Metabolomics and Genetics of Rare Endocrine Disease: Adrenal, Parathyroid Glands, and Cystic Fibrosis

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Abstract Recent advances in metabolomic technologies and methodologies have identified significant metabolites related to rare endocrine disease conditions of the adrenal gland (hyperaldosteronism, primary adrenal insufficiency), parathyroid (hypoparathyroidism), and cystic fibrosis. Metabolomic profiling combined with genomics is increasingly being employed for improving understanding, clinical diagnosis, and management of these clinically challenging conditions. Advances in gas and liquid chromatography combined with tandem mass spectrometry (GC/LC–MS/MS) techniques have improved the profiling of steroid metabolites. Significant alterations in levels of these metabolites demonstrate the potential to serve as specific markers of disease, help in their stratification, and contribute toward moving to personalized medicine.

Keywords Rare endocrine disease · Genetic disease · Metabolomics · Primary aldosteronism · Primary adrenal insufficiency hyperaldosteronism · Parathyroidism Pheochromocytoma · Paragangliomas · Cystic fibrosis

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Abbreviations

ATP1A1	ATPase Na+/K + -transporting subunit alpha 1
ATP2B3	ATPase plasma membrane Ca2+ transporting 3
CACNA1H	Calcium voltage-gated channel subunit alpha 1 H
CACNA1H	Calcium voltage-gated channel subunit alpha 1 H
CASR	G protein-coupled calcium-sensing receptor
CCND1/PRAD1	Cyclin D1
CDKN1C	Cyclin-dependent kinase inhibitor 1C
CFTR	CF transmembrane conductance regulator
CHD7	Chromodomain helicase DNA binding protein 7
CLCN2	Chloride voltage-gated channel 2
CSDE1	Cold shock domain-containing E1
CTNNB1	Catenin beta 1
CYP11B2	Cytochrome P450 family 11 subfamily B member 2
DAX-1 (NR0B1) SF-1	Nuclear receptor subfamily 0 group B member 1
DLST	Dihydrolipoamide S-succinyltransferase
FH	Fumarate hydratase
GATA3	GATA binding protein 3
GCM2	Glial cells missing transcription factor 2
GNA11	G protein subunit alpha 11
H3F3A	H3.3 histone A
HIF2A	Hypoxia-inducible factor 1 subunit alpha
HRAS	HRas proto-oncogene, GTPase
IDH	Isocitrate dehydrogenase (NADP(+)) 1
IRP1	Iron regulatory protein
KCNJ5	Potassium inwardly rectifying channel subfamily J member 5
MAML3	Mastermind-like transcriptional coactivator 3
MDH2	Malate dehydrogenase 2
NF1	Neurofibromin 1
NR5A1	Nuclear receptor subfamily 5 group A member 1
P450scc/CYP11A1	Cytochrome P450 family 11 subfamily A member 1
PHD1	Prolyl hydroxylase 1
POLE1	DNA polymerase epsilon, catalytic subunit
PTH	Parathyroid hormone
RET	Ret proto-oncogene
SAMD9	Sterile alpha motif domain containing 9
SDHx	Succinate dehydrogenase complex iron-sulfur subunit B
SEMA3E	Semaphorin 3E
SGPL1	Sphingosine-1-phosphate lyase 1
SLC25A11d	Solute carrier family 25 member 13
SOX3	SRY-Box transcription factor 3
TMEM127	Transmembrane protein 127
VHL/EPAS	Von Hippel–Lindau tumor suppressor

1 Introduction

Over the course of the past 10 years, research based on metabolomics has grown significantly and emerged as a promising instrument for clinical diagnostics as well as for improving our comprehension of the physiological and pathological processes that are the foundation of study for endocrine-related and rare diseases. In this chapter, we looked at how metabolomics helped diagnose, treat, and follow up rare endocrine disease of the pituitary gland. Beyond the pituitary, metabolomics has also been applied in disease stratification and management and in identifying biomarkers with applications in disease prediction, diagnosis, prognosis, and therapy.

2 Metabolomics of Adrenal Dysfunction

The adrenal glands play an important role in regulation of body homeostasis. Anatomically, they are made up of the cortex and medulla that secrete hormones involved in maintaining electrolyte and mineral balance, control metabolic pathways, provide response to stress (cortisol production in the adrenal cortex and catecholamines in adrenal medulla), and are crucial for sexual differentiation (through producing steroid hormones in the adrenal cortex). Diseases of the adrenal glands result in the resistive synthesis of glucocorticoids, sex hormones, and catecholamines (epinephrine and norepinephrine). Excessive circulating glucocorticoid (cortisol) levels, independent of ACTH, primarily arise from the adrenal gland disease (CS), while increased secretion of corticotropin (ACTH)-dependent cortisol is primarily due to disease of the pituitary and in some cases due to other glands. CS accounts for 15% of the cases while a majority is 70% due to CD and other causes including ectopic production is 15% [1]. The associated genetic defects and metabolite changes related to endogenous and exogenous hypercortisolism were covered in the previous chapter. Adrenal gland dysfunction also results in disorders of aldosterone synthesis (hyperaldosteronism or Conn's syndrome and adrenal insufficiency or Addison's disease), steroidogenesis, and the synthesis of sex hormones. Rare forms of these conditions arise due to germline mutations resulting in benign adrenal tumors or adrenocortical adenomas having an overall incidence in the general population of 1–2 cases per million [2]. Clinical evaluation of these disorders of adrenal steroidogenesis and disease requires measurement of specific hormonal levels, radiological imaging, and histopathology of the biopsy specimens. Advances in GC–MS and LC–MS/MS techniques have greatly improved the diagnosis of these diseases by metabolomic identification and quantification of the steroid metabolome that includes steroid hormones along with metabolic derivatives in bodily fluids (e.g., serum, urine) for clinical (diagnostics and treatment monitoring) as well as research purposes.

