltered amino acid sequence) suggest the variant to be benign. Although, a silent variant may be causative if it produces aberrant splicing, which may be predicted by *in silico* (software) tools that are also incorporated into most bioinformatic pipelines. The ACMG criteria also include more clinical factors such as variant cosegregation in multiple affected family members, and laboratories should liaise with clinicians where appropriate. Another key criterion is the frequency of the variant in control databases. The population frequency threshold to support a variant as being benign versus pathogenic should be considered in comparison to disease frequency. A mutation causing a severe, rare autosomal dominant disorder such as septo-optic dysplasia with midline defects including congenital hypopituitarism should occur extremely infrequently or not at all in population databases. In contrast, a mutation causing a common monogenic disorder such as *GCK* monogenic diabetes may well be found in population databases as these do not strictly consist of purely healthy individuals. Class 5 (pathogenic) and sometimes class 4 (likely pathogenic) variants are considered to be causative of the associated disorder and may be used in further patient and family management. Class 3 variants, also known as variants of unknown significance (VUS), are particularly prevalent and difficult to elucidate in uncommon, less studied genetic disorders such as familial pituitary tumour syndromes where there is comparatively little genetic literature compared to, say, familial breast and colorectal cancer syndromes.[17] Performing a functional assay of the mutated versus wild-type protein products may provide clarification, but this is often limited by access to relevant expertise and sufficient funding. Class 2 (likely benign) and 1 (benign) variants are generally regarded as negative or 'uninformative' results.

Laboratory reports should be written cautiously in a way that allows incorporation of the patient's clinical context. *MEN1* genetic testing is negative in up to 20% of patients with clinical MEN1, defined as two MEN1-related tumours in an individual or one MEN1-related tumour in a first-degree relative of an individual with MEN1.[37] A negative *MEN1* genetic result therefore does not negate a clinical diagnosis of MEN1 and tumour surveillance should be continued. By contrast, a patient with apparently sporadic medullary thyroid cancer is diagnosed with MEN2 if an activating *RET* mutation is detected in their germline DNA and the patient and their family should be managed accordingly.[38]
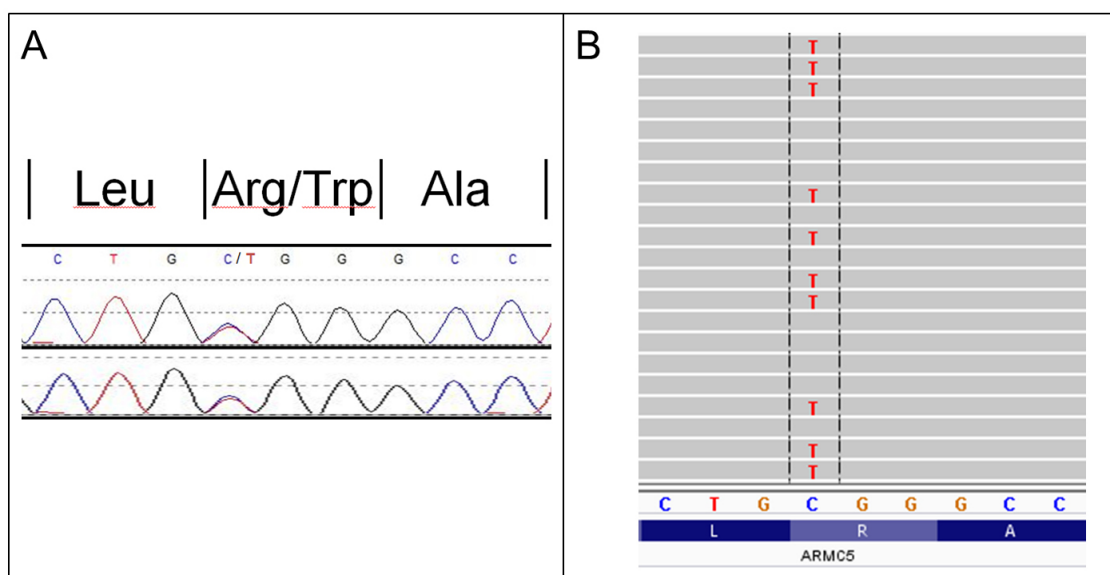
Figure. Pictorial examples of genetic results yielded by different sequencing methods. The same germline *ARMC5* mutation (GRCh37/hg19, Chr16:g.31476121C>T; NM_001105247.1, c.1777C>T, p.Arg593Trp) is shown following Sanger sequencing (A) and next generation sequencing (B).

