JRMO Non-CTIMP Protocol Template

TITLE PAGE

Full Title	Genetics of Endocrine Tumours
Short Title/Acronym	Genetics of Endocrine Tumours
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REC Reference	06/Q0104/133
Chief Investigator	Prof. Marta Korbonits

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1.2 Laboratories and/or technical departments

Bart's Health NHS, Royal Devon & Exeter Hospital NHS, Oxford Medical Genetics Laboratory, University of Birmingham and Cambridge, University of Reading, Beatson Institute Glasgow, Institute of Cancer Research (London), Charite University Berlin, Ingemar Pongratz, Karolinska Institutet, Stockholm, Sweden, University of Antwerp, Histopathology Department, Charing Cross Hospital, London, The Francis Crick Institute, London, Brunel University and University of Duisburg-Essen Germany.

1.3 Central facilities

Royal Devon & Exeter Hospital Genetics Laboratory

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2 GLOSSARY OF TERMS AND ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
ASR	Annual Safety Report
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
DMC	Data Monitoring Committee
EC	European Commission
GAfREC	Governance Arrangements for NHS Research Ethics Committees
ICF	Informed Consent Form
JRMO	Joint Research Management Office
NET	Neuroendocrine tumour
NHS REC	National Health Service Research Ethics Committee
NHS R&D	National Health Service Research & Development
Participant	An individual who takes part in a clinical trial
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SSA	Site Specific Assessment
TMG	Trial Management Group
TSC	Trial Steering Committee

3 SIGNATURE PAGE

Chief Investigator Agreement

The clinical study as detailed within this research protocol **(Version 17, dated 03/03/2021)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Chief Investigator Name: Prof. Marta Korbonits

Chief Investigator Site: Barts and the London, WHRI, QMUL

Signature and Date:  **03/03/2021**

Principal Investigator Agreement *(if different from Chief investigator)*

The clinical study as detailed within this research protocol **(Version 17, dated 03/03/2021)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Principal Investigator Name: Prof. Marta Korbonits

Principal Investigator Site: Barts and the London, WHRI, QMUL

Signature and Date:  **03/03/2021**

4 SUMMARY/SYNOPSIS

Short Title	Genetics of Endocrine Tumours
Methodology	Type of study: observational, non-randomised
Research Sites	48 NHS Trusts
Objectives/Aims	*Brief statement of key primary objectives* To identify genes which are involved in the development of endocrine tumours and tumours related to the endocrine system.
Number of Participants/Patients	Approximately 10,000, recruitment will continue for 20 years.
Main Inclusion Criteria	Subjects: Patients with endocrine tumours or tumours related to endocrine system Controls: patients with other conditions who could serve as controls for patients with endocrine tumours Healthy volunteers: patients with no known condition who can serve as an unaffected control No restrictions on age or gender
Statistical Methodology and Analysis (if applicable)	Descriptive statistics for cohort
Proposed Start Date	14/02/2007
Proposed End Date	Approximately September 2037
Study Duration	30 years

5 INTRODUCTION

Background

The endocrine system comprises a network of cells, glands and tissues which, when stimulated, secrete hormones to regulate numerous physiological processes ranging from metabolism, growth and sexual development to sleep and mood. These glands are often intimately related to the nervous system (neuroendocrine tissue) and are widely distributed all over the body. Tumours arising in endocrine tissue (particularly neuroendocrine glands) are often in surgically challenging sites, and both the local effects of the tumour itself plus the local and / or systemic sequelae of treatment may have long lasting consequences for the patient. Advances in the field of molecular genetics have made it possible, in some cases, to identify specific causative genetic defects for both familial and sporadic tumours. Many of these mutations affect cell signalling pathways relating to cell growth and development, leading to aberrant up regulation of proteins that are thought to be related to tumorigenesis. It is vital to identify which genes are mutated, since recently developed small molecule inhibitors offer a potential adjuvant treatment to the standard approach of surgery and chemo-radiotherapy, and early screening of potentially affected relatives may impact on the clinical course of disease. Many of these tumours are extremely rare, which makes research on an isolated disease very difficult. However, these tumours often have features in common, and discoveries pertaining to a specific tumour can often lead to a breakthrough in a related field. This project targets a number of endocrine and neuroendocrine tumours with a view to identifying novel treatments to improve outcomes in this difficult to treat group.

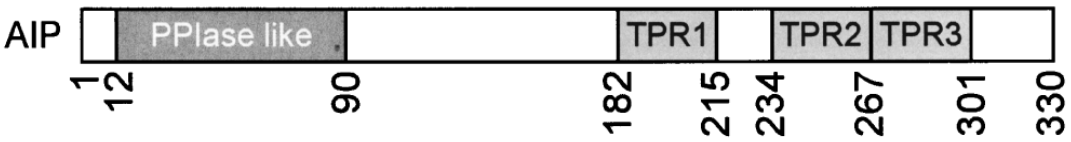
Pituitary

The pituitary gland, a neuroendocrine organ, releases hormones into the circulation in response to neuroendocrine regulation from the hypothalamus. Pituitary tumours comprise around 15% of all intracranial neoplasms, and present with distinct clinical characteristics, usually in terms of local space-occupying effects, or secondary to tumoral hypersecretion or its consequences. Acromegaly and gigantism are due in more than 99% of cases to a somatotroph adenoma, which has been demonstrated to be monoclonal in the great majority of instances[1]. It has been suggested that there are 6 major features of oncogenesis which all need to be present in cases of cancer[2], but only 3 of these (activation of an oncogene, inactivation of a tumour suppressor gene [TSG], inhibition of apoptosis) appear to be relevant to pituitary tumorigenesis; these tumours are usually benign adenomas, and the formation of new blood vessels and the capacity for metastasis are uncommon, although some way of evading senescence may be important. It has therefore been suggested that a very small number of mutations in oncogenes and/or TSG's may be causally responsible for pituitary adenomas. This makes them an excellent model for the early stages of tumorigenesis. However, while much has been established regarding the molecular pathology of these tumours, including extensive studies from our own laboratory[3-7], the initiating mutation or mutations responsible for tumorigenesis have to date defied analysis.