2.1 Hyperaldosteronism (Conn's Syndrome)

Primary aldosteronism (PA) is characterized by inappropriate and excessive secretion of the adrenal steroid hormone and aldosterone (hyperaldosteronism), the main mineralocorticoid hormone responsible for salt and water reabsorption, as well as increased potassium and proton secretion from the kidneys. Among the primary causes of PA is aldosterone-producing adenomas that present clinically as secondary endocrine hypertension. The nonneoplastic rare causes of PA (5%) are due to familial hyperaldosteronism (FH I–IV) and bilateral adrenal hyperplasia (BAH) [3, 4]. The advent of next-generation sequencing (NGS) technology and its wider application determined a largely genetic basis for the rare (5%) causes of PA through the identification of overlapping set of genes carrying numerous disease-causing germline mutations. These genome-wide association studies (GWAS) identified germline variants in CACNA1H (encoding a subunit of T-type voltage-gated calcium channel, CaV3.2), KCNJ5 (potassium inwardly rectifying channel subfamily J member 5), CYP11B2 (encoding aldosterone synthase), CACNA1D (calcium channel voltage-dependent L-type alpha-1D subunit), and CLCN2 (encoding voltage-gated chloride channel ClC-2) [5–8]. Metabolomic approaches and metabolome profiling using gas chromatography–mass spectrometry (GC–MS) and ultra-HPLC–tandem mass spectrometry (UHPLC–MS/MS) have comprehensively profiled the steroid metabolite profiling in sera and urine of patients and have found applications in the areas of personalized medicine for diagnostic purpose and prediction of prognosis. Coupling metabolomics together with genomics using GWAS provides a platform for employing integrated OMICS toward understanding and identifying the clinical phenotype. These genetic variations with their resulting metabolic alterations have created the metabolic phenotypes termed "genetically determined metabotypes" that is now being considered as a diagnostic feature [9]. Clinically, the characteristic presentation of PA is an increase in blood pressure, that is, hypertension along with disturbances in the electrolyte levels. When compared to patients with primary hypertension, these patients are at a higher risk of developing cardiovascular and kidney disease [10, 11], making an early diagnosis vital. Aside from these complications, there is also the need to differentiate between the different PA subtypes and unilateral or bilateral disease as clinical management of both conditions differs; the former is managed surgically while the latter is managed medically.

A metabolomic approach using liquid chromatography with tandem mass spectrometry (LC–MS/MS) successfully quantified adrenal steroids. The multi-steroid signatures associated with steroid biosynthesis and metabolism disorders showed a high level of diagnostic accuracy to differentiate between PA and adrenal hyperplasia that have similar presentations. Levels of cortisol derivatives (18-hydroxycortisol and 18-oxocortisol) were elevated in patients with PAs, carrying KCNJ5 mutations, in comparison to those with adrenal hyperplasia. Urinary 18-hydroxycortisol showed a high accuracy in distinguishing between the two conditions [12]. On the other hand, patients with adrenal hyperplasia showed elevated levels of plasma dehydroepiandrosterone (DHEA), DHEA-S, cortisol, and corticosterone [13].

Targeted metabolomic analysis by LC-MS/MS using a 32-metabolite steroid panel that included 11-deoxycorticosterone, aldosterone, cortisol, 11-deoxycortisol, 21-deoxycortisol, corticosterone, progesterone, 17-hydroxyprogesterone, 18-oxocortisol, 18-hydroxycortisol, cortisone, pregnenolone, androstenedione, DHEA, and DHEA-S was also used. Levels of 18-oxocortisol and 18-hydroxycortisol were found to have distinctively higher levels in cases of FH I and III compared to controls and also showed a high level of correlation with histological features of adenoma. In addition, mutations in *CTNNB1*, coding for β-catenin, have been identified in 2–5% of cases with aldosterone-producing benign adenoma. A somatic mutation in *CLCN2*, coding for the chloride channel ClC-2 (chloride channel protein 2) mutated in familial hyperaldosteronism type II (FH-II) and early-onset PA, has recently been identified. Previous studies have shown that CYP11B2 can convert 11-deoxycortisol efficiently to 18-hydroxycortisol and 18-oxocortisol, while CYP11B1 can synthesize only 18-hydroxycortisol [5, 14, 15] (Table 1).

