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Insights from exome sequencing in common and rare human endocrine disorders

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Abstract

Exome sequencing has emerged in recent years as a rapid and effective tool for the elucidation of genetic defects underlying both rare and common human disease. Increased availability and decreased costs of next generation sequencing has enabled researchers worldwide to use this approach not only in individual patients with rare diseases, but also to screen larger cohorts or populations for genetic determinants of disease. Within the field of endocrinology, exome sequencing has led to significant advancements in our understanding of numerous disorders including adrenal disease, growth and pubertal disorders, type 2 diabetes, as well as a multitude of rare genetic syndromes with prominent endocrine involvement. In this review, we aim to provide an overview of these recent new insights and discuss the role that exome sequencing is expected to play in endocrine research and clinical practice in the coming years.

I Introduction

Ever since the completion of the Human Genome Project over a decade ago, techniques for large scale genomic analysis have continued to evolve at an exponential rate, allowing high-throughput screening to shift from a largely individual gene approach to genome wide analyses of disease causing determinants. An important advancement has been the advent of whole exome sequencing (WES) over the last 5 years. WES is a highly effective form of high-throughput genetic analysis, in which more than 90% of the coding DNA of an individual is sequenced. This is accomplished by using a variety of “exome capture” techniques in which DNA probes that specifically target all of the exons in the genome are used to extract the protein coding portion from the remainder of the genomic DNA. This exome represents approximately 1% of the entire genome thus allowing a much more cost effective approach to mutation identification as opposed to whole genome sequencing. The targeted DNA is then sequenced using next-generation sequencing technologies, and the patient’s sequence is compared to the human reference genome for the identification of genetic variants. As many disease causing mutations are located in the protein coding regions, exome sequencing will readily identify these mutations. However, a substantial number of pathologic genetic aberrations in the regulatory regions of the genome as well as in exons that are not well captured will be missed by exome sequencing. Despite these limitations, when used appropriately, WES has proven to be very successful in identifying

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novel genes and disease pathways. One of the earliest examples is provided by the exome sequencing studies in Kabuki Syndrome (KS). In this poorly understood genetic syndrome of multiple congenital malformations with wide phenotypic variability, exome sequencing effectively identified pathogenic *MLL2* gene mutations in over 50% of patients¹.

The impressive advancement in our understandings of Kabuki syndrome is a striking example of how exome sequencing can discover the cause of rare monogenic diseases (diseases in which a single genetic variant within an individual explains the phenotype). Within endocrinology, exome sequencing has identified the genetic etiology of a number of rare syndromes with prominent endocrine components. Other applications of exome sequencing within the field of endocrinology include the study of large, population-based cohorts to identify more common, low-risk genetic variants that contribute to common polygenic diseases such as type 2 diabetes. Finally, exome sequencing has played a critical role in identifying both germline and somatic mutations leading to endocrine neoplasia (Figure 1). In this review, we will discuss the most prominent advancements resulting from each of these three approaches and then will discuss potential benefits and pitfalls of implementing WES into clinical practice. In preparation of this manuscript, we searched Pubmed for articles containing the MESH terms "endocrinology" or "endocrine" and "exome sequencing" or "next-generations sequencing" up until Nov 1st 2014 and the associated references in the articles of interest.

II Rare endocrine disorders: identification of novel genes and pathways

In recent years, WES has been extensively used to decipher the underlying biological mechanisms of a wide spectrum of endocrine diseases. Most of these reported endocrine disorders are rare, and apart from their clinical description and management, relatively little was known regarding specific etiologic factors or causative genes. WES-based studies involving one or more affected members from the same family or from a number of different families have produced remarkable new insights into gene function (Table 1).

Pubertal disorders and hypogonadism

For many decades the biological mechanisms that underpin the onset and timing of puberty have been studied, as well as the genetic etiology of its associated conditions leading to either precocious or delayed/incomplete pubertal development. It is generally accepted that hypothalamic GnRH neurons act as a pulse generator, but the specific genetic determinants that govern the individual timing of puberty were largely unknown. Last year, Abreu et al. performed whole exome sequencing in 40 patients from 15 different families with familial central precocious puberty (CPP) and identified loss-of-function mutations in the imprinted gene makorin RING-finger protein 3 (*MKRN3*) as the causal genetic factor². Functional evidence was provided by the demonstration of decreasing *MKRN3* mRNA expression in murine hypothalami prior to the onset of puberty further supporting a role for *MKRN3* as a repressor of pubertal onset. A recent follow up study confirmed the clinical significance of *MKRN3* mutations in a large independent cohort of 215 CPP patients³ and the question arises whether differential *MKRN3* expression among children can explain normal variation in pubertal timing. Based on these findings, targeted sequencing of *MKRN3* should be

rapidly introduced into clinical practice in cases of familial central precocious puberty and should be considered in isolated cases of CPP.