Early work established that some 30% of patients with acromegaly had one of 2 mutations of the alpha-subunit of the receptor-associated G protein, leading to constitutive activation, but it has been difficult to show that this has relevant biological consequences. We have identified a partial failure of the feedback regulation on somatotroph tumours, but no mutations of the relevant genes have been recorded[6, 8]. We and others have also explored the possibility that there are somatic mutations in sporadic somatotroph tumours of genes

identified in some hereditary syndromes associated with acromegaly (MEN-I, Carney syndrome), but these appear to be extremely rare[9]. However, we have collaborated with a Chicago group who have been seeking to identify the gene responsible for familial acromegaly, a very rare dominant condition[10-14]. Some 46 families have been described worldwide over the last 40 years, and from a cohort of 8 families a region on chromosome 11q13 has been identified: we have sought to use data from our microarray studies[15] to pinpoint the abnormal gene in these patients, but so far unsuccessfully. Our recent work has suggested that there are abnormalities of the cell cycle in pituitary adenomas, especially down-regulation of the cyclin-dependent kinase inhibitor p27 and activation of cyclin E, and that this is secondary to a putative abnormal growth factor receptor(s), but specific mutations of these receptors are absent[16].

In 2006 a Finnish group have identified a dominant gene of very low penetrance which appears to segregate with familial somatotroph and prolactin-secreting tumours[17]. This lies at or near position 11q13, but it remains unclear as to whether this is indeed the same gene identified in our families with a much more strongly penetrant condition. The gene codes for AIP (= aryl hydrocarbon receptor interacting protein, also known as XAP2 hepatitis B virus X-associated protein 2 or ARA9 = AhR (aryl hydrocarbon receptor)-activated protein 9. AIP has 330 amino acids and has a PPIase-like domain FKBP12 and four tetratricopeptide repeats (TPRs), probably important for protein-protein interactions.



AIP is a putative activating partner for the aryl hydrocarbon receptor, probably increasing the function of AhR. AhR has been linked to the induction of hepatic detoxifying gene products in response to environmental toxins such as dioxin[18]. However, an additional function appears to be regulation of the cell cycle, suppressing cyclin E and increasing expression of p27[19]. The AhR also has been shown to interact with cyclic AMP. In this latter instance, the interaction with cAMP appears to compete with the dioxin-dependent pathway, such that AhR has enhanced transport into the nucleus with transcriptional effects quite separate to those stimulated by dioxin and related ligands. As cAMP is an important second messenger in somatotroph tumours, this may be the relevant pathway underlying the apparent activity of AIP as a tumour suppressor.

There is therefore *a priori* reason to believe that AIP may indeed function as a TSG in pituitary adenomas, and loss of heterozygosity for 11q13 has been seen in tumours in familial cases[17]. However, the initial Finnish data was based on only 3 families, and we now plan to investigate our entire cohort of families with acromegaly and prolactinomas, as well as further families that we are in the process of collecting. We have contacted many endocrinologists throughout the UK, and we have identified a small number of additional families. Finally, in the initial study some of the patients were apparently isolated cases of acromegaly, but presenting at an unusually young age, especially with gigantism. In such cases mutations of the AIP gene have also been recorded.

Recent data suggest that AIP is in the secretory vesicles and released into the circulation and our preliminary data suggests that it can be measured in the serum. Therefore, we would like to compare circulating AIP levels in patients with familial and sporadic pituitary adenoma. In addition, since AIP is expressed in other tumours and organs such as the heart, circulating AIP levels could be relevant to conditions affecting other organs. Therefore, we would like to collect samples from patients with other endocrine, cardiological and oncological conditions as well as healthy volunteers for comparison.

Novel data has suggested that AhR plays an important role in the development of a recently identified cell type the Th17 lymphocytes. Lack of AhR function in mice results in impaired Th17 differentiation with reduced levels of IL17A and IL22[20]. This has effect on the innate immune function of the individual. We would like to study the function of Th17 cells in patients with AIP mutations and appropriate controls, especially since this system also plays a role in the response to environmental toxins.

There are increasing data suggesting that environmental factors can influence the manifestation of various neoplastic conditions as well as genetic diseases. This could be particularly relevant to pituitary disease, as AIP is a known co-chaperone of AhR, the receptor for the environmental toxin dioxin and other organohalogens. Two recent independent studies from northern Italy[21] and from Sicily[22] suggested this in humans, and more recently corresponding data has been published in cats[23], who are prone to develop acromegaly. In addition, environmental changes can alter methylation pattern of the DNA, and abnormal methylation has already been shown to influence pituitary tumorigenesis[24]. In view of these data we would like to collect DNA, serum and environmental pollutant samples from patients with pituitary disease. As controls we would like to use their spouses or other adult relatives living in the same household and a separate control group with no known pituitary disease. We will collect blood samples for DNA, hormone and organohalogen measurements and household dust (collected from hoover bag contents).