Hyperaldosteronism is suggested to cause inflammation and metabolic dysregulation and contribute to development of cardiovascular disease. Metabolomic profiling in patients with Conn's disease revealed significant alterations in levels of triglyceride concentrations, large VLDL particles with urate concentrations, and derivatives of the linoleic acid metabolism pathway [16]. Steroid profiling has also revealed high production of the "hybrid" cortisol metabolites 18-hydroxycortisol and 18-oxocortisol in patients with rare, familial forms of PA associated with specific genetic errors (*CYP11B1*/*CYP11B2* hybrid gene, *KCNJ5* mutations) [8, 17, 18]. The levels of these hybrid metabolites also served as markers to differentiate between PA and BAH. Specifically, the 18-oxocortisol/cortisol ratio in adrenal vein samples and urinary 18-hydroxycortisol levels showed sufficient diagnostic accuracy to distinguish APAs from BAHs of patients [19, 20]. In addition to the clear elevation of plasma 18-oxocortisol in PAs, increased levels of plasma cortisol, corticosterone, DHEA, and DHEA-S were documented in patients with BAH [13]. Differences between the metabolite levels among the varying subtypes were also demonstrated using in situ metabolomics and demonstrated that levels of 18-oxocortisol and 18-hydroxycortisol negatively correlate with the CYP11B1. The steroid profiles were also correlated with their respective genotypes. The PAs carrying KCNJ5 mutations presented significantly higher levels of 18-oxocortisol in both adrenal vein and peripheral plasma samples than all other PAs, while PAs harboring ATPase mutations displayed the highest peripheral concentrations of aldosterone, cortisol, 11-deoxycorticosterone, and corticosterone. At the same time, patients with CACNA1D-mutated PAs had lower aldosterone and corticosterone concentrations than all other groups [21]. Distinct molecular signatures between KCNJ5- and CACNA1D-mutated PAs involving metabolites of steroidogenesis as well as purine metabolism KCNJ5 carriers displayed significantly higher levels of 18-hydroxycortisol and 18-oxocortisol when compared to CACNA1D carriers. Activation of purine metabolism was observed in KCNJ5 mutant APAs, with a significant increase in adenosine monophosphate and diphosphate [14].

Table 1 The different endocrine glands with the associated disease, the gene locus, and the major metabolites altered with the disease

Endocrine gland	Hormones	Disease	Genes and chromosomal locus	Metabolomic platform and approach	Major significant metabolites	References
Adrenal gland	Aldosterone	Primary hyperaldosteronism	*KCNJ5*, *CACNA1D*, *ATP1A1*, *ATP2B3*, *CTNNB1*, *CYP11B2*, *CLCN2*, and *CACNA1H*	*LC–MS/MS (targeted) and steroid profiling using GC-MS and LC–MS/MS (untargeted)*	*18-Hydroxycortisol and 18-oxocortisol*, *tetrahydro-18-oxocortisol*, *aldosterone*, *cortisol*, *11-deoxycorticosterone, and corticosterone (↑)* *Long-chain acylcarnitines C18:1, C18:2, ornithine, and spermidine*	
	ACTH decrease	Adrenal insufficiency	*DAX-1 (NR0B1) SF-1, NR5A1, CDKN1C, SAMD9, POLE1, P450scc/ CYP11A1, and SGPL1*	*GC-MS, LC-MS/MS, 1HNMR, and steroid profiling (targeted)*	*Total cortisol metabolites, 11β-HSD1 11β-HSD2, and 5α- and 5β-reductase (↓)*	[17, 24, 27]
		Pheochromocytoma/ paraganglioma	*NF1, RET, TMEM127, HRAS, SDHx, FH, IDH, H3F3A, HIF2A, MDH2, PHD1, IRP1, SLC25A11d, DLST, VHL/EPAS, and CSDE1 MAML3*	ESI–LC–MS/MS (targeted), *LC–MS*	*Succinate, citrate, isocitrate, cis-aconitate (↑), fumarate, and 2-hydroxyglutarate (↓)* *2-Oxoglutarate/malate carrier and glutamic-oxaloacetic transaminase 2 (↓)*	[29, 38]
Parathyroid	PTH	Hypoparathyroidism	*GCM2, SOX3, CHD7, SEMA3E, GATA3, PTH, CASR, and GNA11*	*UHPLC–MS (untargeted)*	*Adenosine, inosine, hypoxanthine, guanosine, and xanthine (↓) N-acetyl aspartate*	[45]

Pancreas		Cystic fibrosis	*CFTR*	*GC/LC–MS (untargeted) UHLC–MS/MS and GC–MS (untargeted)*	*Sorbitol and cholesteryl esters Purine nucleotides, adenosine, inosine, hypoxanthine, and guanosine (↑) Oxidized glutathione, S-lactoylglutathione, S-nitrosoglutathione, and ophthalmate (↓)*	[21, 49, 50, 55–57]

