Regulation of Cortisol Secretion in Humans: Relation to Vasopressin Action at the Adrenals in Macronodular and Micronodular Adrenocortical Tumours; and Well-Being in Addison’s Disease

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Abstract

The hypothalamic-pituitary-adrenal (HPA) axis exhibits tight physiological regulation on a circadian and ultradian basis in humans. Key central regulators include the peptides corticotrophin-releasing hormone (CRH) and arginine vasopressin (VP), acting at the pituitary, and at peripheral structures relevant to the HPA axis and other components of the stress system. Altered regulation has many causes, frequently related to tumorigenesis, and can lead to disease due to an excess of the HPA axis end-organ hormone cortisol, as in Cushing’s syndrome (CS), or cortisol deficiency, as in Addison’s disease. More subtle alterations of HPA axis function have been associated with many diseases. It may be that a lack of normal circadian and ultradian regulation leads to altered well-being.

Studies of three families with the rare cause of cortisol excess, ACTH-independent macronodular adrenal hyperplasia (AIMAH) revealed that adrenal function could be directly stimulated by an aberrant response to exogenous vasopressin (VP; VPs-AIMAH). In addition, it appeared possible to define subtle forms of adrenal dysregulation or early tumour formation, short of clinical CS, thereby expanding the range of phenotypic expression of this disorder for the first time, and further highlighting the familial nature of VPs-AIMAH. Studies of germline DNA, as well as expression of genes potentially relevant to the VP response in adrenal tumours, did not reveal any abnormality to explain heritable VPs-AIMAH. A SNP-based linkage study in the largest (seven affected) family revealed a single potential locus (LOD score 1.83) leading to sequencing of a number of positional candidates. Further studies have included gene expression studies of the familial AIMAH tumours, the most extensive of these studies internationally, in vivo stimulation studies of adrenal steroid intermediates, and finally whole exome capture and next-generation sequencing, all of which has led to increased knowledge in the AIMAH field, but without final gene/mutation identification to date.
Parallel studies examined VP responses in a convenience sample of patients presenting with adrenocortical hormone hypersecretion states or incidentally discovered adrenal tumours, and an attempt to simultaneously examine the negative predictive value of nocturnal salivary cortisol (NSC) sampling to detect the prevalence of mild CS in patients with type 2 diabetes mellitus led to the conclusion that aberrant VP responses are less frequent in adrenocortical tumours, the NSC has a low false positive rate compared with other screening tests, and that mild CS is not prevalent in local diabetes cohorts, consistent with more recent international data.

Finally, a study aimed at determining the importance of circadian and ultradian HPA axis responses was embarked upon in patients with Addison’s disease, a patient group with an unmet need relating to poor well-being. Dose-response dynamic biochemical studies established the feasibility of continuous subcutaneous hydrocortisone infusion (CSHI) to produce physiological ultradian responses to daily life stress. The feasibility of longer term CSHI was studied in a randomised, double-blind, placebo-controlled clinical trial. Recruitment rates have led to this study being adopted at a multicentre level. Ultimately, this study will address the question of the importance of cortisol rhythmicity and responsiveness to well-being in humans.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Lucia Gagliardi and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Lucia Gagliardi

March 2011
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Publications

Peer-reviewed publications arising during candidature:

**Original work**


**Review articles/Book chapters**


**Letters**


**Submitted**

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Abbreviations

ACA  adrenocortical adenoma
ACC  adrenocortical carcinoma
ACT  adrenocortical tumour
ACTH  adrenocorticotropic hormone
AIMAH  ACTH-independent macronodular adrenal hyperplasia
ATP  adenosine triphosphate
AVPR1A  vasopressin receptor type 1A
AVPR1B  vasopressin receptor type 1B
AVPR2  vasopressin receptor type 2
CAH  congenital adrenal hyperplasia
cAMP  cyclic adenosine monophosphate
CBG  corticosteroid-binding globulin
cDNA  complementary DNA
CNC  Carney complex
CNV  copy number variation
CRF  corticotrophin-releasing factor(s)
CRH  corticotrophin-releasing hormone
CS  Cushing’s syndrome
CSHI  continuous subcutaneous hydrocortisone infusion
CV  coefficient of variation
CYP11A1  cholesterol side-chain cleavage enzyme
CYP11B2  aldosterone synthase
CYP17A1  17α-hydroxylase
CYP21A2  21-hydroxylase
dbSNP  SNP database; National Centre for Biotechnology Information
DEG  differentially expressed genes
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DHEA/S</td>
<td>dehydroepiandrosterone/sulphate</td>
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<tr>
<td>DNA</td>
<td>deoxyribose nucleic acid</td>
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<tr>
<td>DST</td>
<td>dexamethasone suppression test</td>
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<tr>
<td>FC</td>
<td>fold-change</td>
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<tr>
<td>FDR</td>
<td>false-discovery rate</td>
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<tr>
<td>GIP</td>
<td>gastric-inhibitory polypeptide</td>
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<tr>
<td>GPCR</td>
<td>G-protein coupled receptor</td>
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<td>GSEA</td>
<td>gene set enrichment analysis</td>
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<tr>
<td>HbA1c</td>
<td>glycosylated haemoglobin</td>
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<tr>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>IM</td>
<td>intramuscular/ly</td>
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<tr>
<td>INDEL</td>
<td>insertion/deletion</td>
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<tr>
<td>IPA</td>
<td>Ingenuity Pathway Analysis</td>
</tr>
<tr>
<td>I.U.</td>
<td>international units</td>
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<tr>
<td>IV</td>
<td>intravenous/ly</td>
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<tr>
<td>LOD</td>
<td>logarithm of the odds</td>
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<tr>
<td>LOH</td>
<td>loss of heterozygosity</td>
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<td>MARA</td>
<td>motif activity response analysis</td>
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<tr>
<td>MAS</td>
<td>McCune-Albright syndrome</td>
</tr>
<tr>
<td>MEN1</td>
<td>multiple endocrine neoplasia type 1</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
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<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
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<tr>
<td>NFAI</td>
<td>non-functioning adrenal incidentaloma</td>
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<tr>
<td>NGS</td>
<td>next-generation sequencing</td>
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<tr>
<td>NPL</td>
<td>nonparametric linkage</td>
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<tr>
<td>NSC</td>
<td>nocturnal salivary cortisol</td>
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<tr>
<td>OMIM</td>
<td>Online Mendelian Inheritance in Man</td>
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</table>
PCA  principal components analysis
PCR  polymerase chain reaction
PD-VP  physiologic dose vasopressin
PKA  protein kinase A
POMC  pro-opiomelanocortin
PPNAD  primary pigmented nodular adrenocortical disease
PRKAR1A  protein kinase A regulatory subunit 1A
RNA  ribonucleic acid
RR  reference range
RT-qPCR  reverse transcription-quantitative PCR
SCS  subclinical Cushing’s syndrome
SF-1  steroidogenic factor-1
SF-36  short form-36 (health survey)
SNP  single nucleotide polymorphism
SNV  single nucleotide variant/variation
Sp1  transcription factor Sp1
StAR  steroidogenic acute regulatory protein
3βHSD  3β-hydroxysteroid dehydrogenase
T2DM  type 2 diabetes mellitus
TGFβ  transforming-growth factor β
UCSC  University of California, Santa Cruz
UFC  urinary free cortisol
VP/-s  vasopressin/-sensitive
WEC  whole exome capture