We plan to screen all known families with a history of acromegaly and/or prolactinomas, as well as other pituitary tumour families to be identified in the UK, for mutations of the AIP gene or other genes causing endocrine tumours. As the data suggest that early onset, aggressive, seemingly sporadic cases of acromegaly or prolactinoma could also be caused by germline mutations, we will include patients with clinically sporadic but early-onset aggressive disease.

Pheochromocytoma / Paraganglioma

Some of the signalling pathways that may prove important in growth of pituitary tumours are currently being studied in other neouroendocrine tumours (NETs). Our group has investigated these pathways in pheochromocytoma (PCC - a tumour of the adrenal gland) and demonstrated that combined treatment with small molecule inhibitors against proteins involved in the mTOR signalling pathway was able to inhibit tumour growth *in vitro* using mouse tumour cell lines and *in vivo* using a mouse pheochromocytoma model [25-27]. We now plan to focus on paraganglioma (PGL - related to pheochromocytoma but arising in extra-adrenal paraganglia). The genetics of familial and sporadic PCC/PGL is complicated and at present mutations in at least 15 genes have been linked to the disease[28]. These mutations are grouped into those affecting the tyrosine kinase signalling pathways leading to mTOR

activation (typical in PCC) and those affecting energy metabolism and hypoxia signalling including mutations in the succinate dehydrogenase complex (SDH) subunit proteins A-D (PGL)[28]. Distinct clinical phenotypes related to the different causative germline and somatic mutations have been identified and the syndromes they cause are well defined. We plan to focus initially on two syndromes – PGL1 (caused by mutations in SDH-D) and PGL4 (mutations in SDH-B). PGL 1 is associated with multiple (usually benign) head and neck PGL (H&N PGL) as well as PCCs. Although the tumours are slow growing, significant morbidity occurs as the tumour burden increases in size. H&N PGL arise close to the skull base and often may compromise cranial nerves or major vessels due to pressure effects. Surgery to treat them carries major risks of damaging these vital structures, and for patients with bilateral disease alternatives such as gamma knife radiotherapy are usually indicated but are unlikely to ultimately halt the disease process. Patients with PGL4 typically show early onset disease, can develop PCC or PGL, have a higher risk of malignancy and are at risk of additional neoplasms including thyroid tumours and renal cell carcinoma. In cases of aggressive malignant or metastatic disease, survival remains poor despite recent advances in chemotherapy. We have established a method of generating primary culture of PGL and plan to use this to investigate treatment combinations with a view to describing novel treatment options for these patients.

Adrenal cortical adenoma and carcinoma

Benign or malignant adrenal cortical adenomas secreting steroid hormones (cortisol, androgens, aldosterone, deoxycortisol etc) or having no identifiable secretory activity are relatively commonly diagnosed. They can be part of a genetic syndrome such as the classical Carney complex, McCune-Albright, MEN1 or p53-related syndrome or can harbour germline or somatic mutations in ARMC5, potassium channel, ATP- or PKA-related pathways[29-32]. The genomic landscape of somatic mutations of adrenocortical carcinomas has also been recently characterised[33, 34]. These mutations can influence clinical course and severity, sensitivity for treatment and prognosis as well as predisposition to other family members. We will study these tumours for the currently known gene variants and will exploit the identification of new genetic alterations both at germline and somatic level. We have established a method of generating primary culture of adrenal specimens (tumours and normal adjacent) and plan to use this to investigate adrenal stem cells, cancer-stem cells and to test novel treatments options through in vitro and in vivo experiments.

Olfactory neuroblastoma

Olfactory neuroblastoma (ONB) is a rare neuroendocrine tumour affecting all ages with no race, sex or geographical predilection. It arises high in the nasal cavity at the site of olfactory mucosa, usually close to the cribriform plate. Initial symptoms are very non-specific and patients typically have a drawn out investigation schedule, resulting in many cases being locally advanced at the time of definitive diagnosis[35]. Long term studies have shown that patients with advanced local disease, orbital involvement, neurological involvement and regional or distant metastatic disease have a very poor prognosis with currently available treatments[36]. When the disease occurs in children, it is almost always advanced at the time of diagnosis, and the consequences of surgery and / or radiotherapy to the developing mid-face and brain can be significant – including visual disturbance, craniofacial abnormalities and pituitary hypofunction[37]. Population studies have highlighted the risk of radiation induced second primary tumours and this is greatest in patients undergoing treatment as a child or young adult[38]. ONB has a tendency to recur even in localised cases and can

present as recurrence or metastatic disease even 20 years after initial treatment, therefore patients require lifelong surveillance. Cytogenetic characterisation studies and comparative genomic hybridisation studies have highlighted regions of the genome that are frequently involved in ONB. Although many genes map to these regions, a subsequent whole genome sequencing revealed mutations in genes coding for signalling pathway proteins related to the mTOR pathway when the somatic genetic mutations were run through ingenuity pathway analysis (IPA). An unrelated study also demonstrated up-regulation of the Hedgehog signalling pathway in ONB tumours. As yet there have been no studies focused on targeting the mTOR pathway in ONB – we plan to study this with a view to defining new treatment regimens that can be used as an adjunct to currently available methods. We will be testing novel small molecule inhibitors against ONB, which if effective may lead to human trials in cases of inoperable metastatic disease. We may expand this area of study to include other NETs which show mutations / upregulation of the relevant signalling pathways, including PGL, MTC and pituitary. One major benefit of studying nasal tumours is that it is easily possible to obtain control tissue samples from unaffected patients for comparison purposes, making it easier to identify genes specifically upregulated in the tumour.