ATP1A1 ATPase Na+/K + -transporting subunit alpha 1, *ATP2B3* ATPase plasma membrane Ca2+ transporting 3, *CACNA1D* calcium voltage-gated channel subunit alpha 1 D, *CACNA1H* calcium voltage-gated channel subunit alpha 1 H, *CACNA1H* calcium voltage-gated channel subunit alpha 1 H, *CASR* G protein-coupled calcium-sensing receptor, *CDKN1C* cyclin-dependent kinase inhibitor 1C, *CFTR* CF transmembrane conductance regulator, *CHD7* chromodomain helicase DNA binding protein 7, *CLCN2* chloride voltage-gated channel 2, *CSDE1* cold shock domain containing E1, *CTNNB1* catenin beta 1, *CYP11B2* cytochrome P450 family 11 subfamily B member 2, *DAX-1 (NR0B1) SF-1* nuclear receptor subfamily 0 group B member 1, *DLST* dihydrolipoamide S-succinyltransferase, *FH* fumarate hydratase, *GATA3* GATA binding protein 3, *GCM2* glial cells missing transcription factor 2, *GNA11* G protein subunit alpha 11, *H3F3A* H3.3 histone A, *HIF2A* hypoxia-inducible factor 1 subunit alpha, *HRAS* HRas proto-oncogene, *GTPase, IDH* isocitrate dehydrogenase (NADP(+)), *1IRP1* iron regulatory protein, *KCNJ5* potassium inwardly rectifying channel subfamily J member 5, *MAML3* mastermind-like transcriptional coactivator 3, *MDH2* malate dehydrogenase 2, *NF1* neurofibromin 1, *NR5A1* nuclear receptor subfamily 5 group A member 1, *P450scc/CYP11A1* cytochrome P450 family 11 subfamily A member 1, *PHD1* prolyl hydroxylase, *POLE1* DNA polymerase epsilon, catalytic subunit, *PTH* parathyroid hormone, *PTPN22* protein tyrosine phosphatase non-receptor type 22, *RET* Ret proto-oncogene, *SAMD9* sterile alpha motif domain containing 9, *SDHx* succinate dehydrogenase complex iron sulfur subunit B, *SEMA3E* semaphorin 3E, *SGPL1* sphingosine-1-phosphate lyase 1, *SLC25A11d* solute carrier family 25 member 13, *SOX3* SRY-Box transcription factor 3, *TMEM127* transmembrane protein 127, *UPLC–MS/MS* ultra-PLC–tandem mass spectrometry, *VHL/EPAS* Von Hippel-Lindau tumor suppressor

2.2 Primary Adrenal Insufficiency

Primary adrenal insufficiency (PAI), a deficiency of glucocorticoid and mineralocorticoid production, is a relatively rare life-threatening condition due to autoimmune disorders and enzymatic defects. The patients clinically present with skin and mucous membrane hyperpigmentation, craving for salt, failure to thrive, depression, and fatigue. PAI is caused due to pathology within the adrenal glands which results in stimulation of the hypothalamo–pituitary axis and the renin–angiotensin–aldosterone system regulatory feedback loop. PAI is typically diagnosed by measuring levels of ACTH and proopiomelanocortin peptides, which are elevated in addition to inappropriately low cortisol secretion. A delayed diagnosis of PAI is linked with an adverse quality of life and raises the patient's risk of an adrenal crisis that might be fatal. Recent studies have implicated several genetic mutations in the pathophysiology of the disease. These include defects in the nuclear receptors DAX-1 (NR0B1), steroidogenic factor-1 (SF-1/NR5A1), CDKN1C and SAMD9 or loss of POLE1, P450scc/CYP11A1 insufficiency, and sphingosine-1-phosphate lyase-1 (SGPL1) defects [22]. Treatment of PAI conventionally is modeled around corticosteroid replacement therapy that is conventionally administered three times a day [23].

Metabolomic studies in PAI are limited in the literature. In a study, metabolite profiling was carried out in the sera or urine for disease identification and stratification and for evaluating optimal replacement therapy. The natural circadian rhythm of cortisol cycle cannot be entirely replicated by current glucocorticoid replacement regimens, which leads to either over- or under-replacement. The urinary cortisol metabolome was assessed to determine optimal cortisol replacement in patients with PAI. The metabolic profile of patients using two hydrocortisone replacement therapies were compared, namely, the once-a-day dual-release hydrocortisone (DHC) and three-times-a-day hydrocortisone (TID-HC) therapy. In the 24-h urine samples, total cortisol metabolites decreased after DHC therapy compared to TID-HC and were more in line with the usual control levels. 11-β-Hydroxysteroid dehydrogenase (11β-HSD) type 1 activity dropped with DHC compared to TID-HC therapy, whereas 11-β HSD2 activity fell with TID-HC but returned to normal with DR-HC. Moreover, 5α- and 5β-reduced metabolites were decreased with DR-HC compared to TID-HC. Patients undergoing traditional TID-HC replacement treatment with enhanced 11β-HSD1 activity exhibits significant alterations in the urine cortisol metabolome, which may explain the adverse metabolic profile in patients with PAI. Its shift toward normalcy with DHC therapy might serve as an indicator for more favorable metabolic outcomes [17, 24]. Aside from providing means for optimizing therapeutic dosage, the metabolomic approach was also used to measure the effects of glucocorticoid therapy and identify biomarkers related to its action. Serum metabolic profiling was also undertaken in PAI patients, using GC–MS and LC–MS, during glucocorticoid therapy and after its withdrawal to assess response to therapy. The differentially expressed metabolites identified were amino acids (tyrosine, tryptophan, asparagine), malic acid, lactic acid, and uracil. The metabolism of tryptophan, which modulates mood and energy homeostasis, is regulated by

glucocorticoids through the kynurenine pathway. Metabolomic analysis was able to identify that administering high doses of glucocorticoid, especially after a treatment of 10-week treatment, resulted in decreased tryptophan levels by influencing the kynurenine pathway [25, 26]. Hence, metabolomics assisted in assessing the therapeutic effects, allowing for the individualization of approaches and optimization of glucocorticoid therapy.