At the other end of the spectrum of pubertal disorders, WES has also produced important insights into the genetics of delayed puberty and hypogonadism. Kallman syndrome, the combination of hypogonadotropic hypogonadism and anosmia, is caused by defects in a number of genes which play a role in the migration of GnRH neurons from the olfactory placode to the hypothalamus at an early fetal age. Kotan and colleagues used WES, combined with autozygosity mapping and candidate gene screening, to demonstrate homozygous loss-of-function mutations in the *FEZF1* gene in 4 affected individuals from 2 independent families⁴. Strong functional evidence is provided by previous murine studies, showing that *FEZF1* is involved in olfactory receptor neuron migration into the CNS⁵. Similarly, four reports have recently described novel mutations in a number of genes (*PNPLA6*, *RNF216/OTUD4*, *CHIP*) leading to the neurodegenerative Gordon Holmes syndrome, which is characterized by hypogonadism and severe ataxia^{6–9}. *RNF216*, *OTUD4* and *CHIP* are all involved in the ubiquitination process linking disordered ubiquitination to delayed puberty. *PNPLA6* plays a role in intracellular phospholipid homeostasis and mutations in this gene appear to interfere with gonadotropin release⁸. Other WES-based studies have identified novel causative genes in familial cases of female gonadal dysgenesis and primary or premature ovarian failure after puberty^{10–13}. Taken together, these findings are expanding our understanding of the hypothalamic-pituitary-gonadal axis and the networks of genes involved in pubertal control and gonadal development.

Familial Glucocorticoid Deficiency

Until recently, only mutations in the ACTH receptor, encoded by *MC2R*, and its accessory protein, encoded by *MRAP*, had been identified as genetic causes of familial glucocorticoid deficiency (FGD). However, using WES, various research groups have now found a number of new pathways that cause the same FGD phenotype, including those involved in the mitochondrial detoxification of reactive oxygen species (ROS). In a consanguineous family with seven affected patients, WES demonstrated a novel homozygous mutation in the thioredoxin antioxidant system (*TXNRD2*)¹⁴. Functional evidence was provided by a *TXNRD2* knock-down adrenocortical cell line (NCI-H295R), displaying a marked increase in ROS production and increased susceptibility to oxidative stress as compared to control cells. Very similar results have been reported for mutations in the nicotinamide nucleotide transhydrogenase (*NNT*) gene, which is crucial for the production of sufficient amounts of NADPH, required for adequate redox reactions by p450 enzyme systems in the adrenal cortex¹⁵. Moreover, in a genetically isolated Irish population with FGD, exome sequencing identified the first human mutation in *MCM-4* (minichromosome maintenance-deficient 4) as a cause of their adrenal insufficiency, short stature and natural killer cell deficiency¹⁶. The MCM4 protein is involved in DNA replication and its deficiency is known to lead to cell cycle disturbances, genomic instability, increased cancer risk and developmental defects. For the studies implicating *NNT* and *MCM4*, the same research group leveraged inbred populations using a technique called homozygosity mapping to identify candidate regions of the genome that harbored the causal mutations. They then performed target sequencing of all the exons in those regions as opposed to whole exome sequencing. These

studies provide important additional insights into basic adrenal physiology and highlight the increased susceptibility of the human adrenal gland to oxidative stress.

Various rare endocrine disorders

Several discoveries of novel genes and causative pathways have been described in individual patients or families with rare genetic endocrine conditions. Through a collaborative study in a large cohort of well-phenotyped patients, loss-of-function mutations or deletions in the *IGSF1* gene were identified by WES as the cause of a novel X-linked disease, consisting of short stature, central hypothyroidism and macro-orchidism¹⁷. This Ig superfamily member 1 (*IGSF1*) protein is a plasma membrane glycoprotein, which is expressed in the anterior pituitary gland and may serve an important role in protein trafficking to the cell surface, as suggested by various cell line studies. Interesting results also emerged from a recent study in the thyroid field, in which exome sequencing was performed in a consanguineous family with thyroid dysgenesis. Surprisingly, a homozygous missense mutations was identified in the *SLC26A4* ("Pendrin") gene, which until then had only been associated with a thyroid dysmorphogenesis phenotype, thereby expanding the clinical spectrum of *SLC26A4* gene mutations¹⁸.