Medullary thyroid carcinoma

Medullary thyroid carcinoma (MTC) is a rare malignant tumour arising from the calcitonin-producing parafollicular cells (C cells) of the thyroid accounting for 5–10% of all thyroid cancers and for 13.4% of all thyroid cancer-related deaths. MTC can occur in a familial setting in 25% of cases, either as MTC alone (familial MTC, fMTC) or associated with pheochromocytomas and/or primary hyperparathyroidism, configuring the multiple endocrine neoplasia type 2 syndrome (MEN2). Most MEN2 families (about 98%), as well as fMTC kindreds (90-95%), bear germline mutations in the proto-oncogene *RET*, that encodes a receptor tyrosine-kinase involved in the regulation of cell proliferation, growth, differentiation, migration, survival, and apoptosis[39]. Up to 5-10% of fMTC families do not have any detectable *RET* mutation and, in these cases, the disease-causing gene(s) are yet to be identified. Moreover, somatic *RET* mutations are found in approximately 40% of sporadic MTC, while somatic *RAS* mutations have been found in 12.9% of sporadic MTC (meta-analysis of 14 studies)[40]. The best treatment option in MTC is surgical resection; however, biochemical cure can be achieved only in 30-40% of patients with regional lymph node metastases, and this severely affects the prognosis of these patients[41]. As medical treatment options are quite limited, a better understanding of the pathways involved could provide new drug targets for MTC.

Insulinoma and insulinomatosis

Insulinomas are pancreatic NETs arising from β cells and represent the most common cause of pancreatic hyperinsulinaemic hypoglycaemia in adults[42]. Approximately 10% of insulinomas are multiple, and half of these are associated with the MEN1 (multiple endocrine neoplasia type 1) syndrome[43, 44]. In a large insulinoma series, 5% of patients presented with insulinomatosis, defined as multicentric insulin-secreting tumours without clinical and genetic evidence of MEN1[45]. Insulinomatosis can arise in a familial setting[45, 46], and has been reported to be associated paradoxically with the occurrence of diabetes within the same family[5]. The pathogenesis of insulinomatosis is currently unknown. Through our network of collaborators, we have been collecting DNA samples from patients with either familial or sporadic insulinomatosis, and we aim to employ next generation sequencing to identify the

disease-causing mutations in these patients. As we believe that the diabetes observed in some family members may recognise the same genetic cause as the insulinomatosis, we also aim to study the features of these patients' diabetes and screen the unaffected family members for the presence of hyperglycaemia or impaired glucose tolerance. The understanding of the molecular mechanisms underlying the pathogenesis of insulinomatosis could reveal novel pathways involved in neuroendocrine tumorigenesis and β cell proliferation.

Craniopharyngiomas

Craniopharyngioma (CP) is a neuroepithelial intracerebral neoplasm arising from the pituitary and is associated with significant morbidity and mortality. Located in or just above the sella turcica, CPs are difficult to remove surgically because of their infiltrative behaviour, which affects surrounding vital structures including the hypothalamus, visual pathways and cranial nerves. Treatment for these tumours is currently limited to surgery with or without radiotherapy, both of which are associated with significant adverse effects. Most tumours have a high recurrence rate after resection, and all tumours have significant morbidity and mortality. The disease and/or treatment-related consequences are in themselves life-threatening or life-reducing. CPs are subdivided into two types, the adamantinomatous craniopharyngioma (ACPs), which affect mainly children (90% of cases) and the papillary form (PCPs), which affect both young adults and adults. Histologically and molecularly, these two subtypes are different: ACPs present with keratin nodules, epithelial differentiation, cholesterol deposits and have cystic appearance. The molecular mechanisms and the causative mutations for ACPs remained elusive making it difficult to identify novel treatment strategies for ACPs tumours. Recently somatic mutations in the phosphorylation sites that promote degradation of β-catenin were observed in 65-70 % ACPs[47]. These mutations lead to strong nuclear accumulation of β-catenin, however whether β-catenin has a primary role in these tumours remains unclear. PCPs consist of squamous cells and they are less differentiated tumours compared to ACPs. Although equally aggressive and leading to high morbidity, nothing is known about the molecular aetiology PCPs, in part, due to the lack of a suitable animal model and the lack of identification of causative mutated genes. In 2014, a human exome-sequencing study has identified the activating mutation V600E in the *BRAF1* oncogene in a cohort of PCP patients[48]. We plan to use tumour tissue to identify of novel genes causative of both ACPs and PCPs by using the protocols specified herein.

In addition to the above specific studies we may need to assess tumour tissue from other endocrine tumours – particularly those related to the diseases outlined above. Data from our pituitary study which started in 2006 revealed that some of the families with familial acromegaly might have a second tumour. Among others these have included thyroid, breast, testis, adrenal, adipose tissue and tumours related to the endocrine system (non-specified tumour and hamartomas). PCC, PGL and MTC are also linked to a number of other tumours, and the number of associated tumours may increase as the genetic background is studied further. The rare tumours ONB and CP do not have any known associated tumours as yet, but this may change over the course of this study. It is therefore essential to include these other tumours in our study, since this data will contribute to our overall understanding of pathogenesis of endocrine tumours.

Population Study

A special additional pituitary study has been initiated. We have established that one particular gene abnormality is prevalent in patients with Irish origin, especially from the Tyrone, Northern Ireland area[49]. Our current Ethics permission allows screening of all patients with acromegaly or prolactinomas in Northern Ireland. We would like to screen the general population of Northern Ireland overall and specifically the Tyrone area. For the overall general population, we would like to include general population samples from Ireland and compare it with general population samples from Scotland and England so we can establish the background frequency for the R304X mutations. These screens will be performed according to the ethics permission valid for the general population cohorts we will collaborate with.