2.3 Metabolomics of Pheochromocytoma

Pheochromocytomas (PCC) and paragangliomas (PPGLs) are a group of rare heterogeneous neuroendocrine tumors that arise from either the adrenal medullary chromaffin cells or from outside the adrenal gland in the neural crest cells (sympathetic and parasympathetic paraganglia). Most PPGLs are benign tumors, with an incidence of approximately of one per million population per year [27]. The characteristic clinical phenotype of these patients is associated with features of excess circulating catecholamine levels due to increased synthesis or release. The presenting signs and symptoms classically range from a triad consisting of sweating, headaches, and palpitations to nonspecific symptoms such as weight loss, nausea, tiredness, or flushing [27, 28]. The diverseness and nonspecificity of the clinical manifestations, heterogeneity of these tumors regarding the age of presentation, and differences in their location make an early clinical diagnosis of PCC difficult [29]. PCC and PPGLs have the highest degree of heritability, where PPGLs carrying a germline mutation account for 30–40%. More than 20 susceptibility genes with varying mutations have been identified as predisposing factors to this condition, placing it among the rare genetic endocrine conditions. The metabolic phenotypes in PCC are based on the affected specific gene/protein [30] that determines the secretory profile, molecular features, metabolic changes, clinical outcomes, and potential for malignancy [31].

PCC and PPGLs have been classified based on their inheritance, multiple endocrine neoplasia type 2 (MEN2), familial Von Hippel–Lindau (VHL) syndrome and less commonly neurofibromatosis type 1 (NF1)) or sporadic [30], or by molecular pathway subtypes, kinase signaling subtype (RET, transmembrane protein 127 (TMEM127), mutations in the NF1, and HRAS genes), pseudohypoxia (Von Hippel–Lindau (VHL/EPAS)-related and tricarboxylic acid cycle (TCA)-related mutations, and Wnt-altered subtype (CSDE1 somatic mutations and mastermind-like transcriptional coactivator 3 (MAML3) fusion genes)). The TCA-related PPGL subtype consists of tumors having mutations in the succinate dehydrogenase subunits A–D (SDHx), fumarate hydratase (FH), and isocitrate dehydrogenase (IDH). In addition, other genetic mutations in the H3F3A, malate dehydrogenase 2 (MDH2), PHD1, IRP1, SLC25A11, and DLST were identified with a lower frequency and have not yet been included in the Cancer Genome Atlas. PCC and PPGLs are also classified into clusters based on their secretory profile as adrenergic and noradrenergic clusters. The noradrenergic pseudohypoxic phenotype (secreting

norepinephrine and normetanephrine (NMN)) constitutes cluster 1. It includes tumors with SDHx mutations, along with VHL, FH, (MDH2), hypoxia-induced factor (HIF2α), and IDH mutations and the newly identified SLC25A11 [32]. Tumors with the adrenergic phenotype (secreting epinephrine and metanephrine (MN)), which are associated with abnormal kinase signaling pathways and include mutations in the genes rearranged during transfection (RET), NF1, TMEM127, kinesin family member 1B (KIF1B), and MYC-associated factor X (MAX), make up cluster 2. Cluster 3 is associated with the Wnt signaling pathway; it includes somatic mutations of cold shock domain-containing E1 (CSDE1) and mastermind-like transcriptional coactivator 3 (MAML3) fusion genes [28, 32].

The gold standard for diagnosis relies on biochemical measurements of urinary or plasma products of the catecholamine degradation, noradrenaline (MN), adrenaline (NMN), and dopamine (methoxytyramine (MTY)) [33]. Measurements of plasma-free MN have been proven in several independent investigations to have diagnostic sensitivity surpassing 96% and specificity between 85 and 100%. An alternate method with a comparable degree of diagnostic sensitivity is provided by urinary-fractionated MN [28]. The measurement of plasma and urine MN by LC–MS/MS is presently widely accepted in the USA and many other laboratories as the gold standard approach [28, 34]. In addition to laboratory measurement, all patients with documented PCC and PPGLs should have genetic determination of PPGL phenotype as part of the diagnostic panel. It is very common to find mixed phenotypes of both adrenergic and dopaminergic secreting tumors in comparison to either adrenergic or dopaminergic ones. Each of these phenotypes has been linked to mutations in different genes. The genetic mutations in TMEM127 gene have been associated with only the adrenergic tumors while mutations in the KIF1B, MAX, RET, and NF1 genes have been associated with tumors with adrenergic mixed phenotype. On the other hand, it is known that extra-adrenal PPGLs having noradrenergic and dopaminergic phenotypes have mutations in PHD1/PHD2, HIF2A, SDHx, SDHAF2, FH, and IDH genes. The majority of PPGL tumors with HIF2A and VHL-mutated are typically noradrenergic while predominantly dopaminergic secreting tumors are known to be commonly associated with SDHx mutations [33]. Although confirmatory, genetic testing can be complex and, in many cases, unavailable at all centers. This potentially leads to delayed or inconclusive diagnosis [30, 35, 36]. Due to the probable increased risk of metastatic illness in these patients, it becomes crucial for an effective clinical management to distinguish early on between tumors with underlying germline mutations and those that are sporadic [37]. In these instances, quantifying metabolites can help verify functionality and identify underlying mechanisms and factors for germline or somatic mutations in patients with unresolved genetic testing results.