III Insights into common endocrine disorders

Common disorders such as type 2 diabetes, hyperlipidemia, and metabolic syndrome are highly polygenic, meaning that variants in multiple genes influence an individual's risk of developing these disorders. Typically, these genetic variants are common in the population, found in >5% of individuals, and have very small individual effects. To date, major advances have been made in identifying these genetic polymorphisms through genome wide association studies (GWAS). GWAS identify regions of the genome, referred to as loci, which are associated with the condition of interest and can highlight relevant biological pathways. However, in most cases, GWAS do not identify the actual causal risk variant, nor do they assess variants of lower frequency. It is in this context that WES has been able to advance our understanding of common endocrine disorders via two approaches. First, WES allows for the identification of lower frequency coding variants which may only be present in subsets of the population. Second, through sequencing of individuals with an extreme presentation of a common disorder, rare variants with large effects in a specific gene may be found which will provide insights into the underlying pathophysiology of the common disorder. From a public health perspective, an increase in the understanding of the genetic basis of familial dyslipidemia and associated cardiovascular risks, metabolic syndrome and type 2 diabetes could have a major impact on health policy in terms of both primary and secondary prevention in high-risk patient groups (Table 2).

Cardiovascular endocrinology and dyslipidemia

Large scale exome sequencing has been performed for the identification of low frequency genetic determinants of elevated LDL-cholesterol. This approach has successfully identified a number of novel genes that were not previously linked to the risk of cardiovascular disease. As an illustration, in a large, multicenter cooperative effort, known as the NHLBI Grand Opportunity Exome Sequencing Project, exome sequencing was performed on the

DNA of more than 2,000 persons, with a quarter of those subjects falling in either the highest (>98th) or the lowest (<2nd) LDL-cholesterol percentiles. This was followed up with genetic analysis of an additional 1300 persons and genotyping of over 50,000 persons for selected genes¹⁹. This study revealed an important role for low-frequency genetic variants in the *PNPLA5* gene which had not previously been associated with lipid abnormalities. Additionally, there was an increased burden of rare variants in three known lipid genes (*PCSK9*, *LDLR*, and *APOB*). In general, these rare variants had much larger effects on LDL values (~24–116 mg/dl) than the common variants identified by GWAS and together explained a considerable proportion of the LDL variability in this population.

Additionally, insights gained from the extremes of a common disorder may improve our understanding of the underlying pathophysiology as well as open up novel therapeutic avenues. For example, Rosenthal et al combined linkage analysis with exome sequencing data from 16 individuals in a large 5 generation family to identify a novel missense variant in *SLC25A40* as a contributing genetic factor causing severe hypertriglyceridemia²⁰. They then used data from the NHLBI Exome Sequencing Project to validate that individuals with rare nonsynonymous variants in this gene had increased triglyceride levels. *SLC25A40* encodes a solute carrier localized to the mitochondria and its role in triglyceride regulation is currently unknown. Musunuru and colleagues used exome sequencing in a family with hypobetalipoproteinemia, who were negative for the common *APOB* mutations²¹, and identified compound heterozygous nonsense mutations in the *ANGPTL3* gene. This gene was known to be involved in inhibition of lipoprotein and endothelial lipase, leading to elevated plasma HDL cholesterol and triglyceride levels in rodents. The human cases can provide insight as to the potential consequences of using this gene as a therapeutic target. The prototypical example of this is the *PCSK9* gene. Although the initial studies did not use WES but different genomic analysis tools, the finding that loss-of-function mutations in *PCSK9* were associated with an extremely favorable lipid profile, including a low LDL, and lower risk of coronary heart disease has led to the rapid development of a new class of cholesterol-lowering, anti-PCSK9 therapeutics.²²

Type 2 diabetes

In this related field, a large cohort study used WES in 1,000 patients with metabolic phenotypes including type 2 diabetes (T2D), obesity and hypertension, in comparison to 1,000 healthy controls²³. The authors were able to identify two common coding variants that were associated with small but significant changes in risk for T2D. Lohmueller et al. performed exome sequencing in 2000 Danish individuals, half of whom had T2D, and did not identify any genes with an increased burden of rare nonsynonymous variants in patients with diabetes²⁴. These two studies argue that it is very unlikely that rare coding variants in a small number of genes play a major role in the genetic underpinnings of T2D in the general population. However, the deCODE group successfully used whole genome sequencing of 2,630 Icelanders followed by imputation into the larger Icelandic population in order to discover a low-frequency variant in *CCND2* which reduced the risk of T2D by half. *CCND2* encodes a cell cycle regulator that plays a critical role in controlling pancreatic β cell mass. Additionally, they found two missense variants in *PAM* and a frameshift in *PDX1* which increased the risk of T2D²⁵.

The remarkably high incidence of T2D among certain Latino populations has prompted researchers to employ genome-wide approaches to search for determinants of T2D risk among people from various ethnic backgrounds. In a large collaborative study, authors from the SIGMA type 2 consortium performed WES on 3756 people from Mexican or US Latino descent, approximately half of whom were known to have T2D²⁶. The authors demonstrated a strong association between T2D and a low frequency missense variant in hepatocyte nuclear factor 1-alpha (*HNF1A*), a pancreatic transcription factor known to cause the MODY-3 variant of diabetes. Interestingly, multi-ethnic replication studies showed that this specific variant is exclusive to the Latino population and increases the risk of developing T2D by more than five-fold. Patients with this variant were clinically indistinguishable from the remaining patients with T2D. Although preliminary, this study provides a wonderful example of how exome sequencing studies can generate results that may effectively guide follow-up studies on screening practices in high risk groups for common diseases with a heavy burden on population health. Additionally, these patients may benefit from sulfonylurea treatment which has been demonstrated to be beneficial in patients with classic MODY-3.