For the local area screening subjects will be recruited via GP surgeries and via leaflets in public places and churches. GPs will be notified about the study in advance. We envisage that this screening will identify a number of carriers who could be formally referred to appropriate genetic clinic and we hope (and have data already from identified families) early disease recognition will lead to easier treatment and better cure.

6 TRIAL OBJECTIVES

- Primary Objectives

- To identify genes which are involved in the development of endocrine tumours and tumours related to the endocrine system
- To establish the phenotype of patients with genetic alterations in endocrine tumours compared to patients with no such changes and to normal controls
- To study family members of patients, screening for genetic alteration and signs of development of disease
- To establish the mechanism of effect of such genetic alterations

- Secondary Objective

- To identify potential therapeutic targets and test them in vitro and in vivo where possible
- To assess the anti-tumour efficacy of novel small molecule inhibitors for 3 purposes:
    - To establish new treatment options for advanced disease
    - To establish a novel adjuvant treatment to complement the current gold standard for localized disease
    - To improve understanding of the complex system of signalling pathways involved in oncogenesis of many endocrine tumours
- To establish animal models where we can further explore the genetic basis of these tumours, and test our in vitro findings

- Primary Endpoint

- New genes identified in families or sporadic patients
- Disease phenotype established
- Mechanism of effect of novel genes identified

- Secondary Endpoint

- New drug target proven and taken forward to human trial stage
- New disease model established

7 METHODOLOGY

Following patient consent, clinical notes, images and investigations are reviewed, patient's symptoms recorded using questionnaires (see appendix for headache questionnaire) and symptom scores as appropriate.
Samples will either be processed in the laboratory at Barts or handled anonymously in one of the collaborating departments listed below. Patients either recruited prospectively to the study or retrospectively when the samples will be anonymised, so that there is no link between the data we generate and the patients themselves.

Patient tumour samples will be collected for DNA, RNA and protein analysis including gene and protein expression, next generation sequencing techniques (e.g. whole genome sequencing, whole exome sequencing, RNAseq and DNA methylation studies).

In the future and independent of our current study aims, it is likely that analysis of a person's whole DNA genetic code will become much more feasible in research, due to rapidly advancing technology. We may wish to do this with the samples of this study and link the results to clinical data. This may provide some useful knowledge about how genes and medical conditions are connected in more general terms. Any such analysis would be carried out on the samples only in an anonymised form, meaning that the samples etc. are identified only by a code number, so the research teams would not know patient identity. Data will not be returned to patients as identity would not be known to the researchers. There is a very small chance that patient could be identified as the donor of that sample if the DNA was analysed again for some other reason separately from our study, and the genetic codes compared. We consider this to be extremely unlikely to occur without patient knowledge and approval. Our data will not be used for forensic testing.
We would like to study (for gene sequence and expression changes) blood or saliva samples and sporadic or familial tumours of these organs as well as skin, bowel, liver, bone, brain, prostate, kidney, lung, pancreas, lymphoid tissue for expression of AIP and other related endocrine tumorigenesis genes. We would also like to use hair and urine samples to perform innovative hormone assessment. Organohalogen exposure will be measured from nail clippings. For patients with familial insulinomatosis (including unaffected carriers), we would like to collect blood samples both in the fasting state and after stimulus with a standard oral glucose load (75 grams) and a mixed meal, in order to investigate their glucose metabolism and screen these subjects for the presence of diabetes mellitus. These tests are very well established and extensively used in clinical practice. Age-, gender- and BMI-matched healthy volunteers will also undergo the same studies with the purpose of comparing the results to those obtained in the patients. Immunohistochemistry will be performed on paraffin embedded tumours and control tissue to demonstrate up regulation of target proteins in the tumour cells. DNA and RNA will be extracted from formalin fixed tissues and subjected to PCR for mutational analysis. RNA may also be subjected to RT-PCR and qPCR to confirm expression of activated forms of key proteins of interest, which can then be targeted with siRNA techniques and / or transfection studies to further explore their role in the disease process. From selected patients cell lines will be created according to standard NHS routine in accredited NHS laboratories (appropriate consent form from the

laboratory will be signed by the patient and filed in notes). We will also generate primary cultures direct from tumour tissue, to investigate protein expression and evaluate response to targeted treatments using western blot analysis of protein expression and cell viability / apoptosis / cell cycle assays. Where primary culture proves very difficult and with samples from very rare tumours, patient derived xenografts in SCID / Nude mice will be generated, by fully trained individuals in an approved facility with appropriate personal, project and site licensing as required by Home Office guidelines, to facilitate propagation of tumour cells for evaluation of responses to treatments. If appropriate, immortalisation of the cell line may be attempted to facilitate certain experiments and longer-term research. Serum/plasma samples will be analysed for novel circulating DNA, RNA, metabolites and proteins, which could act as tumour markers for the disease. Peripheral blood cells will be studied in immunological experiments. From selected patients, skin biopsies may be taken to examine the mosaic nature of the disease or to gain patient-derived fibroblast for study. This procedure is commonly used in clinical and research practice.

In addition, where appropriate (e.g. pituitary, MTC, PGL syndromes) affected family members will be identified, as well as first-degree relatives both affected and unaffected. Patients identified with genetic abnormalities will be referred to appropriate clinical services. At the same time, all apparent mutations or polymorphisms of novel genes will be tracked in a cohort of at least 100 germline blood samples from normal volunteers for assessment of the background gene frequency in order to assess their relationship to disease status.