Metabolomic studies have helped to bridge this gap by identifying metabolites that have not only helped in detailing the metabolic pathways affected by the disease but also to differentiate between the phenotypes, stratify the disease, and propose metabolites that are amenable to diagnostic applications. The tumor metabolomic profile distinguishes these different subtypes of tumors to classify patients with PGLs as sporadic or hereditary. Untargeted metabolomic approaches

aided in profiling the disease pathology and associating the changes with the different variants of genetic mutations. On the other hand, targeted metabolomic approaches using the identified metabolites have also been studied to determine their impact on metabolism and utilize them as diagnostic markers in clinical laboratories [29] for monitoring therapeutic response potential conversion metastases [38]. Surgical resection of the PPGL with normalization of catecholamine levels was associated with significant changes in the metabolites. Following surgery levels of glycerophospholipids (phosphatidylcholine diacyl (PC aa) 42:0, phosphatidylcholine acyl-alkyl (PCae) 42:5, PCae (44:5), and PCae (44:6) and hexoses were lower, while levels of amino acids (biogenic amines), namely, histidine and creatinine, were demonstrated to be higher [29]. The metabolomic profile in each of the different genetic variants of these tumors was also deciphered. Around 15–25% of all PCC/PPGLs were linked to defects in the Krebs cycle enzymes, SDH, FH, MDH, and IDH, with SDH faults, being the most frequent. The 2-oxoglutarate/malate carrier, glutamic-oxaloacetic transaminase 2, and others have more recently been linked to hereditary PPGL as regulators of mitochondrial metabolites [36]. The PCC/PGLs associated with mutations in the pseudo hypoxic cluster (cluster 1) were associated with the hypoxia-inducible factor (HIF) signaling pathway and involved mutations in genes encoding the HIF2A, succinate dehydrogenase subunits or their assembly factors (SDHx [SDHA, SDHB, SDHC, SDHD]), succinate dehydrogenase complex assembly factor 2 (SDHAF2), Von Hippel–Lindau tumor suppressor (VHL), and egl-9 prolyl hydroxylases 1 and 2 (EGLN1/EGLN2). The pathogenic mutations in these genes lead to an accumulation of their related metabolites, that is, succinate, fumarate, or 2-hydroxyglutarate, which in turn were responsible for tumor development.

Distinct differences were noted in metabolites and pathways related to oxygen sensing, hypermethylation, DNA repair, and overexpression of certain transporters and receptors; notably Krebs cycle enzymes have been through the genetic investigations in PCC/PGL tumors [39–41]. Metabolomic profiling identified differential regulation of metabolites between the various genetic causes of PCC/PGL. Metabolomic analysis of PCC/PGL arising from mutations in SDHx revealed a decrease in activity of SDH (mitochondrial electron transport chain complex II) enzyme and other TCA cycle metabolites, including fumarate glutamate and aspartate with elevated succinate levels [37]. The ratio of two metabolites, succinate to fumarate, was determined as a novel metabolic marker to detect paraganglioma with underlying SDHB/D mutations [38, 42]. Moreover, in tumors linked to SDHx mutations, glutamate levels and ATP/ADP/AMP values were shown to be lower with modest but significant changes in levels of histidine, threonine, and lysoPC (C28:0) [32, 42]. Significant correlations were also noted between plasma MN and total urine catecholamine levels with the sum of detected hexoses (reflecting glucose) which were found [33], along with the increase in levels of glutamine [40]. Significant alterations were noted in isocitrate, cis-aconitate, and citrate levels in patients with mutations in FH. In contrast, IDHx mutations were characterized by higher citrate, isocitrate, and cis-aconitate levels [37]. These differences could serve as potential biomarkers for early diagnosis of disease.

3 Metabolomics of Parathyroid Dysfunction

The parathyroid glands are four small pea-sized glands behind the thyroid gland secreting parathyroid hormone (PTH). The primary endocrine glands maintain calcium and phosphorus homeostasis with other hormones, including vitamin D and fibroblast growth factor (FGF23). PTH regulation occurs mainly between three organs, the intestine, kidney, and bone. A complex interplay occurs between PTH, active vitamin D (1,25(OH)2D), and calcium sensor receptors (CaSRs) that maintain serum calcium concentration within a narrow physiological range to maintain mineral homeostasis. Dysfunction of the parathyroid glands occurs as a primary disease of the gland or secondary to other diseases such as chronic kidney disease. Parathyroid dysfunction results in inappropriate parathyroid hormone (PTH) production, resulting in abnormal calcium homeostasis. Phenotypically, it can manifest as either an increase, hyperparathyroidism, or a decrease, hypoparathyroidism, in circulating PTH levels. Primary parathyroid dysfunction is relatively rare compared to secondary causes and can be seen as an isolated condition or component of a complex endocrine syndrome. Hypocalcemia and hyperphosphatemia are the characteristic hallmarks of primary hypoparathyroidism, which is caused by inadequate quantities of circulating parathyroid hormone.