Height and growth disorders

Population-based genome wide association (GWA) studies have identified over 400 genomic loci associated with height, highlighting multiple known and novel pathways involved in human growth²⁷. However, these common genetic variants do not account for all of the decrement in height seen in individuals with significant short stature²⁸. It is to be expected that by identifying well-phenotyped families with one or more family members affected by severe short stature of unknown origin, WES will unravel novel mechanisms in the highly heterogeneous group of idiopathic short stature (ISS) patients. An example of this approach was a recent study which investigated patients with familial ISS with an advanced bone age, as opposed to a delayed or normal bone age which is more commonly seen in the ISS population. WES of multiple family members from three affected families revealed novel heterozygous mutations in the *ACAN* gene. The *ACAN* gene encodes aggrecan, a key proteoglycan in the extracellular matrix of the growth plate, which plays a significant role in linear skeletal growth²⁹. Notably multiple common variants in *ACAN* have been associated with height in the general population indicating that both common and rare variants in the same gene may have wide ranging effects on growth²⁷.

Osteoporosis

WES was used in a detailed study of five families with X-linked osteoporosis and fractures and identified pathogenic variants in the plasmin 3 (*PLS3*) gene as the cause³⁰. The *PLS3* gene is involved in the formation of filamentous actin bundles and interestingly it was shown that a different *PLS3* genetic variant resulted in an Osteogenesis Imperfecta type 1A phenotype. In the same study, analysis of a population of elderly Dutch persons indicated that heterozygous female carriers of this rare variant had an approximately two-fold increased fracture risk³⁰. Again, this demonstrates the concept that variants with different effect sizes in the same gene will lead to a range of phenotypes. The functional relevance of *PLS3* mutations in X-linked forms of osteoporosis has recently been confirmed by different authors³¹. The exact mechanism of how defects in *PLS3* cause osteoporosis and increased

fracture risk is unknown, but it may be related to altered mechanosensing by osteocytes leading to effects on bone remodeling.

IV Endocrine Neoplasia

Somatic mutations in specific genes are often the inciting events that lead to tumor formation. Individual tumors accumulate multiple mutations and it can be difficult to decipher the mutations critical to tumor development. However, WES and whole genome sequencing have dramatically increased our understanding of tumor biology including some forms of endocrine neoplasia. The now standard approach is to perform paired exome or genome sequencing of both tumor cells and unaffected lymphocytes (or normal biopsy tissue) from the individual patient. This allows comparison of the tumor DNA to normal control DNA from the same individual thus highlighting the specific somatic mutations found within the tumor cells. When paired sequencing is done on a cohort of individuals with the same type of tumor, mutations in common genes can be identified. These genes are likely to be critical to the underlying pathophysiology of the specific tumor type.

In addition to the identification of somatic mutations within tumor specimens, exome sequencing has been able to identify a number of novel germline mutations (mutations present in the sperm or egg prior to conception which are thus present in all cells of the body), which place an individual at increased risk for endocrine tumor formation later in life. Tumors arise when a second, somatic hit occurs in the unaffected allele, as can be seen in many hereditary cancer syndromes. Alternatively, germline mutations can lead to a chronic disease state from conception onwards by constitutive activation of cell signaling machinery.

Adrenal hypercortisolism

In a field characterized by low-patient volume and remarkable tumor heterogeneity, WES has allowed for the collective study of considerable numbers of adrenocortical tissues, which has led to a number of fundamental new insights. Over the past year, four major studies performed WES on adrenal tumor-blood pairs in a large number of patients with adrenal Cushing's syndrome and identified a critical role for genetic mutations involving the cAMP dependent Protein Kinase A catalytic subunit (*PRKACA*)^{32–35}. Adrenal cortisol production was already known to be under cAMP-mediated control, but these studies independently showed that mutational activation of this pathway leads to overt Cushing's syndrome. Similarly, two studies reported inactivating mutations (germline and somatic) in the armadillo repeat containing 5 (*ARMC5*) gene in the majority of studied patients with bilateral macronodular adrenal hyperplasia (BMAH) in both the familial and isolated forms^{36, 37}. These are crucial advancements in our basic understanding of adrenocortical disease and will undoubtedly steer translational research towards the development of selective, tumor-targeted therapies in the near future.