Tissue samples:

Affected patients:
Samples collected will include blood, serum / plasma, peripheral lymphocytes, saliva, hair, urine, teeth, bones, nail clippings and tumour tissue (fresh, frozen and formalin fixed / paraffin embedded).

Wherever possible, samples will be collected by designated GCP-trained members of the study based at Queen Mary University of London or Bart's and the London Hospital Trust. Where this is not possible, permission is given by the chief investigator for samples to be collected by designated GCP-trained staff from the collaborating institutions listed in this document.

Control samples:
For comparison studies healthy volunteer blood and saliva samples (as well as hair, urine and tissue samples) will also be collected and subjected to the same analysis as detailed above. Since many endocrine disorders affect multiple systems of the body, we would also like to collect DNA/RNA/protein, saliva and serum samples from patients with other endocrine, cardiological and oncological conditions for comparison. Samples will be treated as already detailed above. To study adenomatous pituitary or other tumour tissues comparison with normal tissue is essential: as some of these tissues, especially normal pituitary tissue, is very difficult to access during surgery, the only source of such material is from autopsy or cadaveric specimens. This is therefore required for our studies. For other tumour types studied, control tissues samples are also required. This would apply to all body

sites involved in the above project (including but not limited to, thyroid, breast, testis, adrenal, adipose tissue, skin, bowel, liver, bone, brain, prostate, kidney, lung, pancreas, lymphoid tissue and head and neck sites). Where possible, we want to use fresh adjacent normal tissue / marginal biopsies from surgical resections (performed for non-NET related reasons) or where appropriate and in cases which will not result in harm to the patient, to obtain control tissue specimens from patients undergoing surgery for non-related conditions (for example biopsy of nasal mucosa in patients undergoing nasal reconstruction surgery) or a small biopsy from a healthy volunteer[24]. The tissue will be processed in an identical manner to the tumour tissue processing detailed above. Patients who would be suitable to act as control sample donors are identified either by the nature of their operation (e.g. nasal surgery for correction of nasal deformity but not to treat underlying pathology) and approached either at clinic review, pre-assessment or on the day of surgery to explain the nature of the study and request permission to use some of their tissue sample as control. A similar process may apply to patients undergoing thyroid and other endocrine gland surgery for non-malignant indications. In such cases the patients are identified as suitable by their surgical team and recruited by their team who will also gain consent. In the case of children, parental consent will be obtained **before** any approach is made to their child. In cases where control blood or saliva samples are required patients are identified when they attend non-endocrine medical / surgical clinics (such as cardiology) if they need tests anyway as part of their work up for their underlying condition. They are recruited to the study by the consultant's team they are being treated by, and if they consent, additional samples are taken for the study on the same day that they attend for their routine tests. In each case the donor is asked to sign appropriate consent forms and given an information leaflet explaining the remit of this study (**please see 'Gaining consent SOP'**). They are also provided with contact information and are able to withdraw consent at any stage should they change their mind.

Collaborating clinical departments:

Tissue accrual:

The patients will be recruited from Endocrinology Departments from collaborating NHS Trusts (Bart's Health, UCLH, Royal Free and University College School of Medicine, St George's Healthcare, Guy's and St Thomas' Hospital, Great Ormond Street Hospital, Mid Yorkshire Hospitals, Norfolk & Norwich University Hospitals, Nottingham University Hospitals, University Hospital Southampton, Southend University Hospital, Newcastle upon Tyne Hospitals, Oxford University Hospitals, University Hospital of North Staffordshire, Northern General Hospital Sheffield, Salford Royal Manchester, Aberdeen Royal Infirmary, Plymouth Hospitals, Leeds General Infirmary, Cambridge University Hospitals, University Hospitals Birmingham, University Hospitals Bristol, Lothian Edinburgh, Liverpool (Aintree Hospitals, Alder Hey Children's, Royal Liverpool Children's Hospitals, Liverpool Women's), University Hospitals of Leicester, Belfast HSC, King's College Foundation Trust Hospital, Royal Devon and Exeter, Imperial College Healthcare, Derby Hospitals, Cardiff University School of Medicine, The Ipswich Hospital, Medway Maritime Hospital, East and North Hertfordshire, North Bristol, Great Western Hospital Swindon, Barking Havering & Redbridge University Hospitals, Central Manchester University Hospitals, Countess of Chester NHS Foundation Trust, Lancashire Teaching Hospitals NHS Foundation Trust, University Hospitals Dorset NHS Foundation Trust, Sheffield Children's NHS Foundation Trust, Ninewells Hospital and Medical School, The Walton Centre, The Christie NHS Trust,

University Hospitals Coventry and Warwickshire and West Hertfordshire Hospitals NHS Trust).

Analysis:
At the start of this project all the genetic screening of patients was performed in our research laboratory at Bart's. However, since that time, for the AIP gene the molecular genetics laboratory at the Royal Devon & Exeter Hospital, a CPA accredited NHS/Research laboratory, has set up a comprehensive service and we collaborate with them on the analysis of the samples. Most genetic testing of known or novel genes and areas will be performed at the CPA accredited molecular genetics laboratory in Exeter or at our research laboratories at Bart's and Oxford. Additional analysis of anonymous samples may also be undertaken by a specialist laboratory (or laboratories), as required. These sites may include our collaborators Dr Elaine Harper at Syntaxin, Oxford, Prof Eamonn Maher at the University of Birmingham and Cambridge, Prof Rainer Cramer at the University of Reading, Prof Eyal Gottlieb and Dr Simone Cardaci at the Beatson Institute Glasgow and the Institute of Cancer Research, London, Prof Christian Strasburger, at Charite University Berlin and Ingemar Pongratz, Karolinska Institutet, Stockholm, Sweden. Dr. Adrian Covaci, Toxicological Center, University of Antwerp, Charing Cross Hospital, Prof. Charles Swanton, The Francis Crick Institute in London, Dr Michael Themis, Brunel University and Prof. Ralf Kuppers, University of Duisburg-Essen. Craniopharyngiomas may be assessed by specialist pathologists Professor Teresa Ribalta Farres (Barcelona Childrens' Hospital) and Associate Professor Gaetano Bulfamante (Universita Degli Studi Di Milano)