Management

Result disclosure closes the loop of genetic counselling and should be performed by the requesting clinician. The category of genetic variant(s) found and implications for the patient and their family should be addressed. In all cases, genetic results should be conveyed in terms of current knowledge but genotype-phenotype relationships are often uncertain. The risk of disease associated with a particular mutated gene may fall with time as initial estimates are often based on large families with high penetrance which were amenable for discovery studies. In addition, some disorders where gene testing is negative may later be suitable for repeat testing as further causative genes are identified, as has occurred in PPGL (now associated with at least 26 genes influencing mitochondrial oxidative metabolism or tumour growth regulation), FHH (classically due to *CASR* mutations but now also due to *GNA11* and *AP2S1* mutations) and MEN4 (an MEN1-like disorder due to *CDKN1B* mutations).

In the case of a VUS, the affected patient should be advised that the variant does not explain their disorder currently, that testing family members for the VUS will not clarify whether they have inherited the disorder, and that only further research can discern whether the VUS is a benign polymorphism or a causative mutation. Such research includes segregation studies, but a larger body of evidence is required to upgrade a VUS to a class 5 (pathogenic) or even a class 4 (likely pathogenic) variant. It is critical that family members understand that any such testing is for the purpose of segregation analysis rather than predictive testing. Failing to convey this message may lead family members to incorrectly think they are no longer at risk of having the disorder simply because they have tested negative for the VUS and they may thus abandon potentially lifesaving surveillance. Another challenge is incidental findings in genes unrelated to the phenotype in question, which is a risk that increases with the number of genes tested. An informed pretest decision by the patient as to whether they wish to know such results is imperative.

Finding the culprit mutation provides the opportunities of family testing and reproductive planning. 'Cascade testing' refers to testing for a known familial mutation in at-risk family members, starting with first-degree relatives and proceeding to second-degree relatives if the linking relative has the mutation. Alternatively, family testing may be *ad hoc* if linking relatives are deceased, unavailable or uninterested in genetic testing, or if expedited testing is required because of emerging clinical features or for family planning. Because of issues surrounding consent, confidentiality and contact tracing, clinical genetics services remain the hub for family testing. The chance of offspring inheriting genetic disorders depends on inheritance pattern. In couples where each partner is heterozygous for a mutation associated with an autosomal recessive disorder such as APECED due to *AIRE* mutations, 25% of children will be homozygous for the mutation and develop the disorder, 50% of children will be heterozygous carriers and 25% will be neither affected nor carriers. In autosomal dominant disorders such as *AIP*-related FIPA, children of an affected individual with a heterozygous mutation will have a 50% chance of inheriting the mutation, although not all individuals with the mutation will be affected due to variable penetrance (the chance of a disease manifesting or not in an individual with the mutation). X-linked dominant disorders such as X-linked hypophosphataemic rickets due to either hemizygous or heterozygous *PHEX* mutations will be inherited by all daughters and no sons of an affected man and by half of all daughters and sons of an affected female. Imprinting refers to silencing of an allele depending on the parent of origin. An example of maternal imprinting is *SDHD*-related PPGL where 50% of offspring will be affected if born to an affected father, or unaffected carriers if born to an affected mother. Mitochondrial DNA mutations – such as m.3243A>G which may cause maternally inherited diabetes and deafness – are exclusively inherited from the maternal side and disease manifestation depends on overall and tissue-specific mutant load. However, mitochondrial disorders may also arise due to nuclear gene defects with either autosomal recessive or dominant inheritance. The health consequences for an individual carrying the causative mutation depends on penetrance as well as expressivity (the degree of disease manifestation in an individual with the mutation).

An alternative to genetic testing of family members in familial endocrine disorders is phenotypic screening. This is ideal where the test is simple and highly sensitive and the disorder highly penetrant – for instance, serum calcium screening in first-degree relatives of a person with FHH. In contrast, cascade testing for a known familial *ARMC5* mutation has superseded the multistep dynamic testing that was previously used in familial BMAH. Detecting subclinical ACTH-independent cortisol rises in response to varying stimuli was proposed to predict which family members would develop overt BMAH-related Cushing's syndrome; however, these cumbersome tests were later found to correlate poorly with mutational status.[39] Whether to perform genotypic or phenotypic screening also depends on family structure. If an older patient with no children is suspected to have *HNF1A* or *HNF4A* monogenic diabetes, phenotypic screening by substitution of insulin with a sulphonylurea with close monitoring by an experienced endocrinologist may be a quick, inexpensive and safe option. If this older patient had several lean children and grandchildren with diabetes, then a monogenic diabetes gene panel in one individual followed by targeted sequencing of the familial mutation (if found) would

be more efficient in determining the aetiology of diabetes in each affected family member.