Primary hyperaldosteronism

Similarly, WES has transformed our understanding of primary hyperaldosteronism, the most common cause of secondary hypertension accounting for approximately 10% of all

hypertensive patients³⁸. Primary hyperaldosteronism is caused by either classical Conn's syndrome (adenoma) or bilateral adrenal hyperplasia of the zona glomerulosa. In a field hard pressed for increased understanding of the underlying biology since the description of the syndrome by Dr Conn himself in 1953, a similar WES approach was used as in adrenal Cushing's syndrome which identified somatic mutations in the *KCNJ5* gene in 40% of all cases of Conn's syndrome³⁹. The *KCNJ5* gene encodes the inwardly rectifying potassium channel Kir3.4 in the adrenal zona glomerulosa cells which plays a crucial role in aldosterone biosynthesis. Constitutional activation of this channel leads to increased intracellular calcium levels and ultimately to adrenal hyperplasia or adenoma formation. When present as a germline gain-of-function point mutation, the same *KCNJ5* mutation leads to familial hyperaldosteronism type III with bilateral adrenal hyperplasia, childhood hypertension and severe hypokalemia from an early age onwards^{40, 41}. Apart from *KCNJ5*, several other related genes including *ATP1A1*, *ATP2B3* and *CACNA1D*, have been implicated in the same pathophysiologic process of ion channel dysfunction leading to primary hyperaldosteronism^{42, 43}.

Other neuroendocrine tumors

Outside the adrenal gland, WES-based studies have also provided additional insights in the pathophysiology of other endocrine tumors, including parathyroid adenoma, non-functioning pituitary adenoma, pheochromocytoma, paraganglioma and insulinoma (Table 3)^{44–48}. *DICER1* is a member of the ribonuclease III family involved in microRNA generation and when mutated, *DICER1* is known to cause a well-described hereditary cancer syndrome. Sahakitrungruang et al. performed paired blood-tumor WES in an infant with a pituitary blastoma causing Cushing's Disease⁴⁹. In this study the authors found a novel germline frameshift mutation in *DICER1* together with an additional somatic missense mutation in the pituitary tumor cells hence extending the spectrum of the *DICER1* cancer predisposition syndrome to include pituitary blastomas.

V Translating exome sequencing into clinical practice in endocrinology

The current use of exome sequencing in clinical endocrinology practice is quite limited, though we believe that this is sure to change in the near future. In order to understand the potential uses and limitations of exome sequencing in clinical practice, it is useful to review the benefits and pitfalls of interpreting exome sequencing data. The primary benefit of exome sequencing as opposed to candidate gene sequencing is that it offers an unbiased approach in which ~20,000 genes are tested simultaneously. Clinicians are often faced with patients whom they suspect have a genetic etiology of their disorder, yet the clinician is unable to identify a specific syndrome or candidate gene. In such cases, exome sequencing can be particularly beneficial. These patients may represent milder forms of known diseases or novel disorders. For example, using WES, we recently identified a known pathogenic mutation in *FAM111A* causing Kenny-Caffey Syndrome in a patient presenting with short stature⁵⁰. Kenny-Caffey syndrome is marked by short stature, impaired dentition, myopia, and severe neonatal hypocalcemia. This patient never presented with the cardinal symptom of hypocalcemia and thus this diagnosis was never considered despite the presence of the other features of Kenny-Caffey. WES readily made the diagnosis. This example also points

to the utility of WES in sporadic cases as opposed to familial cases with multiple affected family members. Many of the rare disease research studies presented above focused on familial cases as these are the low hanging fruit for gene discovery. However, many diagnoses and novel insights into disease pathophysiology can be made with WES of single patients. In those cases, detailed clinical phenotyping is essential as clinicians and investigators from different groups will need to compare genotype and phenotype data in order to facilitate novel gene discovery.

On the other hand, one must be very cautious with the interpretation of WES data. WES identifies tens of thousands genetic variants in each individual. The majority of these variants are commonly found in the population and thus are unlikely to be causal of rare diseases or have large effects on common diseases. Due to large scale sequencing efforts, there are now publicly available databases where one can look up the population frequency of a genetic variant. Commonly used databases include the 1000 Genomes Project (<http://browser.1000genomes.org/>), the NHLBI exome variant server (<http://evs.gs.washington.edu/EVS/>), and the recently launched Exome Aggregation Consortium browser (<http://exac.broadinstitute.org/>). However, despite the availability of these sequencing databases, each individual will still have many rare and novel genetic variants that will require interpretation. For example, in one study using data from the 1000 Genomes Project, each individual of European ancestry had on average 112 novel variants including 68 missense variants and 1 nonsense variant⁵¹. Many of these novel variants will fall in genes that could theoretically be linked to a patient's disease but are actually benign polymorphisms. Therefore, rigorous assessment is needed of each variant prior to concluding that the variant is contributing to the patient's condition. Additionally, while large scale sequencing efforts are underway across the globe, many ethnicities are currently underrepresented in the public databases making their exome data even more challenging to interpret.