Inclusion Criteria
Patients with endocrine tumours
Family members of patients with endocrine tumours
Patients with other diseases serving as controls for endocrine patients
Individuals who are asymptomatic but are at risk of carrying a genetic mutation
Healthy volunteers serving as controls for endocrine patients
Samples from autopsy with appropriate anonymization
Samples from historical cases with appropriate anonymization

All ages are included. Paediatric patients (age 6-15) have special Information sheets and Consent forms and parental agreement is also requested. Patients under the age of 6 will have parental agreement only. Next of kin will sign the patient consent forms on the behalf of the deceased patient.

Exclusion Criteria
Subjects who are not willing to sign the informed consent and do not fit the inclusion criteria.
Patients that cannot understand the consent forms or PIS in English, unless a qualified translator is present
Patients lacking capacity to consent

8 STUDY DESIGN / PLAN – STUDY VISITS

Patients are contacted via their treating physicians (in or outpatient), via their family members or patients contact the researcher via patient website.

- Inclusion and exclusion criteria assessed.
- Patients meeting the inclusion and exclusion criteria are given information about the study and are given an appropriate information sheet about the study
- Relevant patient contact details are taken
- Patients are given opportunity to ask questions and raise any issues
- Patient gives informed consent
- Patient sample is taken (blood, saliva, tissue etc)
- Patients notes, images and investigations are reviewed, patients' symptoms recorded, using questionnaires/symptom scores as appropriate
- Patients and their doctors are informed of their result, and if there is any risk to their relatives arises, possibility of contact to family members is discussed

Schedule of Assessment (in Diagrammatic Format)

	Initial visit				Intervention	Follow up
Assessment	Screening endocrine patients	Control patients	Normal volunteers	Family member of affected patient	Treatment of endocrine patients	Follow up visits
History and / or physical	x	x	x	x	x	x
Weight / height	x	x	x	x	x	x
Imaging (MRI/CT/X-ray)	x			If indicated	x	x
ECG	x	x	x	x	x	
Saliva	x	x	x	x		x
Urine	x	x	x	x	x	x
Blood tests	x	x	x	x	x	x
Biopsy / FNA	x	x	x	If indicated		x (if recurrence)
Fresh tissue samples		x	x	If indicated	x	x (if recurrence)
Fresh frozen samples		x	x		x	X (if recurrence)
Adjacent normal tissue samples		x			x	
Hair samples	x	x	x	x		
Nail clippings	x	x	x	x		

Samples will be studied for DNA, RNA, protein content, hormone levels, plus tumour tissue will be processed to commence primary cell culture, extract RNA / DNA and next generation sequencing analysis.

End of Study Definition

This is an observational study which observes patients for the remainder of their lifespan to enable us to establish the natural history of these rare diseases. Many of these tumours are known to recur or are associated with other tumour development, hence there is a need for long-term follow-up. Patients will remain in the study indefinitely unless they withdraw.

9 STATISTICAL CONSIDERATIONS

We have an established collaboration with Dr Jonathan Bestwick, a statistician at the Wolfson Institute.

- Sample Size

This is a nationwide observational study on endocrine tumours and their related novel diseases, where classical sample size calculations cannot be performed. We have setup a large, international patient cohort and plan to collect samples and patient outcome data for the next 30 years at least.

- Method of Analysis

- Descriptive demographic and treatment data will be analysed with group comparison tests (two-sample or multi-sample, parametric or nonparametric) and correlations will be calculated.

Regular interim analysis will be performed throughout the duration of this research, and at the end of individual projects (PhD, post doc – i.e. every 2-4 years).

10 ETHICS

Patients and suitable control subjects will be identified as outlined above and approached either in clinic or via their primary physician, to request that they enrol in the study. Some patients contact us directly via the website or after receiving information regarding our study by other clinicians. They are given information leaflets outlining what our study aims to achieve, and what we will do with the samples we take. They will understand that they can refuse or change their mind at any time. They will also be informed that they can access the results but will be made aware that these are long term studies and that results may take years. We will explain that nothing we do will interfere with their diagnosis / staging or with the subsequent treatment that they may require. Control patients will also be provided with information regarding our study, plus an outline of why we need healthy tissue for comparison and reassurance that they should not suffer any adverse outcome as a result of being included in this study. Patients or family members detected as carrying oncogenic mutations will be referred to an appropriate genetic counselling service, and followed up as appropriate. The same will be done for any family member unexpectedly diagnosed with a tumour during the study.

11 SAFETY CONSIDERATIONS:

This is a well-established observational study and there have been no safety issues related to this study. Any adverse events arising will be handled as detailed below.

12 DATA HANDLING AND RECORD KEEPING:

- Confidentiality

Information related to participants are kept confidential and managed in accordance with the Data Protection Act, NHS Caldecott Principles, The Research Governance Framework for Health and Social Care, and the conditions of Research Ethics Committee Approval.

- Record Retention and Archiving

Archiving of study data will follow Barts Health rules according to requirement of the Research Governance Framework and Trust Policy for the 20 years following data collection.