Reproductive options include expectant management, adoption, prenatal testing and preimplantation genetic diagnosis (PGD). In autosomal recessive disorders, PGD may be used to select and transfer unaffected embryos: these may be either homozygous for the wild-type allele (25% of embryos, preferable as they are neither affected nor carriers) or heterozygous for the mutation (50% of embryos, especially in couples with a low yield of viable embryos). In autosomal dominant disorders, only homozygous wild-type embryos (50% of embryos) can be selected. Although used less frequently, preimplantation sex selection can be sufficient for patients with X-linked disorders, either selecting male embryos of men with an X-linked dominant disorder or female embryos of women with an X-linked recessive mutation. Three-parent IVF using the affected woman's oocyte nucleus, an unaffected donor oocyte and the partner's sperm is an emerging option for women with a pathogenic mitochondrial DNA mutation. These options are especially pertinent in endocrinology as the underlying disorder may cause infertility and thus independently necessitate assisted reproductive technologies. For instance, secondary hypogonadism may be caused by MEN1 or FIPA, and premature ovarian insufficiency may relate to Turner's syndrome (45,X), an *FMR1* premutation, or an *AIRE* mutation resulting in APECED.

Future Directions

Ongoing technological advancements are reducing the cost of sequencing, which should increase the uptake of genetic testing in endocrine disorders. In our laboratory, the cost of WES has already decreased from A\$4500 in 2015 to A\$2000 in current practice. This is different to the consumables cost which is closer to A\$300 and sometimes, misleadingly, advertised to the public. Regardless, these costs should not be considered prohibitive if the results would influence patient management or guide family testing. It is useful to compare sequencing costs against other typical costs in the evaluation of a patient – for example, A\$600 for a brain MRI or A\$1000 for an overnight inpatient admission. Unlike these latter costs, the cost of NGS is distributed across a family because, if it reveals the causative mutation in the proband, other members may be evaluated by merely sequencing one amplicon. Genetic testing may ultimately be subsidised by the Medicare Benefits Scheme (MBS) or private health cover as it is in the United States.[40] As of 1 November 2017, germline *BRCA1/2* genetic testing is MBS-funded in select women with ovarian, fallopian tube or primary peritoneal cancers. This was instigated by the availability of olaparib, a poly ADP ribose polymerase (PARP) inhibitor which is funded by the Pharmaceutical Benefits Scheme for women with tumour recurrence and a known germline *BRCA* mutation as *BRCA*-mutated tumours are highly dependent on PARP-mediated DNA repair pathways. The increasing functionality of genetic testing should provide similar funding avenues.

Less than one in five (3,280/19,580) genes are associated with a Mendelian phenotype and 25% (798/3,280) of these genes can cause two or more phenotypes, some with mixed inheritance patterns.[41] These figures are expected to evolve due to emerging phenotypic extensions whereby genetic disorders are shown to have a spectrum of clinical manifestations, and allelic series, which is the molecular corollary referring to different mutant alleles within a gene giving rise to a range of phenotypes. International collaborative databases should enhance our understanding of genotype-phenotype correlations and genotyping may ultimately be performed early in clinical evaluation to save patients from the time-consuming, costly and sometimes invasive diagnostic odyssey that complete phenotyping entails. In the paediatric setting, WES has already been shown to reduce clinical costs, increase diagnostic yield and alter patient management compared to traditional diagnostic methodologies.[42]

As mentioned, even NGS may fail to demonstrate the molecular cause of a clearly genetic disorder, which may reflect intronic, polygenic or epigenetic changes that are frequently missed by NGS.[28] NGS may also miss indels and mistake pseudogenes for one another due to the employment of 'short reads' of DNA pieced together. Long-read sequencing (Table 2) is the latest sequencing technology whereby reads of up to 1 MB in length allow elucidation of CNV, pseudogenes and triplet repeats and determination of whether variants are *in cis* (on the same allele) or *in trans* (on alternate alleles, as required for an autosomal recessive disease to manifest). Long-read sequencing also performs reasonably well in GC-rich genes as DNA amplification is unnecessary and it utilises a more durable polymerase enzyme compared to standard NGS platforms. In addition, the real-time data of long-read sequencing may reveal delays in nucleotide binding as an indicator of methylation or other epigenetic changes.[43]

Somatic genetic testing of tumour DNA extracted from affected tissues may guide the management of some malignancies – such as the use of BRAF and MEK inhibitors in patients with melanomas with a somatic *BRAF* mutation – but it is yet to determine the management of endocrine tumours. Although, the recent discovery of somatic *CTNNB1* and *BRAF* mutations in adamantinomatous and papillary craniopharyngiomas, respectively, heralds the use of tumour genetic testing in endocrinology to guide pharmacotherapy with novel beta-catenin inhibitors and vemurafenib.[44,45]

Conclusions

There is a genetic basis to many endocrinopathies, including both neoplastic and metabolic disorders. Close liaison between endocrinologists, biochemists, clinical geneticists and genomic pathologists is required in the identification of endocrine genetic disorders. If a genetic aetiology is considered possible, the plausible genetic causes and the utility of genetic testing should be determined. If the results of genetic testing would inform the assessment or management of the patient or their family, genetic testing should be arranged and tailored to the type of genetic abnormality in question. As we discover the genetic basis of more endocrine disorders, the utility of genetic testing will rise and scientists and clinicians will increasingly be faced with the issue of genetic testing in endocrinology.

Competing Interests: None declared.

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