For optimal interpretation of WES data, it is extremely helpful to have parental samples in addition to the patient's sample. This is referred to as trio sequencing. Parental samples are often available for pediatric patients but are much more difficult to obtain in adults. Trio sequencing allows for the identification of *de novo* versus inherited variants. It also allows one to determine if two variants in the same gene are present on different copies of the chromosome and potentially causing a recessive disorder (i.e. each variant was inherited from a different parent) as opposed to both being inherited from a single parent. These types of analyses are particularly beneficial for rare disorders where the genetic variant(s) is expected to segregate with the disease phenotype in the family. That being said, the actual penetrance of many "disease causing" genetic mutations is unknown. A striking example is provided by Flannick et al. who sequenced seven genes known to cause maturity-onset diabetes of the young (MODY), a monogenic form of diabetes, in population bases samples from the Framingham and Jackson Heart Studies⁵². They found that approximately 1.5% of individuals carried reportedly pathogenic mutations in these genes despite the majority of carriers being euglycemic with no sign of diabetes. As WES becomes more ubiquitous, many reportedly pathogenic genetic variants will be found and prognostication based on these variants will be quite difficult until a larger evidence base is amassed.

In addition to identifying variants in genes related to the patients' phenotype of interest (i.e. the reason the test was ordered in the first place), WES has the potential to uncover incidental findings such as a cancer predisposition or potential latent cardiomyopathy. Due to these concerns, in 2013, the American College of Medical Genetics (ACMG) published a list of 57 genes for which they believe that clinical labs should report incidental findings⁵³. This recommendation has been quite controversial and practice varies with regards to reporting of incidental findings⁵⁴. Yang et al. found that 3% of their patients who underwent WES had medically actionable incidental findings in genes recommended for reporting by the ACMG.⁵⁵

Despite these limitations, WES is a powerful tool that is rapidly changing the diagnostic approach for patients with rare disorders suspected to have a genetic etiology. Its use has been most widespread in the diagnosis of children with neurodevelopmental issues or multiple congenital anomalies and most series report a diagnostic yield of roughly 25%^{55–58}. With the expected improvements in next-generation sequencing techniques, data analysis, and the increased availability of human genome data, this percentage may very well rise in years to come. Clearly, the clinical utility of this approach will vary depending on the specific patient characteristics. For example, WES was able to establish a likely diagnosis in over a third of cases with a 46, XY disorder of sexual development, a highly heterogeneous condition which is often a diagnostic dilemma⁵⁹. Another potential area where WES could be useful is in the diagnosis of children with severe short stature for whom a molecular diagnosis may impact treatment decisions and provide for improved prognostication^{50, 60, 61}. We anticipate that WES use in clinical endocrinology will continue to expand but its use will be primarily focused on patients with undiagnosed rare conditions.

An alternative application of WES is to replace gene panel diagnostic testing for disorders in which there are multiple implicated causal genes. For example, in a study of 31 hereditary pheochromocytoma/paraganglioma cases, WES was superior in establishing the causative gene defect than the more traditional approach of sequential testing of candidate genes, partially because of the poor phenotype-genotype correlations in some of these tumors⁶². Other potential areas where WES may prove to be more efficient than sequential candidate gene testing include Noonan syndrome, MODY, neonatal hyperinsulinism and idiopathic hypogonadotropic hypogonadism, but those studies have not yet been performed. However, one must realize that WES does not always provide uniform sequencing coverage of the desired genes while targeted diagnostic panels have been validated to ensure adequate coverage of all targeted exons. Additionally, WES does not allow for robust determination of copy number variants (deletions and duplications) and targeted analysis for a candidate gene may still be necessary.

Additionally, tumor sequencing is rapidly becoming ubiquitous in the treatment of cancer with the hope that molecular characterization of tumors will allow clinicians to optimize selection of chemotherapeutic regimens⁶³. These advances are sure to influence the treatment of endocrine malignancies and the insights gained from the research studies reviewed above will assist in developing targeted therapies. Finally, we believe that the role of WES in common endocrine disorders remains quite limited. The majority of the genetic determinants to these disorders are common variants found outside of the exome and thus

WES will provide limited prognostic information. While there are some notable exceptions, such as the *HNF1A* variant found in the Latino population, the lack of data surrounding variant penetrance and difficulties with interpretation of exome data in the setting of common disease make it unlikely that WES will become a standard part of the routine endocrine evaluation. However, in the more distant future, as whole genome sequencing becomes cheaper and larger well phenotype populations of patients are studied, genetic risk profiles for common disease will likely be commonplace.