- Anonymization of samples:

All human tissue material, whether cellular or non-cellular, will be handled according to the requirements of current legislation. Samples will be link-anonymised (if consent permits) or anonymised, with the link-file kept is a secure location with access restricted to authorised staff, in accordance with ethical approval. All material transferred, stored or exported will only be with appropriate consent or ethical approval in anonymised form, with the identity of the donor withheld. In the event of a finding of potential clinical significance to donors or their relatives this will be notified to the originating institution using the anonymised code. In such an event, a decision will be made by the originating institution team as to whether the identity of the donor should be determined using the link file for notification through the 'normal care' or research team as appropriate.

- The General Data Protection Regulation (GDPR) (Regulation (EU) 2016/679):

All data will be handled in compliance with current legislation such as the Data Protection Act, in accordance with Ethical Approval, or with any subsequent legislation such as the proposed General Data Protection Regulations of the European Union or successor body as applicable to the UK in the event of future developments.

13 LABORATORIES (if applicable)

Refer to Methods section in Methodology.

- Central/Local Laboratories

At the start of this project all the genetic screening of patients was performed in our research laboratory at Bart's. However, since that time, for the AIP gene the molecular genetics laboratory at the Royal Devon & Exeter Hospital, a CPA accredited NHS/Research laboratory, has set up a comprehensive service and we collaborate with them on the analysis of the samples. Most genetic testing of known or novel genes and areas will be performed at the CPA accredited molecular genetics laboratory in Exeter or at our research

laboratories at Bart's and Oxford. Additional analysis of anonymous samples may also be undertaken by a specialist laboratory (or laboratories), as required. These sites may include our collaborators Dr Elaine Harper at Syntaxin, Oxford, Prof Eamonn Maher at the University of Birmingham and Cambridge, Prof Rainer Cramer at the University of Reading, Prof Eyal Gottlieb and Dr Simone Cardaci at the Beatson Institute Glasgow and the Institute of Cancer Research, London, Prof Christian Strasburger, at Charite University Berlin and Ingemar Pongratz, Karolinska Institutet, Stockholm, Sweden. Dr. Adrian Covaci, Toxicological Center, University of Antwerp, Charing Cross Hospital, Prof. Charles Swanton, The Francis Crick Institute in London, Dr Michael Themis, Brunel University and Prof. Ralf Kuppers, University of Duisburg-Essen. Craniopharyngiomas may be assessed by specialist pathologists Professor Teresa Ribalta Farres (Barcelona Childrens' Hospital) and Associate Professor Gaetano Bulfamante (Universita Degli Studi Di Milano)

- Lab Procedures

Routine clinical care: DNA sequence determination, routine hormone level investigations, imaging studies, urinalysis, surgical intervention / biopsy

Non-Routine clinical care: RNA expression determination, protein expression determination, circulating novel hormone level determination, in vitro tissue culture, next generation sequencing, DNA sequence determination, sample collection (serum, blood, hair, nail, urine, saliva).

- Data Preparation and Collection
  - Sample labelling will be according to individually generated study codes.
  - We have an established set of tumour specific databases to record samples as they arrive / leave our facility via an in and out log.
  - Samples are kept according to nature of the sample (room temperature, -20 or -80 freezers)
  - Samples are stored in dedicated cabinets and temperature monitored freezers

14 PRODUCTS, DEVICES, TECHNIQUES AND TOOLS

Devices

N/A

Techniques and interventions

N/A

Tools

N/A

Medicinal product

N/A – This is an observational study and therefore any procedures we perform on these tissues will not impact the patient in any way.

15 SAFETY REPORTING

Adverse Events (AE)
We have established protocols for reporting adverse events should they arise. Any clinical adverse event arising secondary to this study will be reported via the standard reporting system of the relevant trust, and also reported to the PI.

Serious Adverse Event (SAE)
As above, but in this case the R&D office will also be notified.

Urgent Safety Measures
N/A

Annual Safety Reporting
We perform an annual audit to review any safety and adverse events arising, and will alter protocols if necessary to minimise future risk. If this is required R&D will be informed.

Overview of the Safety Reporting responsibilities
The CI/PI has the overall pharmacovigilance oversight responsibility. The CI/PI has a duty to ensure that safety monitoring and reporting is conducted in accordance with the sponsor's requirements.

The study coordinator and PI are responsible for safety reporting.

16 MONITORING & AUDITING

The local HTA committee regularly monitors our activities and record keeping
SOPs are kept updated for all activities. GCP training is up to date for all study personnel and all follow GCP rules.

17 TRIAL COMMITTEES

The trial management committee consists of:
Professor Sian Ellard, Professor Ashley Grossman and Professor Marta Korbonits

18 FINANCE AND FUNDING

The study has received funding from MRC, Wellcome, BTLC, Pfizer, NIHR, The Royal Society, Mexican government grant scheme and we plan to apply for funding for research council and charity grants and investigator initiated pharmaceutical funding in the future.

19 INDEMNITY

Queen Mary University London is the sponsor of this study and provided the necessary indemnity.

20 DISSEMINATION OF RESEARCH FINDINGS:

Study results are disseminated in scientific literature (JCEM, NEJM, Clinical Endocrinology, Trends of Endocrinology and Metabolism, Journal of Endocrine Investigation, etc), via the media (BBC), via patient website (www.fipapatients.org), patient meetings and via talks on conferences (ENDO, ECE, BES, Pituitary meeting) and teaching courses organised in the UK and abroad.

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22 APPENDICIES

Please do not include the Participant Information Sheet and Consent Form; these should be stand-alone documents