3.1 Hypoparathyroidism

Incidental hypoparathyroidism is a rare disorder with an estimated prevalence of 0.25 per 1000 individuals. Hypoparathyroidism is clinically characterized by decreased parathyroid hormone levels resulting in hypocalcemia that directly impacts calcium and phosphorus homeostasis and the bone. It can occur as an isolated condition or as part of a complex endocrine syndrome. The most common cause of hypoparathyroidism is transient postsurgical hypoparathyroidism resulting from a functional impairment or surgical resection of parathyroid glands after acute manipulation during neck surgery. Other causes include impairment of PTH action, pseudo-hypoparathyroidism, and genetic causes. Numerous somatic or germline mutations have been identified, leading to dysgenesis of the parathyroid gland or an inability of the parathyroid glands to secrete PTH. These include mutation in the PTH gene, GCM2, SOX3, CASR, GNA11, TBX1, CHD7, GATA3, and TBCE [43, 44]. Rare genetic defects involving the transient receptor potential ion channel (TRMP6) and tight-junction gene claudins 16 and 19 have been identified, resulting in the abnormal homeostasis of magnesium leading to hypoparathyroidism (23). The cause of low magnesium levels is attributed to nutritional deficiencies or chronic diseases, including T2DM, hypertension, and renal conditions, which either decrease secretion of PTH or increase resistance to the actions of PTH in the bone and kidneys. Metabolomic studies evaluating changes in the metabolites

within hypoparathyroidism are limited. A single study by Paprocka et al. in children with hypoparathyroidism identified alterations in N-acetyl aspartate by 1H magnetic resonance spectroscopy [45]. Further metabolomic studies are needed to identify the different metabolites altered with hypoparathyroidism.

4 Exocrine Pancreatic Dysfunction: Metabolomics of Cystic Fibrosis

Metabolomics has also been important in studying the pathology of cystic fibrosis, which leads to endocrine-related complications of the pancreas. Cystic fibrosis is a lethal autosomal recessive disorder arising from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene expressed in the apical membranes of various epithelial cells. It is a cAMP-regulated channel that conducts ATP and regulates several apical membrane-associated channels, including the sodium, chloride, and potassium channel along with regulating release of bicarbonate. The disease represents an example of a monogenic defect with over 2000 mutations that leads to characteristic multisystemic disease. Besides the characteristic pulmonary manifestations, patients with CF show endocrine defects in the pancreas and the reproductive system. The most common cause of the pathology is blockage of endocrine ducts due to the thickened secretions. CF mutations can be grouped as those causing severe or mild disease and are further categorized as one of six classes; classes I–III represent severe disease, while mild mutations are classes IV–VI.

Recently, metabolomics has been utilized as an invaluable tool to study the changes in the metabolic profiles in CF, understand the pathophysiology, and elucidate the different metabolic pathways altered with CF [46, 47]. The gold standard for initial newborn screening is a measurement of immunoreactive trypsinogen (IRT) in dry blood spots (DBSs), followed by targeted CFTR mutation analysis and confirmation with abnormally elevated sweat chloride. Our laboratory identified significant differences in 26 metabolites involved in peroxisomal, amino acids, sorbitol, glycolysis, and mitochondrial metabolic pathways. A distinct and interesting finding was the decrease in the osmolyte sorbitol in adult patients with CF patients compared to healthy controls. In order to maintain correct cellular activities and cell survival, organic osmolytes are crucial for regulating cell volume and fluid balance. The perturbation in the sorbitol pathway was identified as a causative factor for the mucoviscidosis [48]. A reduction in the sorbitol levels, and glycerol phosphorylcholine, another osmolyte, was noted in an untargeted metabolomic analysis of primary human airway epithelial cell culture in CF patients. Additionally, significant alterations were noted in the purine nucleotides, adenosine, inosine, hypoxanthine, and guanosine, which may regulate cellular responses via purinergic signaling. Reductions were also seen in metabolites related to glutamate, including oxidized glutathione levels, in *S*-lactoylglutathione, *S*-nitrosoglutathione, and ophthalmate [49].

Metabolomic profiling identified differences in patients with different grades and severity of the disease and between the functional classes of CF. Distinct metabolites were identified that related to clinical phenotype and lung function. We identified specific metabolites between the different CF functional classes using chemical isotope-labeled LC–MS-based metabolomics. The metabolomic profile was assessed between CF and controls, between the different mutation classes of CF, and specifically among classes III and IV. Significant alterations were seen in glutathione, glutamine, glutamate, and arginine metabolism, amino acids, and di- and tripeptides. The significant metabolites include gamma-glutamylglutamic acid, 1-aminopropan-2-ol, cystathionine, ophthalmate, and serotonin. An above-average FEV1% level of lung function was associated with decreased glutamic acid and increased guanosine levels. Metabolomic profiling, between the three analyses, demonstrated alterations in several amino acids and dipeptides governing glutathione metabolism and identified two metabolites in common between the analyses. These metabolites, namely, 3,4-dihydroxymandelate-3-O-sulfate and 5-aminopentanoic acid, could serve as biomarkers for CF [50].