VI Conclusions

WES has allowed for a quantum leap forward in our understanding of endocrine pathophysiology. Through elucidating the genetic etiology of rare endocrine disorders, WES has provided insights into the biological pathways underlying all areas of endocrinology and opened up many new pathways for research. In the short term, these insights will be extremely beneficial for the diagnosis of rare endocrine disorders. More importantly, though, these discoveries will pave the way for novel therapeutic strategies in common endocrine disorders as well as in endocrine neoplasia. Gene discovery is just the first step in the pathway towards improved patient care and there is still much to be done to realize the full potential of these initial genetic insights.

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Key Points

1. Exome sequencing in rare familial endocrine syndromes has led to the identification of numerous causal genes and unraveled new biological pathways.
2. Through the collaborative study of large, population-based cohorts, exome sequencing has identified more common, low-risk genetic variants that contribute to common polygenic diseases such as type 2 diabetes and hyperlipidemia.
3. Exome sequencing of neuro-endocrine tumors has provided novel insights into their underlying pathogenesis, in particular implicating increased protein kinase A activity in the majority of adrenal Cushing's syndrome cases and somatic ion channel defects in primary hyperaldosteronism.
4. With the decreasing costs, increasing availability and high diagnostic yield, the use of exome sequencing in endocrine patient care is expected to increase rapidly in the coming years.

A

B

Figure 1. Approaches for the use of exome sequencing in endocrine disorders

A: Exome sequencing can be used in individual families with rare endocrine conditions for the identification of novel causal genes. Pictured here is a representative pedigree of a dominantly inherited syndrome.

B.: In common endocrine disorders, exome sequencing is used to identify low frequency variants in the population associated with disease. A common strategy pictured here is to select individuals from the extremes of the population distributions for a given trait, i.e. very high and low LDL levels, who have a higher probability of carrying genetic variants with larger effects.

C: To identify the genetic etiology of various endocrine tumors, exome sequencing is performed on paired blood and tumor specimens from individuals with a specific tumor type. Somatic variants not present in the germline are identified which contribute to the underlying pathophysiology of the given tumor.

Exome Sequencing studies in rare endocrine disorders: individual patients or family-based studies reporting novel genes

Table 1

Author	Year	Ref	Journal	Gene	Organ	Most prominent endocrine features
Abreu	2013	2	NEJM	<i>MKRN3</i>	HPG	Central precocious puberty
Kotan	2014	4	AJHG	<i>FEZF1</i>	HPG	Hypogonadotropic hypogonadism (Kallmann)
Shi	2014	6	AJHG	<i>CHIP</i>	HPG	Ataxia and hypogonadism
Synofzik	2014	7	Brain	<i>PNPLA6</i>	HPG	Gordon Homes syndrome
Topaloglu	2014	8	JCEM	<i>PNPLA6</i>	HPG	Gordon Holmes syndrome
Margolin	2013	9	NEJM	<i>RNF216/OTUD4</i>	HPG	Hypogonadotropic hypogonadism and ataxia
Zangen	2011	10	AJHG	<i>PSMC3IP/HOP2</i>	Ovary	Female gonadal dysgenesis
Caburet	2014	11	NEJM	<i>STAG3</i>	Ovary	Premature ovarian failure
Kasipillai	2013	12	JCEM	<i>eIF4ENIF1</i>	Ovary	Primary ovarian insufficiency
Vries	2014	13	JCEM	<i>SYCE1</i>	Ovary	Primary ovarian insufficiency
Prasad	2014	14	JCEM	<i>TXNRD2</i>	Adrenal	Familial glucocorticoid deficiency
Meimaridou	2012	15	Nat Gen	<i>NNT</i>	Adrenal	Familial glucocorticoid deficiency
Hughes	2012	16	JCI	<i>MCM4</i>	Adrenal	Adrenal failure, SS, NK cell deficiency
Samuels	2013	64	JCEM	<i>ACTH (bioinact)</i>	Adrenal	Glucocorticoid deficiency
Sun	2012	17	Nat Gen	<i>IGSF1</i>	Pituitary	Central hypothyroidism, testicular enlargement
Tegtmeyer	2014	65	NEJM	<i>PGM1</i>	Pituitary	CDG with hypogonadotropic hypogonadism, growth arrest
Webb	2013	66	Brain	<i>ARNT2</i>	Pituitary	Hypopituitarism, postnatal microcephaly
Kuhnen	2014	18	JCEM	<i>SLC26A4</i>	Thyroid	Congenital hypothyroidism / thyroid dysgenesis
Dauber	2012	67	JCEM	<i>CYP24A1</i>	Bone	Infantile hypercalcemia
Dauber	2012	68	JCEM	<i>NIN</i>	Growth	Microcephalic primordial dwarfism
Hanson	2011	69	AJHG	<i>CCDC8</i>	Growth	3M syndrome
Nilsson	2014	70	JCEM	<i>ACAN</i>	Growth	SS, accelerated bone maturation
Kerns	2014	71	JCEM	<i>CDKN1C</i>	Growth	IUGR, SS, early-adulthood diabetes
Al-Maawali	2014	72	EJHG	<i>PRPS1</i>	Growth	IUGR, diabetes insipidus, retinal dystrophy
Bashamboo	2014	73	HMG	<i>FOG2</i>	Testis	46, XY Disorder of Sexual Development

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Author	Year	Ref	Journal	Gene	Organ	Most prominent endocrine features
Stitzel	2013	74	ATVB	<i>LIPA E8SJM</i>	Lipids	Hypercholesterolemia
Gillis	2014	75	JMG	<i>TRMT10A</i>	Multiple	Abnormal glucose metabolism, SS
Dauber	2014	76	HMG	<i>SLC35C1</i>	Multiple	Fucosylation defect with SS and developmental delay

HPG: Hypothalamic, pituitary, gonadal
CDG: Congenital disorder of glycosylation
NK: Natural Killer
SS: short stature
IUGR: Intra-uterine growth restriction

Table 2

Exome sequencing studies in common endocrine disorders

Author	Year	Ref	Journal	Highlighted Gene	Disease / Trait
Lange	2014	19	AJHG	<i>PNPLA5, PCSK9, LDLR, APOB</i>	LDL-cholesterol
Rosenthal	2013	20	AJHG	<i>SLC25A40</i>	Hypertriglyceridemia
Musumuru	2010	21	NEJM	<i>ANGPTL3</i>	Familial combined hypolipidemia
Peloso	2014	77	AJHG	<i>ANGPTL8, PAFAH1B2, COL18A1, PCSK7</i>	HDL-cholesterol, triglycerides, risk for CHD
Albrechtsen	2013	23	Diabetologia	<i>CD300LG, COBLL1, MACF1</i>	Common metabolic phenotypes (T2D, obesity, hypertension)
Lohmueller	2013	24	AJHG	<i>none identified</i>	T2D: low frequency variants
Steinthorsdottir	2014	25	Nat Gen	<i>CCND2, PAM, PDX1</i>	T2D: low-frequency variants
Consortium, S.T.D. et al.	2014	26	JAMA	<i>HNF1A</i>	T2D: low-frequency variant (Latino populations)
Moltke	2014	78	Nature	<i>TBC1D4</i>	T2D (Iceland)
Du	2014	79	HMG	<i>C5orf22, SPRYD7, GH2</i>	Height (African American population)
Van Dijk	2013	30	NEJM	<i>PLS3</i>	Osteoporosis
Laine	2014	31	JBMR	<i>PLS3</i>	Osteoporosis

T2D: Type 2 diabetes, CVD: cardiovascular disease

Table 3

Exome sequencing studies in (neuro)endocrine tumors

Author	Year	Ref	Journal	Highlighted Gene	Disease
Goh	2014	32	Nat Gen	<i>PRKACA, CTNNB1, GNAS</i>	Adrenal CS
Sato	2014	33	Science	<i>PRKACA</i>	Adrenal CS
Cao	2014	34	Science	<i>PRKACA, DOTIL, CLASP2</i>	Adrenal CS
Beuschlein	2014	35	NEJM	<i>PRKACA</i>	Adrenal CS
Assie	2014	36	NEJM	<i>ARMC5</i>	Macronodular adrenal hyperplasia
Gagliardi	2014	37	JCEM	<i>ARMC5</i>	Bilateral macronodular adrenal hyperplasia
Assie	2014	80	Nat Gen	<i>ZNRF3, CTNNB1, TP53, CDKN2A, RB1, MEN1, DAXX, TERT, MED12</i>	Adrenal cancer
Choi	2011	39	Science	<i>KCNJ5</i>	Primary hyperaldosteronism
Beuschlein	2013	42	Nat Gen	<i>ATP1A1, ATP2B3</i>	Primary hyperaldosteronism
Azizan	2013	43	Nat Gen	<i>ATP1A1, CACNA1D</i>	Primary hyperaldosteronism
Newey	2012	44	JCEM	<i>MEN1-LOHchr11, POT1</i>	Parathyroid adenomas
Cromer	2012	45	JCEM	<i>MEN1-LOHchr11, EZH2</i>	Parathyroid adenomas
Newey	2013	46	JCEM	<i>none identified</i>	Non-functioning pituitary adenomas
Cao	2013	47	Gene	<i>none identified</i>	Pheochromocytomas/paragangliomas
Cao	2013	48	Nat Comm	<i>YY1</i>	Insulinoma
Sahakitr.	2014	49	JCEM	<i>DICER1</i>	Tumor Predisposition/Infantile Cushing's disease

CS: Cushing's syndrome; CA: carcinoma; LOH: loss of heterozygosity