Moreover, serum metabolomics was employed to evaluate CF bacterial lung illness in the preform post-exacerbation stage and identify which systemically measurably connected pathways were impacted throughout recovery. Bile acids, amino acid metabolites generated from microorganisms, increases in the lipid classes of glycerophospholipid, glycerolipids, cholesterol, phospholipids, and the class of sphingolipids were among the compounds and pathways affected. The resolution of the exacerbation was characterized by alterations of the tryptophan–kynurenine pathway, decreased polyamines, a reduction in lipid markers such as fatty acids (n6/n3), and increased in nitric oxide pathway metabolites [51]. On the other hand, metabolites altered with acute pulmonary exacerbation in CF patients demonstrated lower essential amino acids, L-arginine, and oxoproline levels than healthy controls. This decrease was mainly attributed to the skeletal muscle wasting, poor protein intake, increased amino acid utilization, and decreased intestinal absorption of proteins leading to an overall protein-deficient state [52, 53].

In addition to the derangements in the amino acids, patients with CF also showed abnormal lipid metabolism for most lipid subclasses, with significant plasma elevations in odd-chain and polyunsaturated fatty acyl lipids and a decrease in the plasma levels of several species of lysophosphatidylcholine (18:0, 18:2, 20:3, and 20:5) and phosphatidylcholine (36:5, O-38:0, 38:4, 38:5, 38:6, and P-40:1). Plasma phospholipid signatures were found to discriminate between mild and severe forms of CF. In contrast, levels of phosphatidic acids and diacylglycerols were particularly affected by different genotypic mutation classes. A biomarker panel of five oxidized lipids successfully differentiated patients with reduced lung function. Four species of PC (36:3, 36:5, 38:5, and 38:6) were consistently downregulated in severe vs. mild patients, while sphingolipid SM(d18:0) was significantly increased in all patients [54]. The lung function of CF patients is often assessed through forced vital expiratory capacity (FEV1) measurements using FEV1% or FEV1/FVC ratio. Lipid

fractions of the PUFA (C20:3n-9, C20:5n-3, C22:5n-3, and C22:6n-3) positively correlated with FEV1, along with PC (32:2) and PC (36:4), and oleoyl ethanolamide was negatively correlated with FEV1 progression. Lower PC(32:2), PC(38:5), and C18:3n-3, triacylglycerols higher cholesterol, and cholesterol esters were noted in chronically infected patients [55–57].

Metabolomic analysis was also carried out in other body fluids, including sputum, saliva, sweat, urine, bronchoalveolar lavage fluid, and exhaled breath analysis. These studies were generally aimed at identifying the differences in metabolite patterns to unravel the underlying pathophysiological mechanisms of CF and evaluate the effectiveness of treatment modalities. A recent study identified the changes in the lung microbial composition through untargeted metabolomic analysis of the sputum and exhaled breath. Patients with homozygous Phe508del genotype usually receive treatment with combination therapy lumacaftor and ivacaftor. Lumacaftor targets CFTR class II mutations specifically, while Ivacaftor improves the gating (class III) or conduction (class IV) defect in the mutant channels.

CFTR modulators improve CFTR function significantly by partly restoring the function of the chloride channel and improving transport of epithelial fluid in the airways. Besides improving lung function, treatment with CFTR modulators alters the pulmonary microbiome by reducing the abundance of the bacteria, for example, *Pseudomonas aeruginosa*. Metabolomic analysis by GC-TOF/MS showed changes in concentrations of the metabolite phenyl pyruvate in the sputum. On the other hand, the breath metabolome showed alterations in volatile organic compounds such as 4-ethylbenzanoic acid 2-pentyl ester, suggesting a strong link between oxidative stress and inflammation [58]. Untargeted metabolomic profiling of sweat between carriers and cases showed significant alterations in purine derivatives, organic acids, dipeptides, amino acids, and amino acid derivatives, in affected patients, and alterations in levels of asparagine and glutamine, in asymptomatic patients [59]. Patients with CF also present with lung disease characterized by bronchial inflammation due to chronic bacterial infection. The resulting inflammatory response is predominantly dominated by neutrophils. Metabolomic studies were used to identify and quantify the metabolites in the bronchoalveolar lavage fluid samples from these patients. A targeted metabolomic approach identified and quantified metabolites related to proteins, metabolism of purines, polyamines, and nicotinamide which correlated strongly with the clinical markers and neutrophil counts [60]. In addition to these body fluids, the urine metabolomic profile was also studied. The urinary metabolome in CF although heterogeneous showed metabolic alteration that were distinct when compared to non-CF groups. A targeted metabolomic study in the urine revealed an altered methyl status and oxidative stress in children with CF using NMR. Additionally, a subgroup of these children with pancreatic insufficiency showed a considerable rise of phthalate chemicals in their urine NMR spectra in comparison to children with CF who did not have pancreatic insufficiency [47, 61, 62].

5 Conclusion and Future Perspectives

Metabolomics has slowly made inroads into many aspects of patient care and has shown its relevance in understanding disease pathophysiology, diagnosis, and therapeutic monitoring. It provides a bridge between knowledge accumulated from basic science to clinical research as it considers the individual's metabolic characteristics. Combining the clinical (phenotype) with the metabolomic and genomics data will aid the clinical decision-making process by providing more sensitive and specific analyte panels for diagnostic testing. The potential of this omic approach is to further advance in bringing an era of personalized medicine in endocrinology.

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